Final Report

Review of the risk management practices employed throughout the fish processing chain in relation to controlling histamine formation in at-risk fish species

FS241055

Date: 31.10.2013

Christian James, Simon Derrick, Graham Purnell & Stephen J. James

Food Refrigeration & Process Engineering Research Centre, Grimsby Institute, Nuns Corner, Grimsby, North East Lincolnshire, UK, DN34 5BQ

FSAS Project Officers: Elaine Steele & Lorna McIvor

1. Executive summary

This project was carried out for the Food Standard Agency in Scotland (FSAS) to review the risk management practices employed throughout the fish processing chain in relation to controlling histamine formation in at-risk fish species in Scotland.

Histamine (Scombroid or Scombrotoxin) fish poisoning is a foodborne chemical intoxication associated with the consumption of spoiled fish flesh that has a high histamine content. Symptoms include: rapid-onset headaches, sweating, rash, nausea, vomiting, and diarrhoea. The fish species normally associated with histamine fish poisoning are pelagic fish species (pelagic fish live near the surface and are usually agile swimmers with streamlined bodies) such as mackerel, herring, sardines, pilchards and certain tuna species. The pelagic fish sector represents the largest fish sector in Scotland, by volume of landings.

The objective of this project was to provide a comprehensive review of current risk management practices for controlling histamine, in at-risk fish species, throughout the Scottish fish processing chain. The main aim of the review was to identify key risk areas in the whole chain (from catch-to-fork) and any gaps in the management of these. To achieve this it had three objectives:

1. To critically review relevant literature to identify and quantify key monitoring and intervention steps relevant to the control of histamine throughout the chain.
2. To survey current management and control practices for at-risk fish in key industrial sectors (catching, landing, processing) in Scotland.
3. To compare all literature and measured data to identify any gaps in current knowledge/management procedures and any areas where the sector could benefit from advice or guidance.

This report summarises the outcome of this project.

The literature review concluded that, of the fish species targeted by the Scottish pelagic fishing sector, Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*), horse mackerel (*Trachurus trachurus*), and sardine (*Sardina pilchardus*) are the fish species likely to be “at-risk” of containing high histamine levels capable of causing histamine fish poisoning. However, no literature was identified that has assessed the histamine risk of blue whiting (*Micromesistius poutassou*) or sprats (*Sprattus sprattus*). There is currently no evidence that Scottish Atlantic salmon (*Salmo salar*) represents an at-risk fish species.

The overall occurrence of histamine fish poisoning in Scotland, and the UK as a whole, is low. In recent years tuna has been the suspect vehicle in all outbreaks and the majority have been related to food service. Whilst the occurrence of reported illness caused by histamine from seafood within Scotland, and the UK as a whole, is low, it is believed that the incidence of histamine fish poisoning is vastly under-reported. This is because the range of symptoms, and their severity, caused by histamine fish poisoning mean that consumers do not always inform the authorities or seek medical attention upon being symptomatic.

The industrial survey showed that the current pelagic sector in Scotland primarily consists of a highly integrated industrial operation composed of 26 modern pelagic trawlers and 5 primary processors located in 3 ports. These handle 99.8% of the annual catch of 129,000 tonnes (2010 data; Marine Management Organisation

(MMO), 2011) and 95% of this product is then exported as a frozen product by the primary processors. The modern pelagic trawlers all use refrigerated sea water (RSW) tanks to ensure good temperature control post capture and all primary processors undertake histamine monitoring for all consignments landed. The primary processors efficiently grade, gut and freeze the fish with constant temperature monitoring and the product is normally frozen within hours of being unloaded from the vessel. The other fisheries generally consist of seasonal inshore fisheries, that use a range of multi-role vessels that either supply the primary processors or supply local markets and wholesalers with fresh high quality line caught product.

The industrial survey carried out in this project identified that, within the supply chain, elevated histamine levels were occasionally detected in the product. However, they were usually far below the legal maximal limits and were identified before reaching the retailer/consumer. These were usually associated with mackerel. The elevated levels in mackerel could often be traced back to a breakdown in the controlled supply chain that occurred during the summer season. Thawing by secondary processors was also highlighted as an area of concern. The literature review and subsequent modelling concluded that retail display and domestic storage were also potential hazards.

The key control to histamine formation is temperature. The most important control to prevent histamine formation and accumulation is rapid chilling of harvested fish and maintenance of low temperatures (<2°C) until the point of consumption. The risk from histamine fish poisoning is best mitigated by applying basic Good Hygiene Practices (GHPs) and a HACCP system. Appropriate sampling plans and testing for histamine should be used to validate the HACCP systems, verify the effectiveness of control measures, and detect failures in the system. Testing to ensure that the limits with regard to histamine are not exceeded is a requirement for food business operators (FBOs) as described in EU regulation 853/2004, and the criteria and methods used for determining histamine levels are described in EU regulation 2073/2005.

Based on the current level of knowledge, the conclusion from this project is that there are risks of histamine fish poisoning resulting from the consumption of Scottish caught and processed at-risk fish species, such as mackerel and herring. However, assuming Food Business Operators comply with current regulations and basic Good Hygiene Practices, these risks are low.

Specific recommendations are that:

1. Further education and reference information needs to be provided for company quality assurance (QA) staff with regard to: histamine risk assessment, appropriate sampling, and testing procedures; particularly in the smaller companies sourcing fish from inshore vessels.
2. Similar guidance to that of the US *Fish and Fishery Products Hazards and Control Guidance* (FDA, 2011) should be produced for FBOs that reflect recommended Scottish (UK) fishing and processing practices and controls in the context of EU requirements.
3. The Risk Assessment for histamine conducted by FBOs as part of their HACCP for pelagic species should incorporate different levels of likelihood of its occurrence dependant on the origin/supply of the raw material, i.e. inshore line

caught mackerel has a higher likelihood and therefore a higher risk rating than herring sourced from commercial vessels.

4. The updated HACCP risk assessments should be used in a re-evaluation of sampling plans employed by FBOs on receiving at-risk product.
5. Temperature control measures should be improved for the higher histamine risk fisheries, or more stringent temperature acceptance tests employed at factory reception.
6. The acceptable histamine levels should be revised with respect to position along the supply chain. Lower acceptance limits should be applied earlier in the chain.
7. More targeted guidance could be provided by the FSA, working with the retailers, to consumers on the domestic storage of at-risk fish.
8. The effect of temperatures experienced by product during thawing for secondary processing on the formation of histamine needs further investigation to determine the potential risk to the final product. We would reiterate the Seafood report SR598 (Archer *et al*., 2008) recommendation that a "proper comparison" of the different thawing methods from commercial perspective is made, including methods such as microwave, radio-frequency and ultra high pressure (UHP) thawing.
9. The effect of temperatures experienced by product during retail display on the formation of histamine needs further investigation to determine the potential risk to final product. Further investigation with pelagic species is required in order to provide guidance.
10. To enable more accurate assessment and modelling of the risks in processing Scottish at-risk fish species, a scientific study should be carried out to establish: (1) histidine levels in Scottish sourced at-risk fish species (particularly Atlantic mackerel (*Scomber scombrus*) and Atlantic herring (*Clupea harengus*), but also horse mackerel (*Trachurus trachurus*) sardine (*Sardina pilchardus*), blue whiting (*Micromesistius poutassou*), and sprats (*Sprattus sprattus*); (2) which histamine forming bacteria are present in these species, and (3) what are the characteristics of histamine formation in these species.
11. The role of other biogenic amines, notably cadaverine and putrescine, in "histamine" poisoning needs to be established, and their importance in at-risk fish species of relevance to the Scottish seafood industry. One study (Klausen & Lund, 1986) has reported that while histamine formation in herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) is similar, cadaverine is formed at much higher levels in mackerel compared with herring; and that this may possibly explain why mackerel and not herring are often implicated in incidents of histamine fish poisoning.

2. Glossary

Abbreviations used in the main text	
CCP	Critical Control Point
CFP	Common Fishery Policy
DEFRA	Department of Environment Food and Rural Affairs
EHO	Environmental Health Officer
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FM	Fish Merchant /Wholesaler
FBO	Food Business Operator
FDA	USA Food & Drug Administration
FPO	Fish Producer Organisation
EFSA	European Food Safety Authority
FSA	Food Standards Agency
GHP	Good Hygienic Practices
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
ICES	International Council for the Exploration of the Sea
MMO	Marine Management Organisation
NEAFC	North East Atlantic Fisheries Commission
NOAEL	No observed adverse effect level
PLN	Port of Landing (vessel) Number
PP.	Primary Processor, category I or II
R.	Retailer
RSW	Refrigerated Seawater Tanks
SP.	Secondary Processor, category A or B
SPFA	Scottish Pelagic Fishermen’s Association
SSSP	Seafood Spoilage and Safety Predictor
SSQC	Shetland Seafood Quality Control
TAC	Total Allowable Catches
V.	Vessel, category 1,2,or 3
WHO	World Health Organisation

Units and equivalents

National and International standards, and many publications, express histamine levels using a variety of units. In this report we have tried to keep to mg/kg throughout.

Terms

ppm = parts per million = 1/1,000,000

mg/kg = milligrams/kilogram

mg/100 g = milligrams/100 grams

µg/g = micrograms/gram

mg% = milligrams/100 ml = mg/dl

Equivalents

1 ppm = 1 mg/kg = 1 µg/g = 0.1 mg/100 g

1 mg/kg = 1 µg/g = 1 ppm = 0.1 mg/ 100 g

1 mg/100 g = 10 mg/kg = 10 µg/g = 10 ppm

1 µg/g = 1 mg/kg = 1 ppm= 0.1 mg/100 g

Contents

3. Aims and Objectives of the Investigation

The objective of this project was to provide a comprehensive review of current risk management practices for controlling histamine in at-risk fish species throughout the Scottish fish processing chain.

The main aim of the review was to identify key risk areas in the whole chain (from catch-to-fork) and any gaps in the management of these.

To achieve this it had three objectives:

1. To critically review relevant literature to identify and quantify key monitoring and intervention steps relevant to the control of histamine throughout the chain.
2. To survey current management and control practices for at-risk fish in key industrial sectors (catching, landing, processing) in Scotland.
3. To compare all literature and measured data to identify any gaps in current knowledge/management procedures and any areas where the sector could benefit from advice or guidance.

4. Introduction

Histamine (Scombroid or Scombrotoxin) fish poisoning[1] is a foodborne chemical intoxication associated with the consumption of spoiled fish flesh that is high in histamine. High levels of histamine are formed in spoiled fish flesh from fish species with naturally high levels of histidine (an amino acid common in the protein of certain species of fish). In these fish, histidine decarboxylase, an enzyme formed by certain bacteria, converts muscle histidine into histamine as the fish spoils. The most common bacteria responsible for histamine formation are *Morganella morganii*, *Clostridium perfringens*, *Hafnia alvei* and *Raoultella planticola*. These bacteria are commonly present in the salt-water environment and naturally exist on the gills and in the gut of the live fish. Histamine formation can be very fast, as little as two to three hours at temperatures above 20°C. Once histamine formation has occurred subsequent chilling, freezing or cooking will not destroy it. Histamine formation can continue to occur at refrigeration temperatures. The fish often appears normal and no off odour is present. The illness can cause a variety of symptoms, including rapid-onset headaches, sweating, rash, nausea, vomiting and diarrhoea, that normally lasts a few hours, but is known to continue for up to two days. It is thought that the incidence of histamine fish poisoning is vastly under-reported due to the short-lived effects or misdiagnosis as an allergy.

The fish species normally associated with histamine fish poisoning are pelagic fish species (pelagic fish live near the surface and are usually agile swimmers with streamlined bodies) such as mackerel, herring, sardines, pilchards and certain tuna species. The pelagic sector represents the largest fish sector in Scotland by volume of landings. In the northern waters around Scotland, the main pelagic fish species are Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*) and blue whiting (*Micromesistius poutassou*), with smaller populations of sprat (*Sprattus sprattus*) and Atlantic horse mackerel (*Trachurus trachurus*). Mackerel are by far the most important of these species in terms of both value and size of landings in Scotland. The majority of cases of histamine fish poisoning recorded in the UK are linked to tuna and mackerel.

These issues were factors in the decision for the Food Standard Agency in Scotland (FSAS) to put out a research call to:

Review the risk management practices employed throughout the fish processing chain in relation to controlling histamine formation in at-risk fish species in Scotland.

[1] Both the terms "histamine fish poisoning" or "scombrotoxin fish poisoning" are contentious and a little misleading. Since there is evidence that this form of poisoning is not caused simply by histamine but possibly by a combination of biogenic amines there is a preference by some to refer to this food safety problem as scombrotoxin fish poisoning to avoid an emphasis on the role solely of histamine and to avoid being confused with histamine intolerance and histamine induced adverse effects which have similar symptoms. The term scombrotoxin itself is a little misleading since it derives from its association with species belonging to the Scombridae and Scomberesocidae families (scombroid fish), such as mackerel and tuna. However, other non-scombroid fish, such as sardines, herring, pilchards, marlin and mahi-mahi have been involved in outbreaks of the illness. The problem is referred to as "histamine fish poisoning" throughout this report since the original FSA-Scotland call referred to histamine, as do EU regulations.

The review discussed here focuses on the histamine risk and the risk management practices employed in the pelagic fishing and processing sector in Scotland to control histamine formation.

5. Materials and Methods

The project was structured to look at the key interactions in a methodical manner with work on many objectives carried out in parallel and using material produced in other objectives.

All the work was carried out within an industrially relevant context and aimed to engage with the Scottish Fishing Industry and relevant enforcement officials.

5.1 Objective 01: Literature review

A critical review of all relevant and appropriate literature relating to the control of histamine was carried out, starting with a literature search of peer reviewed publications using Web of Knowledge and non-peer reviewed articles using Google. This review identified the key risk areas concerning the control and management of histamine in the fish processing chain (from catch-to-fork) and was then used to inform a practical survey of the current management and control practices used by the pelagic fishery sector in Scotland when either catching, landing, processing or supplying at-risk species in Scotland. The review sourced information from research and commercial literature and through contacts with fishing and fish processing companies, and researchers in UK, EU, NZ, Australia and USA.

Data sources were allocated a weighting based on the quality of the data and medium of publication. For example; higher weightings were allocated to peer reviewed publications and published Government/official data. 'Grey' information was also included in the literature review phase albeit with a lesser weighting. This inclusion of 'grey' literature allowed the review to access experience built up by industrial practices over many years and data from unpublished sources that could provide sufficient data density for statistical confidence. As anticipated the majority of papers reviewed did not provide sufficient numerical data for a quantitative meta-analysis of the data.

The review also identified suitable data on product temperature histories and growth/survival/death models for histamine producing bacteria in at-risk fish. It also identified suitable microbial growth models, such as the Seafood Spoilage and Safety Predictor (SSSP) ver. 3.1 (http://sssp.dtuaqua.dk/HTML_Pages/Help/English/Index.htm) program (which includes models to predict histamine formation by both *Morganella psychrotolerans* and *Morganella morganii*), that could be used to model histamine formation in fish through the cold-chain. These models were used in Objective 03.

The review also included a review of any physical and chemical interventions that have the potential to reduce histamine contamination.

5.2 Objective 02: Industry survey

The key objectives of this study involved the direct gathering of data (primarily on temperature control, hygienic processing and interventions) from sectors of the Scottish Fish Industry.

The first stage was to map the pelagic fishing sector in Scotland and identify the key companies involved.

To engage with this industry sector, a short document explaining the purpose of the work and the help we would like from fish processers and distributors was agreed

with the FSAS before this was sent to all companies involved with harvesting and processing of the at-risk species. All recipients were contacted by phone shortly after distribution of the document.

This was followed up by site visits and questionnaires that addressed not only the control procedures and practices being implemented by the industry, but also investigated the industry's knowledge and evidence used to validate their controls. In addition the industry's attitudes to, and operation of, the methods used to verify the effectiveness the control measures employed was determined.

5.2.1 Objective 02.01: Survey of catching practice

The primary aim of this survey was to obtain information from companies in order to assess the level of understanding and control of histamine within the catching sector.

The mapping exercise identified two main categories of catching, defined by the vessels used, method of catching, season and classification of fishery:

1. Purpose built pelagic trawlers

   Direct access to these vessels proved difficult, but temperature records for the vessel "Kings Cross" were obtained for seven different fishing days during 2010 and 2012.

2. Inshore line caught mackerel fishery

   In discussions with major fish processing companies a general impression was obtained that the industry felt that inshore line caught mackerel from Scottish waters was a potentially a significant problem source of fish with high histamine levels. Studies were therefore carried out at Peterhead and Shetland to assess operational practices and monitor temperatures of mackerel from these sources. To aid the work a Grimsby processing company agreed to raise a special order for mackerel that could be monitored to provide real data.

5.2.2 Objective 02.02: Survey of on land processing and distribution

The primary aim of this survey was to obtain information from companies in order to assess the level of understanding and control of histamine across the processing sector. The survey however had an important secondary role in that it publicised the project and provided the contact information necessary to allow further discussions in order to get more detailed information.

The survey was produced in a number of formats to facilitate its completion by a wide range of respondents. These included;

- A one-page introduction to the project with links to the survey, available both as a webpage and as down-loadable document. http://tinyurl.com/Histamine-Intro.
- A document based survey that could be either printed out and returned by post or completed on the computer and returned by email. http://tinyurl.com/Histamine-paper-survey.
- An online survey, using the Survey Monkey web based survey design and collection service, that allow respondents to quickly and easily complete the survey on-line, the survey questions were adjusted for individual sectors, e.g.
    - Smokehouses and Secondary Processors: https://www.surveymonkey.com/s/Histamine-Processor.

- All other sectors: www.surveymonkey.com/s/FSA_Histamine_Survey.

The survey was designed to be a first point of contact with the project, in that the information it requested would provide the details necessary to identify those companies that should and could be contacted for follow up investigation via visits or phone calls, whilst at the same time collecting the relevant information from other companies.

5.2.3 Objective 02.03: Survey of industrial attitudes

This element of the survey gathered information on catchers and processors' attitudes to cleaning and monitoring with regard to histamine production, and on the difficulties faced by the fish businesses in relation to controlling and testing of histamine in the catching and processing environment. Key staff were interviewed to determine their attitude to histamine in raw materials and finished products and the steps they had considered/taken to reduce the problem.

5.3 Objective 03: Data review and analysis

The data set obtained from Objective 02 was analysed for any correlations and gaps between measured factors and historical histamine data, and the key risk areas for histamine control in the processing chain and their monitoring and management identified in the literature review carried out in Objective 01. It had been envisaged that Principal Component Analysis (PCA) techniques would be used to seek subtle correlations in the data beyond a simple ordered ranking, however there was insufficient data to do this.

Records of temperature data in different supply chains covering chilled storage, transport, retail display, home transportation and consumer handling were used to augment the experimentally obtained data and predict typical and worst time-temperature histories for the products of interest. A limited series of experiments were also carried out to supplement this data (see Appendix 9, Section 17). All the sets of time-temperature data were used to predict the potential for formation of histamine under these conditions using the Seafood Spoilage and Safety Predictor (SSSP) model, which can be used to predict the formation boundary of histamine depending on storage conditions and product characteristics in fish (see Appendix 10, Section 18).

6. Results and Discussion

6.1 Literature review

The full review of the literature can be found in Appendix 1 (Section 9). Many reviews and risk assessments have been carried out on histamine fish poisoning and a comparison of these reviews and a summary of their main conclusions can be found in Appendix 2 (Section 10).

6.1.1 Risk assessment

It is clear from the published literature on histamine (Lehane & Olley, 1999) that there are three pre-requisites for the elevation of the post-mortem histamine concentration in fish:

1. A sufficiently high content of histidine in the fish (from which histamine is formed).
2. The presence of bacteria capable of producing the enzyme histidine decarboxylase (which reacts with free histidine in the fish to form histamine).
3. Environmental conditions, such as high temperatures, that allows such bacteria to proliferate.

6.1.1.1 The illness

Incubation period: Can range from several minutes to several hours, although the mean incubation period is around one hour.

Duration: Symptoms normally last for 8-12 hours, but can persist for several days.

Symptoms: May include rash, localised skin inflammation, nausea, vomiting, diarrhoea, abdominal cramps, low blood pressure, headache, tingling, flushing and severe respiratory distress. The most consistent sign is a flushing of the face and neck causing heat and discomfort, which can appear similar to sunburn. It is considered to be rarely, if ever, fatal.

Treatment: Treated with antihistamines.

6.1.1.2 Toxicity levels

Histamine is a biogenic amine. The threshold toxic dose for histamine in fish is not precisely known (Taylor, 1986). In most cases, histamine levels in illness-causing fish have been above 200 mg/kg, often above 500 mg/kg (Food and Drug Administration, 2011). A recent FAO-WHO expert meeting (FAO-WHO, 2012) concluded that a dose of 50 mg of histamine, which is the no-observed-adverse-effect level (NOAEL), is the appropriate hazard level. Based on this hazard level and a serving size of 250 g of fish per day, the FAO-WHO expert meeting (FAO-WHO, 2012) calculated that the maximum concentration of histamine in that serving was 200 mg/kg. They concluded that when food business operators apply good hygienic practices (GHP) and a hazard analysis and critical control point (HACCP) system, an achievable level of histamine in fish products should be lower than 15 mg/kg, based on data made available by industry (using a test method with a lower detection limit of 15 mg/kg).

However, there is some evidence that despite a widely reported association between histamine and histamine fish poisoning, histamine alone may be insufficient to cause

food toxicity (Al-Bulushi *et al*., 2009). Putrescine and cadaverine have been suggested to potentiate histamine toxicity (Al-Bulushi *et al*., 2009; Food and Drug Administration, 2011).

6.1.1.3 Histamine formation

Histamine formation occurs in spoiled fish flesh that is high in histidine and contaminated with histamine-forming bacteria (Tao *et al*., 2009). The main bacteria responsible for histamine formation (Table 9) are all commonly present in the salt-water environment. They naturally exist on the gills and in the gut of the live fish (Taylor & Speckhard, 1983; Kim *et al*., 2003a). Histamine-forming bacteria produce the enzyme histidine decarboxylase during growth. This enzyme reacts with free histidine to form histamine, a non-volatile biogenic amine (Al-Bulushi *et al*., 2009). Once the bacterial enzyme histidine decarboxylase has been formed, it can continue to produce histamine in the fish even if the bacteria are not active. Detectable amounts of histamine appear to accumulate only after high levels of bacteria are reached in fish muscle. A mesophilic bacterial count of 6-7 $\log_{10}$ cfu g^{-1} has been found to be associated with 50 mg histamine/kg fish, the US Food and Drug Administration (FDA) maximum allowable histamine level (Du *et al*., 2002; Takahashi *et al*., 2003; Al-Bulushi *et al*., 2009).

Levels of free histidine differ between at-risk fish species and there is evidence of a correlation between levels of histidine and the formation of histamine in different species. The minimum histidine concentration required for bacterial histidine decarboxylase activity is estimated to be 1000 to 2000 mg/kg (Lee *et al*., 2012). There is some evidence that histidine levels in fish species may be related to season, with higher levels of histidine present in the fish during summer when they are at their most active. Seasonal differences in fish muscle tissue pH may also relate to differences in histamine formation (Dalgaard *et al*., 2006).

There is evidence that other biogenic amines, notably cadaverine and putrescine, may also need to be present (Rossi *et al*., 2002; Al-Bulushi *et al*., 2009; Food and Drug Administration, 2011). These are formed in a similar fashion, and again their formation will differ in different fish species. It is believed that differences in cadaverine formation may possibly explain why mackerel (*Scomber scombrus*) and not herring (*Clupea harengus*) are often implicated in incidents of histamine fish poisoning (Klausen & Lund, 1986). These fish can have similar histamine contents, but cadaverine formation is greater in mackerel.

6.1.1.4 Bacterial species responsible for histamine formation

The main bacteria responsible for histamine formation are certain species of *Enterobacteriaceae*, C*lostridium*, *Pseudomonas* and *Lactobacillus.* Enteric (commonly found in the gut of live fish) bacteria have been found to be the most important histamine forming bacteria in fish. *Morganella morganii* has been cited by many recent studies as being of most importance, but this bacteria does not produce toxic concentrations of histamine in seafood chilled to below about 7°C. A number of psychrophilic and psychrotolerant bacteria (such as *Morganella psychrotolerans* and *Photobacterium phosphoreum*) have been identified as significant histamine formers at low temperatures. *M. psychrotolerans* has been identified as being able to form toxic concentrations of histamine in seafood at storage temperatures as low at 0°C (Emborg *et al*., 2005, 2006; Emborg & Dalgaard, 2008a,b).

At least some of the histamine-forming bacteria are halotolerant (salt-tolerant) or halophilic (salt-loving) so are capable of growing and forming histamine in fish products.

A number of the histamine-forming bacteria are facultative anaerobes, such as *P. phosphoreum*, that can grow in reduced oxygen environments (Food and Drug Administration, 2011). A number of studies (Özogul *et al*., 2002b; Jeya Shakila *et al*., 2005b; Emborg *et al*., 2005) have shown that vacuum packing does not inhibit the growth of histamine-forming bacteria and histamine formation. Although some modified atmospheres will inhibit histamine formation.

6.1.1.5 Fish species

A full list of at-risk fish species can be found in Table 7 in Appendix 1 (Section 9). Of the pelagic species targeted by the Scottish fishing industry, Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*) and horse mackerel (*Trachurus trachurus*) have been identified in the published literature as fish species likely to be "at-risk" of containing high histamine levels capable of causing histamine fish poisoning, as have sardine (*Sardina pilchardus*). No literature, however, on the risk of histamine formation in blue whiting (*Micromesistius poutassou*) or sprats (*Sprattus sprattus*) has been identified.

Mackerel is more commonly implicated with incidents of histamine fish poisoning than herring. This could be because while similar amounts of histamine accumulate in herring and mackerel under the same conditions, another biogenic amine, cadaverine, is formed at much higher levels in mackerel compared with herring (Klausen & Lund, 1986). There is evidence that other biogenic amines, notably cadaverine and putrescine, have a role in "histamine" poisoning and may need to be present as well as histamine to cause poisoning (Rossi *et al*., 2002; Al-Bulushi *et al*., 2009; Food and Drug Administration, 2011).

There was confusion and concern within the Scottish fish industry regarding whether North Atlantic salmon (*Salmo salar*) could be a source of histamine fish poisoning. Our literature review has concluded that, to date, there have been no reported incidents of elevated histidine or histamine in fresh North Atlantic salmon (*Salmo salar*), either wild caught or farmed. Two separate studies (de la Hoz *et al*., 2000; Emborg *et al*., 2002) have shown that although histamine can be formed in Atlantic salmon (*Salmo salar*) during storage, the rate of accumulation is slow and high levels are not formed before the fish spoils.

6.1.1.6 Incidence of histamine fish poisoning in Scotland (and UK)

Until the mid-1980s mackerel, especially smoked mackerel, accounted for the vast majority of incidences and outbreaks of suspected histamine fish poisoning in the UK (Taylor, 1986; Bartholomew *et al*., 1987). Since this time there has been significant decrease in the numbers of incidences and outbreaks overall, and particularly those related to mackerel. This has been attributed to improved handling and storage practices, particularly improvements in the cold-chain and the use of low storage temperatures (Taylor, 1986).

Since the ObSurv surveillance system was established in 1996 for all general outbreaks of infectious intestinal disease (IID) in Scotland, only six outbreaks of histamine fish poisoning have been reported in Scotland (see Table 3). Tuna was the suspect vehicle in all outbreaks and the majority related to food service. Whilst the incidence of reported illness caused by histamine from seafood within Scotland,

and the UK as a whole, are generally low, it is believed that the incidence of histamine fish poisoning is vastly under-reported. This is because the range of symptoms and their severity caused by histamine fish poisoning mean that consumers do not always inform the authorities or seek medical attention upon being symptomatic.

6.1.2 Permitted histamine levels in fish

Throughout the world there are regulations limiting the amount of histamine permitted in fish.

In the EU, EC regulation 2073/2005 specifically cites the following fish species as associated with high amounts of histidine: Scombridae (which include Albacore, mackerel, tuna), Clupeidae (Atlantic herring (*Clupea harengus*), Baltic herring (*Clupea harengus membras*), Sardine (*Sardina pilchardus*)), Engraulidae (anchovies), Coryphaenidae (mahi mahi), Pomatomidae (bluefish), and Scombresosidae (saury).

For such species, the legislation stipulates that for a batch to be acceptable nine independent samples from each batch should result in:

1. An average histamine concentration lower than 100 ppm (equivalent to 100 mg/kg or 10 mg/100 g).
2. No more than 2 samples out of the 9 with a concentration of between 100 and 200 ppm.
3. No sample with a histamine content higher than 200 ppm (equivalent to 200 mg/kg or 20 mg/100 g).

The Australia/New Zealand food standards require samples to have less than 200 mg/kg (equivalent to 200 ppm or 20 mg/100 g) histamine (Australian and New Zealand Food Authority (ANZFA), 2010).

The US FDA has a hazard action level of 500 mg/kg (equivalent to 500 ppm or 50 mg/100 g) and a decomposition level of 50 mg/kg (equivalent to 50 ppm or 5 mg/100 g) (Food and Drug Administration, 1995).

The draft Codex Alimentarius decomposition standard for smoked fish requires that products of susceptible species should not contain more than 100 mg of histamine per kg fish flesh (equivalent to 100 ppm or 10 mg/ 100 g). Although when considering hygiene and handling the level is 200 mg/kg (Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission, 2010).

6.1.3 Key controls

The potential for histamine-formation in at-risk fish is dependent on a number of intrinsic and extrinsic factors. Firstly, histidine and histamine-forming bacteria need to be present. Subsequent growth of histamine-forming bacteria and histamine formation will depend on:

1. Species of the fish.
2. Harvest practice.
3. Rate of post-harvest chilling.
4. Storage times and temperature.
5. Storage atmosphere.

6. Handling procedures.
7. Salt (NaCl).
8. pH.

It is clear from what is known about histamine formation that there are three key controls to control histamine formation in at-risk fish species:

1. Rapid cooling of fish immediately after harvest to a centre/core temperature <2°C. Thus preventing the initial growth of histamine-forming bacteria (and the formation of the bacterial enzyme histamine decarboxylase). Unlike bacteria or viruses, histamine is not destroyed by cooking so once formed cannot be easily destroyed.
2. Maintenance of low temperatures (<2°C) until the point of consumption.
3. Hygienic handling of fish from the moment of capture to the point of consumption is also crucial to reduce the formation of histamine.

Currently, time and temperature are the only hurdles (controls) available to the industry to reduce the risk of histamine formation. Other options for reducing the growth of histamine-producing bacteria may be the incorporation of anti-microbial agents in the ice or saltwater/ice slurry used to chill fish. Similar interventions have been adopted for other "foods of animal origin", such as red meat and poultry. EC Regulations 853/2004 and 1333/2008 do allow for the use of approved substances as "processing aids". The BIOHAZ Panel of EFSA recently revised the joint AFC/BIOHAZ guidance on the submission of data for the evaluation of the efficacy of substances for the removal of microbial surface contamination of foods of animal origin.

6.2 Industry survey

The industrial survey was undertaken to map the supply chains for Scottish pelagic fish and fishery products, identify participants, identify the measures undertaken by the industry to control the formation of histamine, and to identify any gaps or areas where improvement could be made.

6.2.1 Mapping exercise

The full results of the mapping exercise can be found in Appendix 3 (Section 11). Details of the current Scottish pelagic fishing fleet can be found in Appendix 4 (Section 12), and pelagic landing data for 2010 in Appendix 5 (Section 13).

The pelagic sector is Scotland's largest fishery in terms of both value and quantity, it primarily consists of a highly integrated industrial operation composed of 26 modern pelagic trawlers and 5 primary processors located in 3 ports, that handle 99.8% of the annual catch of 129,000 tonnes (2010 data), 95% of this product is then exported as frozen product by the primary processors. A wide range of smaller inshore vessels also operate that target a mixed fishery throughout the year that can switch depending on season (usually summer) and quota availability to targeting hand-line caught mackerel. These vessels (<10 m) fish the inshore waters using typically a line of baited hooks, but this is limited by quota to 300 tonnes p.a. (or 0.001% of total mackerel landings in 2010) for Scottish waters (MMO, 2012a).

The pelagic fisheries are both highly seasonal and variable, due to the shoaling nature of the fish species; being largely dictated by their breeding, migration and feeding cycles in the various fishing areas.

6.2.2 Survey of catching practice

The full survey of catching practice can be found in Appendix 6 (Section 14).

The mapping exercise identified two main categories of catching, defined by the vessels used, method of catching, season and classification of fishery, i.e.

1. Purpose built pelagic trawlers
2. Inshore line caught mackerel fisheries

The purpose built pelagic trawlers all use refrigerated sea water (RSW) tanks to ensure good temperature control post capture and all primary processors undertake histamine monitoring for all consignments landed. The primary processors efficiently grade, gut and freeze the fish with constant temperature monitoring and product is normally frozen within hours of being unloaded from the vessel.

The other fisheries consist of seasonal inshore fisheries, that use a range of multi-role vessels that either supply the primary processors or supply local markets and wholesalers with fresh high quality line caught product.

6.2.3 Survey of on-land processing and distribution

The full survey of on-land processing and distribution can be found in Appendix 7 (Section 15).

Despite follow up phone calls and reposting of emails, the initial response to the survey was poor, with only six companies completing the online or paper based survey. However, this figure includes a response by SPFA on behalf of all their 28 member vessels. Follow up contact with some of the non-responding companies indicated that whilst they have pelagic fish on their Seafood Scotland database entry, they in fact only retail such product and as such did not employ specific controls for histamine. Others who were contacted made it clear that they were not interested in, or too busy to participate in the survey. Because of the relatively low response from industry, the focus of the survey was shifted to a greater reliance of obtaining the required information directly via phone calls and meetings with the companies and with the survey forms completed by the interviewer. In addition, some non-Scottish companies that both source product from Scottish suppliers and are key players in the supply of pelagic fishery products to the UK retailers were also included in the survey. In total, data from 15 processing companies were collected.

The survey identified two distinct supply chains of Scottish pelagic fish and fish products to the UK markets. The first supply chain consisted of companies that are supplying the major retailers (SP.A) and as such has tight control of product quality and storage conditions throughout the supply chain. The second involved secondary processing (principally of smoked product) by smaller companies (SP.B) that sold via local retail outlets or online/mail order.

Whilst the large primary processors and some larger secondary processors take responsibility for histamine testing of product on reception of the fish, many of the remaining sectors rely on visual inspection of product quality and knowledge of where and when product was caught to decide on product safety.

The industrial survey found that the pelagic processing industry is predominantly supplied with frozen product by the primary processors. The larger secondary processors supply the major retail chains and, as such, their controls and monitoring for histamine were found to be very rigorous to meet the required specifications. Smaller secondary processors, including the traditional smoke-houses producing kippers and smoked mackerel, were found to rely on supplier assurance from the primary processors and did not undertake their own checks of histamine levels in their raw materials or products.

The HACCP analysis for histamine undertaken by the companies indicated that the majority of processors, whilst aware of histamine as a potential hazard, felt that the likelihood of it occurring during their operations was low. The exceptions being the larger secondary processors who acknowledged that monitoring histamine and temperature was essential throughout their supply chain. Those companies that did undertake their own histamine assays generally used the rapid test kits produced by Neogen, and tested samples from every consignment received (details in Appendix 8, Section 16).

The industrial survey identified that, within the supply chain, elevated histamine levels were occasionally detected in the product. However, they were usually far below the legal maximal limits and were identified before reaching the retailer/consumer. In each case, the elevated levels in mackerel could be traced back to a breakdown in the controlled supply chain that occurred during the summer season. This demonstrated that the controls within the supply chain are effective at identifying and dealing with potential issues before they become a safety issue and that for the larger processors; at least, product traceability allows the identification of the source and any other potentially unsafe product.

6.2.4 Survey of industrial attitudes

The key points identified from the online survey, subsequent visits and discussions with stakeholders in the Scottish pelagic seafood industry were:

1. The general perception of the industry is that the necessity for histamine control, although important for product safety, is undertaken primarily to meet customer requirements because their industrial experience suggests the likelihood of histamine occurring in their product is low to non-existent.
2. The consensus of the industry is that the risk assessment for histamine, as part of their HACCP plan, results in a level of control that does not require a Critical Control Point in order to ensure product safety.

6.2.5 Overall

The key points identified from the on-line survey, subsequent visits and discussions with stakeholders in the Scottish pelagic seafood industry are:

1. HACCP plans in all sectors had only a single risk assessment for histamine, whereas the likelihood of its occurrence is largely dependant on the product temperature history, i.e. source of raw materials and the nature of the product (frozen or fresh).
2. Evidence to support the risk assessment for histamine in their HACCP plans, was largely based on experience and advice from enforcement authorities. Only the largest secondary processors relate their assessment to published information.

3. The rapid test kits used by processors were not always the most suitable for the concentrations of histamine likely to be present in the pelagic fish at the point where monitoring took place in the specific supply chain(s). This is because many of the rapid test kits used cannot detect low concentrations of histamine (<50 mg/kg). It is unlikely that measurable quantities of histamine would be detected at some of the points where monitored, even with temperature abuse sufficient to have a significant effect on shelf life, due to the relatively short time periods between capture and landing.
4. The supply chains for the major retail markets had generally adequate control for histamine with monitoring of histamine levels and temperature at all the key points within the chain. There is, however, a small risk of uncontrolled raw materials entering the supply chain at the point of landing.
5. Gaps in the control of histamine were identified at the following points in the supply chain.
    a. The summer hand-line mackerel fishery does not always have adequate temperature control from point of capture to landing.
    b. Landing sites and fish markets do not differentiate between pelagic and other species with respect to the checks and monitoring conducted at the point of landing (Shetland).
    c. Artisanal secondary processors (SP.B) were unlikely to test for histamine in either raw materials or final product.
    d. Artisanal secondary processors (SP.B) did not always have adequate control of product temperature during thawing/tempering of raw materials, resulting in a potential increase in the likelihood of histamine formation.

6.3 Supplementary experimentation and modelling

The literature review and industrial survey revealed a lack of data on the cooling, warming and thawing rates for herring and mackerel under industrial processing conditions. It was hoped that indicative temperatures through the supply could be verified as part of this project, however, ongoing high profile prosecutions over illegal landings made such observation/inspection difficult and limited data was collected. Limited trials were therefore carried out with an industrial company to obtain some data on thawing rates in a commercial operation. In addition, a series of short practical trials were conducted to determine example cooling and warming rates for herring and mackerel under a range of conditions. This data was used to model the effect of temperature changes under a range of hypothetical scenarios on histamine formation using the Seafood Spoilage and Safety Predictor (SSSP).

6.3.1 Experimental evaluations

The rates of temperature change are important to determine the periods at which products remain at the histamine-forming elevated temperatures. A series of practical trials were therefore conducted to determine example cooling and warming rates for herring and Atlantic mackerel under a range of conditions. In addition trials were carried out with an industrial company to obtain some data on thawing rates in a commercial operation. Full details of these trials can be found in Appendix 9 (Section 17).

This limited thawing trial looked at one commercial thawing method used at a SP producing smoked fish products. Since thawing is a very slow process at low

ambient temperatures, high ambient temperatures of up to 25°C were used by the company to enable more rapid thawing during an overnight process. The thawing room was filled with racked fish blocks during the day (mainly the morning) and thawed overnight, the room being emptied in the morning. The temperatures measured showed that this length of time was required, since deep centre temperatures of the fish at the centre of the blocks were still just below 0°C at the end of the process. Whilst centre/core temperatures of fish blocks generally remained low (<1°C) during the thawing process, surface temperatures were a concern and temperatures of up to 11°C were measured in whole fish after thawing. FDA guidance (FDA, 2011) is that previously frozen fish should not be exposed to temperatures above 4.4°C for "more than 12 hours, cumulatively, if any portion of that time is at temperatures above 21°C" or "more than 24 hours, cumulatively, as long as no portion of that time is at temperatures above 21°C". The process used by this company used air temperatures in its thawing operation above 21°C and for more than 12 hours, however the air temperatures measured around the fish blocks were lower, <20°C (due to poor air circulation within the room). Time constraints unfortunately prevented further study.

FDA guidance (FDA, 2011) is that fish should be chilled "as soon as possible after harvest, but not more than 9 hours from the time of death", once chilled fish should not be above 4.4°C for "more than 8 hours, cumulatively, as long as no portion of that time is at temperatures above 21°C".

Mean experimental cooling times for Atlantic mackerel and herring are given in Table 1. A range of times were seen for each method due to variation in fish sizes. A range of times were seen for each method due to variation in fish sizes. As would be expected, Atlantic mackerel being larger and heavier than herring took longer to cool for all methods. At the point of harvest the fish will be at the temperature of the sea it was caught in. This can be as high as 17°C plus in in-shore fisheries in mid summer. The studies carried out have shown that if the initial primary cooling stage is carried out in air or unagitated ice/water, the slowest cooling methods, then cooling times to 4°C will be approximately 60 minutes in Atlantic mackerel and less with herrings.

Table 1. Mean experimentally measured cooling times (centre temperature of individual whole fish from 16°C to <4°C) for Atlantic mackerel and herring cooled under different cooling regimes

Cooling Method	N =	Mean cooling time of core (centre) to <4°C, in minutes (SD)	
		Atlantic mackerel	Herring
Air at 2°C	4	61.6 (1.7)	48 (3.4)
Ice	4	43 (4.1)	21 (6.3)
Ice and water (non-agitated)	4	63 (19.5)	34 (13.5)
Ice and water (agitated)	4	30 (3.5)	-

Mean experimental warming times to >4°C for prechilled (0°C) Atlantic mackerel and herring are given in Table 2. FDA guidance (FDA, 2011) is that once chilled fish should not be above 4.4°C for "more than 8 hours, cumulatively, as long as no portion of that time is at temperatures above 21°C". These results show that within a processing environment, where the ambient air should be expected to be refrigerated, any exposed uniced fish can warm above 4°C in less than 11 minutes if exposed to high air velocities. If the fish is not exposed to high air velocities then

warming will take on average 22 to 24 minutes in herrings and approximately 30 minutes in mackerel. In an unrefrigerated environment (17°C) fish will warm much faster (even under static air conditions) and centre/core temperatures exceed 4°C in approximately 15 minutes.

Table 2. Mean experimentally measured warming times (to >4°C) for Atlantic mackerel and herring warmed under different warming regimes

Cooling Method	N =	Mean warming time from 0°C to >4°C in minutes (SD)			
		Atlantic mackerel		Herring	
		Surface	Centre	Surface	Centre
Static air at 8±°C	2	29.5 (2.1)	31.1 (3.0)	21.5 (2.1)	24.0 (5.7)
Forced air at 8±1°C	2	2.3 (1.8)	15.1 (0.1)	11.4 (1.9)	11.9 (1.2)
Static air at 17±1°C	8	-	14.8 (2.0)	-	15.3 (2.7)

6.3.2 Modelling

The Seafood Spoilage and Safety Predictor (SSSP) v.3.1 was used to predict the expected growth of histamine during a range of hypothetical temperature scenarios representing different fishery practices. Details of this modelling can be found in Appendix 10 (Section 18). The SSSP modelling software was developed by the National Institute of Aquatic Resources (DTU Aqua) at the Technical University of Denmark (DTU) and is available as freeware at http://sssp.dtuaqua.dk/.

The SSSP program considers *M. morganii* and *M. psychrotolerans* growth and the subsequent catalysation of histamine formation. At higher temperatures mesophilic *M. morganii* are responsible for the majority of histamine formation whereas at lower temperatures, growth and histamine formation by the psychrotolerant bacterium *M. psychrotolerans* is more important.

Inspection of the model formulae show that zero growth rates will be achieved below T = 2.8°C and above T= 44.7°C for *M. morganii*, and below T = -5.9°C and above T = 33.1°C for *M. psychrotolerans*. Thus, histamine formation triggered by *M. morganii* could be negated if the fish could be maintained at the temperature of melting ice (0°C), however *M. psychrotolerans* could contribute to histamine at these temperatures.

A number of temperature scenarios, reflecting typical fisheries and supply chains were postulated as a basis for comparison modelling. However, use of the model showed that the model is primarily designed to predict histamine formation during storage and thus makes an assumption that product temperatures can change instantaneously. This means that it is difficult to use it to predict growth during dynamic operations such as chilling, freezing and thawing.

In order to look at such processes, temperature change curves were incorporated into the model by using small time steps of gradually changing temperatures to attempt to simulate the true temperature experience of mackerel and herring throughout the catch to consumption chain. Whilst more accurately reflecting the true temperatures experienced by the histamine triggering organisms, the method is cumbersome and uses the model in a way that may give unexpected results.

This initial modelling suggested that there is no substantial risk of histamine formation in most normal capture to consumption chains provided that the temperature control measures operate correctly. The baseline temperature

scenarios assumed good domestic storage practices to maintain temperatures <6°C, however it has been shown that domestic storage temperatures can be up to 10°C (James *et al*., 2008), the model showed that elevated domestic storage temperatures could lead to potentially critical levels of histamine being formed. It was hoped to model the effects of the thawing process on histamine formation using the SSSP model but unknown anomalies in the model prevented useful outputs from being obtained.

7. Conclusions and Recommendations

The main findings of this project are:

- The overall incidence of histamine fish poisoning in Scotland, and the UK as a whole, is low. In recent years tuna has been the suspect vehicle in all outbreaks and the majority have been related to food service. Whilst the incidence of reported illness caused by histamine from seafood within Scotland, and the UK as a whole, is low, it is believed that the incidence of histamine fish poisoning is vastly under-reported. This is because the range of symptoms and their severity caused by histamine fish poisoning mean that consumers do not always inform the authorities or seek medical attention upon being symptomatic.
- Of the fish species targeted by the Scottish pelagic fishing sector, Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*), horse mackerel (*Trachurus trachurus*), and sardine (*Sardina pilchardus*) have been identified in published literature as fish species as likely to be "at-risk" of containing high histamine levels capable of causing histamine fish poisoning. No literature, however, on the histamine risk of blue whiting (*Micromesistius poutassou*) or sprats (*Sprattus sprattus*) have been identified.
- There was concern within the Scottish fish industry regarding whether North Atlantic salmon (*Salmo salar*) could be a source of histamine fish poisoning. Our literature review has concluded that based on available data, to date, there is no evidence of elevated levels of histidine or histamine in fresh North Atlantic salmon (*Salmo salar*), either wild caught or farmed, and that this species does not represent an "at-risk" species with regards to histamine.
- The pelagic sector in Scotland primarily consists of a highly integrated industrial operation composed of 26 modern pelagic trawlers and 5 primary processors located in 3 ports, that handle 99.8% of the annual catch of 129,000 tonnes (2010 data), 95% of this product is then exported as frozen product by the primary processors. The modern pelagic trawlers all use refrigerated sea water (RSW) tanks to ensure good temperature control post capture and all primary processors undertake histamine monitoring for all consignments landed. The primary processors efficiently grade, gut and freeze the fish with constant temperature monitoring and product is normally frozen within hours of being unloaded from the vessel.
- The other fisheries generally consist of seasonal inshore fisheries, that use a range of multi-role vessels that either supply the primary processors or supply local markets and wholesalers with fresh high quality line caught product.
- The industrial survey identified that, within the supply chain, elevated histamine levels were occasionally detected in the product. However, they were usually far below the legal maximal limits and were identified before reaching the retailer/consumer. These were usually associated with mackerel. In each case, the elevated levels in mackerel could be traced back to a breakdown in the controlled supply chain that occurred during the summer season. This demonstrated that the controls within the supply chain are effective at identifying and dealing with potential issues before they become a safety issue, and that for

the larger processors at least, product traceability allows the identification of the source

- The industrial survey also identified that artisanal secondary processors did not always have adequate control of product temperature during thawing/tempering of raw materials, resulting in a potential increase in the likelihood of histamine formation. A limited trial was carried out looking at one commercial thawing method of concern. This used high ambient temperatures of up to 25°C. Whilst centre/core temperatures of fish blocks generally remained low (<1°C) during the thawing process, surface temperatures were a concern and temperatures of up to 11°C were measured in whole fish after thawing. Time constraints unfortunately prevented further study. It was concluded that further trials are required to establish limits for thawing to avoid/control a rise in histamine during this process.
- The literature review identified few specific studies regarding the impact of retail display on histamine in fish, however survey data on retail display cabinet temperatures identified retail display as an area of concern. The effects of retail display on ice were subject to the SSSP modelling technique. This demonstrated a potential for a rise in histamine levels and a need to reposition and re-ice was concluded.
- The literature review identified few specific studies on the impact of domestic storage on histamine in fish, however, survey data on domestic storage temperatures identified domestic storage as an area of concern. The effects of domestic storage temperatures were subject to SSSP investigation and concluded that there was a potential for a rise in histamine levels during domestic storage.
- The only widely available model for predicting histamine formation during storage in seafoods is The Seafood Spoilage and Safety Predictor (SSSP) v.3.1. This can be used to predict histamine formation by *Morganella psychrotolerans* and *Morganella morganii*. Although in our experience it is a user-friendly software tool that is useful to evaluate the effect of constant or fluctuating temperature storage conditions, it makes an assumption that product temperatures can change instantaneously. This means that it is difficult to use it to predict growth during dynamic operations such as chilling, freezing and thawing where temperatures are constantly changing.
- The key control to histamine formation is temperature. Preventing the initial growth of histamine-forming bacteria (thus preventing the formation of the bacterial enzyme histamine decarboxylase) is vital to control histamine formation in at-risk fish species. Unlike bacteria or viruses, cooking does not destroy histamine. The most important control to prevent histamine accumulation is rapid chilling of harvested fish and maintenance of low temperatures (<2°C) until the point of consumption. Since histamine-forming bacteria may be present in the gut, gills and skin of fish, hygienic handling of fish from the moment of capture to the point of consumption is also crucial to reduce the formation of histamine. Recommended controls can be found in Chapter 7 of the US FDA *Fish and Fishery Products Hazards and Control Guidance* (FDA, 2011); the key temperature controls are reproduced in Appendix 11 (Section 19).

- There are four key stages from catch-to-fork where the fish may be subjected to elevated temperatures with the potential to cause a rise in histamine levels:
    1. Initial cooling of the fish immediately after harvest.
    2. Thawing of previously frozen fish.
    3. Retail display.
    4. Domestic storage.

7.1 Advice and guidance

The emphasis of current regulations and guidance is that all controls should be proportionate to the risk. Since the overall incidence of histamine fish poisoning in Scotland, and the UK as a whole, is low and histamine fish poisoning is not considered to be life threatening, it may be concluded that no specific controls are required and that its control is covered by FBOs applying good hygienic practices (GHP). Nevertheless, there is a requirement in EC regulation 853/2004 for FBOs to ensure that the limits with regard to histamine are not exceeded, thus there is a need for guidance.

In our opinion, there is currently limited specific guidance for the Scottish, and UK, seafood sector regarding histamine (and other risks). There is no repository of advice and guidance for FBOs similar to that for the meat sector (e.g. the FSA Meat Industry Guide (MIG)). The most relevant documents/advice that we have been able to find are the following:

- *The Good Practice Guide for Pelagic Fishermen* produced by The Sea Fish Industry Authority and Seafood Scotland mentions the importance of good temperature control in controlling histamine formation and that "fish held at 0°C poses no problem", but there are few other details or recommendations regarding histamine. Other links to general hygiene guidance and recommendations are on the Sea Fish Industry Authority's website (http://www.seafish.org/industry-support/legislation/hygiene), but these do not specifically address histamine.
- The criteria for histamine testing is described on The Sea Fish Industry Authority's website (http://www.seafish.org/industry-support/legislation/contaminants/histamine) along with links to the relevant regulations, but no advice is provided on how to apply the criteria.
- *Guidelines for the handling of chilled finfish by primary processors* produced by The Sea Fish Industry Authority (1989) cites "the occasional development of scombrotoxin during storage of oily fish" as a hazard and recommends chilled storage at 0°C to +4°C as preventing development of the toxin but contains no more specific data on histamine, and its references are out of date. The same advice is given in the Sea Fish Industry Authority *Guidelines for the handling of chilled fish by retailers* (1987).
- Recommendations for thawing of seafood were published in Seafood report SR598 (Archer *et al*., 2008). Histamine is mentioned in this report but there are no specific recommendations regarding the control of histamine formation during thawing or the risk of using elevated temperatures for the thawing of at-risk fish species. However, it does recommend clearly that product

temperatures are monitored during thawing and that they should be kept as "close to the temperature of melting ice, i.e. as close to 0°C, as possible".

While useful these contain relatively limited information on histamine for FBOs to incorporate into their HACCP schemes.

At present we would recommend that FBOs use the more comprehensive US FDA *Fish and Fishery Products Hazards and Control Guidance* (FDA, 2011) as a basis when developing/reviewing their HACCP schemes. However, this document does contain information that is not relevant to the Scottish (UK) seafood sector, and FBOs need to be aware of the differences between EU and US regulations, practices and controls.

7.2 Recommendations and data gaps

The main aim of this project was to identify any gaps in current knowledge/management procedures and any areas where the sector could benefit from advice or guidance. Specific recommendations are that:

1. Further education and reference information needs to be provided for FBO QA staff with regard to: histamine risk assessment, appropriate sampling, and testing procedures; particularly in the smaller companies sourcing fish from inshore vessels.
2. Similar guidance to that of the US *Fish and Fishery Products Hazards and Control Guidance* (FDA, 2011) should be produced for FBOs that reflect recommended Scottish (UK) fishing and processing practices and controls in the context of EU requirements.
3. The Risk Assessment for histamine conducted by FBOs as part of HACCP for pelagic species should incorporate different levels of likelihood of its occurrence dependant on the origin/supply of the raw material, i.e. inshore line caught mackerel has a higher likelihood and therefore a higher risk rating than herring sourced from commercial vessels.
4. The updated HACCP risk assessments should be used in a re-evaluation of sampling plans employed by FBOs on receiving at-risk product.
5. Temperature control measures should be improved for the higher histamine risk fisheries, or more stringent temperature acceptance test employed at factory reception.
6. The acceptable histamine levels should be revised with respect to their position along the supply chain. Lower acceptance limits should be applied earlier in the chain.
7. More targeted guidance could be provided by the FSA, working with the retailers, to consumers on the domestic storage of at-risk fish.

The assessed risks and controls that were covered can only be as accurate as the data used to inform them. Whilst there is much expert opinion and anecdotal data regarding histamine risk and control, the availability and quality of hard data are lacking in some areas. There is a particular lack of hard scientific data concerning the fish species of concern to the Scottish pelagic sector. As such, we would recommend that:

8. The effect of temperatures experienced by product during thawing for secondary processing on the formation of histamine needs further investigation to determine the potential risk to the final product. We would reiterate the Seafood report SR598 (Archer *et al*., 2008) recommendation that a "proper comparison" of the different thawing methods from commercial perspective is made, including methods such as microwave, radio-frequency and ultra high pressure (UHP) thawing.

9. The effect of temperatures experienced by product during retail display on the formation of histamine needs further investigation to determine the potential risk to final product. Further investigation with pelagic species is required in order to provide guidance.

10. A scientific study is carried out to establish: (1) histidine levels in Scottish sourced at-risk fish species (particularly Atlantic mackerel (*Scomber scombrus*) and Atlantic herring (*Clupea harengus*), but also horse mackerel (*Trachurus trachurus*) sardine (*Sardina pilchardus*), blue whiting (*Micromesistius poutassou*), and sprats (*Sprattus sprattus*); (2) which histamine forming bacteria are present in these species, and (3) what are the characteristics of histamine formation in these species. Such data would enable more accurate assessment and modelling of the risks in processing such species.

11. The role of other biogenic amines, notably cadaverine and putrescine, in "histamine" poisoning needs to be established, and their importance in at-risk fish species of relevance to the Scottish seafood industry. One study (Klausen & Lund, 1986) has reported that while histamine formation in herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) is similar, cadaverine is formed at much higher levels in mackerel compared with herring; and that this may possibly explain why mackerel and not herring are often implicated in incidents of histamine fish poisoning.

8. Acknowledgements

The authors would like to thank those companies, plants, organisations, associations, and professionals who provided their time, assistance, data and opinions for this project.

This project was funded by the Food Standard Agency in Scotland.

9. Appendix 1: Review of literature

Histamine (Scombroid or Scombrotoxin) fish poisoning is a foodborne chemical intoxication associated with the consumption of spoiled fish flesh that is high in histidine. The fish have to initially have a sufficiently high content of free histidine. In these fish, bacterial histidine decarboxylase from histamine-forming bacteria converts muscle histidine into histamine during decomposition. There are three pre-requisites (Lehane & Olley, 1999) for the elevation of the post-mortem histamine concentration in fish:

1. A sufficiently high content of free histidine in the fish.
2. The presence of bacteria capable of producing the enzyme histidine decarboxylase.
3. Environmental conditions, such as high temperatures, that allows such bacteria to proliferate.

9.1 Histamine fish poisoning

The symptoms of histamine fish poisoning (Attaran & Probst, 2002; McLauchlin *et al*., 2006; FAO-WHO, 2012) vary between individual cases but can include:

- A peppery taste sensation
- Tingling of the mouth and lips
- Headaches
- Sweating
- Rash
- Itching of the skin
- Nausea
- Dizziness
- Vomiting
- Diarrhoea

Symptoms normally last a few hours, but can continue for several days. These can begin within minutes of ingestion (Attaran & Probst, 2002). In some cases where there are other underlying medical issues, or the individual is particularly sensitive, the symptoms can be severe enough to require admittance to hospital for medical treatment (McLauchlin *et al*., 2006). It is considered to be rarely, if ever, fatal (FAO-WHO, 2012).

9.2 UK incidence of histamine poisoning related to fish

Until the mid 1980s, mackerel, especially smoked mackerel, accounted for the vast majority of incidents of suspected histamine fish poisoning in the UK (Taylor, 1986; Bartholomew *et al*., 1987). Since this time there has been significant decrease in the numbers of incidents and outbreaks overall, and particularly those related to mackerel. This has been attributed to improved handling and storage practices, particularly improvements in the cold-chain and the use of low storage temperatures (Taylor, 1986).

Between 1976 and 1986, 258 incidents of suspected histamine fish poisoning were reported in Britain (Bartholomew *et al*., 1987). The majority of these incidents involved mackerel (111, 43%, mainly smoked mackerel), followed by tuna (73, 28%)

and sardines (37, 14%). Ten incidents (4%) involved herring (pickled or kippered). The authors noted that although there had been a small number of incidents caused by canned salmon, they questioned whether these were in fact incidents of histamine fish poisoning (since low levels of histamine had been measured) and postulated that they may have been caused by another (undefined) toxin that caused similar symptoms.

Between 1992 and 2009, 71 general outbreaks (an incident in which two or more linked cases experience the same illness) of histamine fish poisoning were reported in England and Wales (between 0 and 10 incidents per year) affecting 336 people (Health Protection Agency, 2010). Analysis of outbreaks of foodborne illness associated with fish or fish products over this 18 year period showed that histamine fish poisoning accounted for 56% of these and that outbreaks occurred more frequently in food service settings (75%) and during the summer months (Health Protection Agency, 2010). The majority (>95%) of incidents were linked to the consumption of tuna (Gillespie *et al*., 2001; McLauchlin *et al*., 2006). In 2010, there were 2 outbreaks and 6 incidents of histamine fish poisoning reported in England and Wales (Health Protection Agency, 2010). One of these was associated with mackerel, while the others were due to the consumption of tuna.

In Scotland, the ObSurv surveillance system was established in 1996 for all general outbreaks of infectious intestinal disease (IID) in Scotland. Surveillance reports are regularly published in Health Protection Scotland's *HPS Weekly Report*. Since the present outbreak surveillance system was established in 1996, only six outbreaks have been identified in Scotland (Table 3), one in 1997, two in 2001, one in 2002, one in 2008 and one in 2009. Tuna was the suspect vehicle in all outbreaks and the majority related to food service. No histamine fish poisoning outbreaks were reported to ObSurv during 2011 (Smith-Palmer, 2012).

Table 3. Reported outbreaks of histamine fish poisoning in Scotland from 1996 to 2011

Year	Location	Number ill	Suspect vehicle	Reference
2009	Shop	4	Tuna	Smith-Palmer & Cowden, 2010
2008	Other	12	Tuna	Smith-Palmer & Cowden, 2009
2002	Restaurant	3	Tuna	Smith-Palmer & Cowden, 2003
2001	Canteen	9	Tuna	Smith-Palmer & Cowden, 2002
	Restaurant	5	Tuna steaks	Smith-Palmer & Cowden, 2002
1997	Private house	1	Tuna steak	Anon., 1997

Whilst the occurrence of reported illness caused by histamine from seafood within Scotland, and the UK as a whole, is generally low, it is believed (FAO-WHO, 2012) that the incidence of histamine fish poisoning is vastly under-reported. This is because the range of symptoms and their severity caused by histamine fish poisoning mean that consumers do not always inform the authorities or seek medical attention upon being symptomatic. In addition, it is often not possible to directly link the reported symptoms to a specific food sample required to undertake laboratory analysis to prove that histamine was the definitive cause of the symptoms. These factors mean that those incidents reported usually involve illness in more than one person who have had consumed food from the same source and or have had symptoms that had required medical assistance.

Worldwide histamine fish poisoning ranks among the most prominent seafood intoxications (Hungerford, 2010). For countries with the highest (reported) outbreak rates (examples are Denmark, New Zealand, France and Finland), numbers range from 2 to 5 cases/year/million people (Dalgaard *et al*., 2008).

It is normally the responsibility of the local Environmental Health Office to investigate the possible causes of incidents where histamine fish poisoning is suspected. However, unless there is direct evidence of culpability by the food business operator (FBO) responsible for the product, sufficient to bring a criminal prosecution, then details of the incident are not necessarily published. In 2005 The Food Standards Agency in Scotland and Health Protection Scotland (HPS) established a Food Surveillance System (FSS) in which the Local Authorities (LAs) enter the results of analysis conducted and which can be used to monitor trends in food safety incidents. Over the years this system has been developed and has been expanded to cover many of the LAs in the rest of the UK. The published reports (UK Food Surveillance System, 2012) however do not provide a sufficiently detailed breakdown of the data to include details on specific chemicals (histamine) that are tested.

Another source of information regarding the occurrence of histamine at levels likely to cause histamine fish poisoning are the inspections and analysis conducted by the relevant authorities during the import and export of pelagic species. The EU Border Inspection Post (BIP) report any products rejected due to histamine levels that exceed the legal maximum (100 mg/kg) to the EU's Rapid Alert System for Food (RASFF Database Portal). Analysis of the reports demonstrates that two consignments in 2009, one consignment in 2010 and one consignment in 2011 were rejected at the port of entry into the UK (from a total of 117 notifications for the same period from all of the EU). All of the affected consignments originated from tropical countries with three of the four consignments being tuna species and the fourth, salted mackerel.

With regard to fishery products sourced from Scotland and exported, the availability of data is dependant on the controls and reporting within the destination country. Between EU member states there are no regulatory inspection points and as such any data relating to histamine levels in exported product are determined by the industry, and therefore not published. The USA Food & Drug Administration (FDA) have only issued a single "Import Refusal Report" for Scottish fish relating to high histamine levels, since 1999. This was for a consignment of canned smoked Brisling sardines, in various sauces, produced by the International Fish Canners of Fraserburgh in 2004 (FDA Import Refusal Report, 2004). This resulted in a FDA Inspector undertaking an inspection of the production facilities in October 2010, with the finding that the HACCP plan did not sufficiently address controls for histamine in either the raw materials or during pre-canning processing (FDA Warning Letter, 2011). The company revised the HACCP plan accordingly and the submitted corrective actions were accepted by the FDA as being now compliant with US Food Safety Regulations (FDA Closure Letter, 2011).

9.3 Histamine formation in fish

The muscle tissues of fresh fish from at-risk species contain very little, if any, histamine (Frank & Yoshinaga, 1987; Rossi *et al*., 2002; Tao *et al*., 2009). Histamine formation (Figure 1) occurs in spoiled fish flesh that is high in histidine and contaminated with histamine-forming bacteria (Tao *et al*., 2009). Histamine formation can be very fast, as little as 2 to 3 hours at temperatures above 20°C

(Clark *et al*., 1999). Histidine is an amino acid common in proteins that acts as an intracellular buffer in the muscles of fish species (Abe *et al*., 1985). The level of histidine in fish species depends on the activity patterns of that particular species. High levels are associated with active "high-speed pelagic swimmers with outstanding sprint capability", such as mackerel (Abe *et al*., 1985). Certain naturally occurring bacteria produce the enzyme histidine decarboxylase during growth. This enzyme reacts with free histidine to form histamine, a non-volatile biogenic amine (Al-Bulushi *et al*., 2009). Detectable amounts of histamine appear to accumulate only after high levels of bacteria are reached in fish muscle. A mesophilic bacterial count of 6-7 $\log_{10}$ cfu g^{-1} has been found to be associated with 50 mg histamine/kg fish, the US Food and Drug Administration (FDA) maximum allowable histamine level (Du *et al*., 2002; Takahashi *et al*., 2003; Al-Bulushi *et al*., 2009).

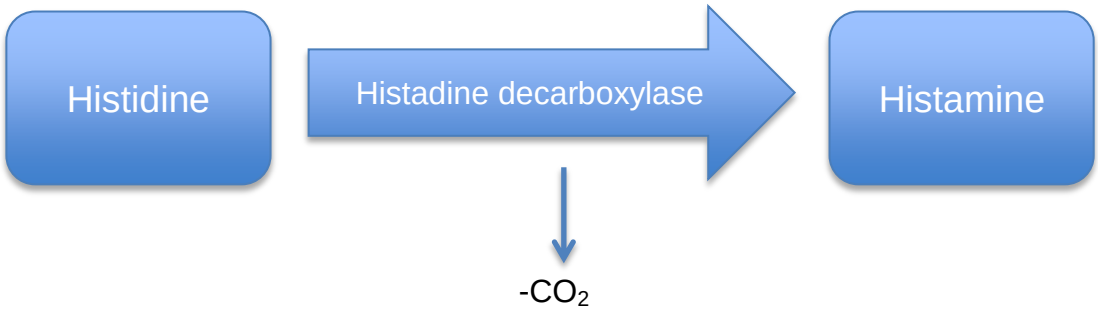

Figure 1. Conversion of histidine to histamine by microbial histidine decarboxylase

Levels of free histidine differ between at-risk fish species (Table 4) and there is evidence of a correlation between levels of histidine and formation of histamine in different species. For example Silvia *et al*. (1998) found higher levels of histamine formation in skipjack tuna (*Katsuwonus pelamis*) in comparison with big-eye tuna (*Thunnus obesus*) to be related to a 50% difference between free histidine levels in the two species. Free histidine is 3 to 5 times higher in fresh mackerel compared with fresh herring (Klausen & Lund, 1986; Mackie *et al*., 1997). The minimum histidine concentration required for bacterial histidine decarboxylase activity is estimated to be 1000 to 2000 mg/kg (Lee *et al*., 2012).

Table 4. Initial histidine levels reported in fish species associated with histamine fish poisoning

Fish species	Histidine (mg/kg)	Reference
Atlantic herring (*Clupea harengus*)	200	Klausen & Lund, 1986
	1233-1760	Mackie *et al*., 1997
Atlantic mackerel (*Scomber scombrus*)	2200	Klausen & Lund, 1986
	3730-3862	Mackie *et al*., 1997
Bigeye tuna (*Thunnus obesus*)	629-1187	Ruiz-Capillas & Moral, 2004
	4910	Antoine *et al*., 1999
Dophinfish, mahi mahi (*Coryphaena hippurus*)	1840 to 3440	Antoine *et al*., 1999; 2001
Yellowfin Tuna (*Thunnus albacares*)	3070 to 10390	Antoine *et al*., 2001
Silver mullet (*Mugil curema*)	1333.9	Millán *et al*., 2003

According to Emborg (2008), there is no clear understanding of how free histidine is distributed in fish. A study of skipjack tuna (*Katsuwonus pelamis*; Frank *et al*., 1981) found that histamine was formed in the anterior part of the fish (the muscle section close to the head) earlier and in higher concentrations than in the other sections. Concentrations were also high in the belly flaps. Histamine concentrations were arranged in a gradually decreasing gradient toward the posterior end of the fish. Higher levels of histidine have been measured in the white muscle (1187.1 mg/kg) of bigeye tuna (*Thunnus obesus*; Ruiz-Capillas & Moral, 2004) compared to the red muscle (629.0 mg/kg). However, a study of Spanish mackerel (*Scomberomorus niphonius*; Middlebrooks *et al*., 1988) did not observe any significant variation in the histamine concentration between different sections.

There is evidence that other biogenic amines, notably cadaverine and putrescine, have a role in "histamine" poisoning (Rossi *et al*., 2002; Al-Bulushi *et al*., 2009; Food and Drug Administration, 2011). Cadaverine is formed by the decarboxylation of the amino acid lysine, via the action of lysine decarboxylase. Putrescine is formed by the decarboxylation of the amino acid ornithine, via the action of ornithine decarboxylase. According to Rossi *et al*. (2002), many bacteria species produce ornithine and/or lysine decarboxylase, however only a few have histidine decarboxylase. In a comparison of biogenic amine formation in herring (*Clupea harengus*) and mackerel (*Scomber scombrus*), Klausen & Lund (1986) found that while similar amounts of histamine accumulated in herring and mackerel, cadaverine was formed at much higher levels in mackerel compared with herring. They concluded that high contents of cadaverine in mackerel might possibly explain why mackerel and not herring are often implicated in incidents of histamine fish poisoning.

9.3.1 Seasonality

Few studies have addressed whether there are intrinsic seasonal differences in histidine levels or in histamine formation in fish.

There is some evidence that histidine levels in fish species may be related to season, with higher levels of histidine present in the fish during summer when they are at their most active. In a survey of Japanese sardine (*Sardinops melanostictus*) in the sea of Hyuga-Nada, by Shirai *et al*. (2002), histidine content was highest in August to September and lowest in March. Similar results have been reported in Japanese jack mackerel (*Trachurus japonicus*) caught in the East China Sea (Osako *et al*., 2004), histidine levels were higher during spring and summer than in autumn and winter. Both studies concluded that since water temperatures are highest in summer and that because summer is a very active season for the fish, the high histidine content at this time may be because histidine has a role in buffering lactic acid which is produced in higher quantities during periods of high activity and under higher temperatures

Seasonal differences in fish muscle tissue pH may also relate to differences in histamine formation. Dalgaard *et al*. (2006) noted that autumn garfish (*Belone belone belone*) have caused more recorded outbreaks of histamine fish poisoning than spring garfish. They postulated that this could be due to the fact that autumn garfish had a lower pH (6.02 ± 0.01) than spring garfish (6.45 ± 0.04), and histamine formation by *P. phosphoreum* in broth with pH 6.07 ± 0.09 was more pronounced than at pH 6.48 ± 0.09. However, this was "not clearly observed" in studies with naturally contaminated garfish.

9.3.2 Toxicity levels

The threshold toxic dose for histamine in fish is not precisely known (Taylor, 1986). In most cases, histamine levels in illness-causing fish have been above 200 mg/kg, often above 500 mg/kg (Food and Drug Administration, 2011). A recent FAO-WHO expert meeting (FAO-WHO, 2012) concluded that a dose of 50 mg of histamine, which is the no observed adverse effect level (NOAEL), is the appropriate hazard level. At this level healthy individuals would not be expected to suffer any of the symptoms associated with histamine fish poisoning. They also concluded that there was no cumulative effect for the ingestion of consecutive meals containing fish, since histamine usually leaves the body within a few hours (histamine is metabolized by naturally occurring enzymes in the body and excreted in the urine).

Table 5. Consumption data of fish products in the UK, fish products in bold are considered as likely to be "at-risk" of containing high histamine levels capable of causing histamine fish poisoning (adapted from Henderson *et al*., 2002)

Fish product	Mean g/day	Medium g/day	Maximum g/day
Cod	82	79	338
Tuna – canned	**75**	**63**	**265**
Tuna – fresh	**97**	**100**	**305**
Haddock	82	70	340
Salmon	107	96	610
Sardine/pilchard	**85**	**80**	**360**
Trout	152	154	460
Mackerel	**101**	**97**	**486**
Herring	**125**	**120**	**370**
Place	129	106	400
Sole	140	145	327
Minimum	75	63	265
Maximum	152	154	610
Median	101	97	360

Based on available data and expert opinion, the FAO-WHO meeting agreed that a serving size of 250 g represented the maximum amount eaten in most countries at a single eating event (FAO-WHO, 2012). Serving size/portion size per consumer is defined in different ways in the literature and UK data shows significant variation between products. The UK Sea Fish Industry Authority (Seafish, 2012) defines one portion of seafood as 140 g. Survey data on the consumption of major fish species in the UK from Henderson *et al*. (2002), presented in Table 5, showed the UK median for fish consumption across species is 258 g/day. The mean consumption for different fish species ranged among fish consumers between 185 g/day and 369 g/day. Among species recognised as frequent histamine affected species herring, fresh tuna, mackerel, sardines and canned tuna are, in decreasing order, the species with the highest portion size per consumer/day.

Based on the hazard level of 50 mg of histamine and the serving size of 250 g, the FAO-WHO expert meeting (FAO-WHO, 2012) calculated that the maximum concentration of histamine in that serving was 200 mg/kg. They concluded that when food business operators apply good hygienic practices (GHP) and hazard analysis critical control point (HACCP), an achievable level of histamine in fish

products should be lower than 15 mg/kg, based on data made available by industry (using a test method with a lower detection limit of 15 mg/kg).

9.3.3 Permitted histamine levels in fish

Throughout the world there are regulations limiting the amount of histamine permitted in fish.

In the EU, EC regulation (No. 2073/2005) specifically cites the following fish species as associated with high amounts of histidine: Scombridae (which include Albacore, mackerel, tuna), Clupeidae (Atlantic herring (*Clupea harengus*), Baltic herring (*Clupea harengus membras*), Sardine (*Sardina pilchardus*)), Engraulidae (Anchovies), Coryphaenidae (mahi mahi), Pomatomidae (bluefish), and Scombresosidae (saury). For such species, the legislation stipulates that for a batch to be acceptable, nine independent samples from each batch should result in:

1. An average (mean) histamine concentration lower than 100 ppm (10 mg/100 g).
2. No more than 2 samples out of the 9 with a concentration of between 100 and 200 ppm.
3. No sample with a histamine content higher than 200 ppm (200 mg/kg or 20 mg/100 g).

Food Standards Australia New Zealand require samples to have less than 200 mg/kg (equivalent to 200 ppm or 20 mg/100 g) histamine (Australian and New Zealand Food Authority (ANZFA), 2010).

The US FDA has a hazard action level of 500 mg/kg (equivalent to 50 mg/100 g) and a decomposition level of 50 mg/kg (equivalent to 50 ppm or 5 mg/100 g) (Food and Drug Administration, 1995).

The draft Codex Alimentarius decomposition standard for smoked fish requires that products of susceptible species should not contain more than 100 mg of histamine per kg fish flesh (equivalent to 100 ppm or 10 mg/ 100 g) although when considering hygiene and handling the level is 200 mg/kg (Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission, 2010).

9.4 Fish species of concern

Histamine fish poisoning is often referred to as scombroid poisoning because of its association with Scombridae fish (a family of fish species that include tuna, mackerel and bonitos), however a range of non-Scombridae fish, particularly dophinfish or mahi mahi (*Coryphaena hippurus*), can also cause histamine fish poisoning.

The fish normally associated with histamine fish poisoning in humans are pelagic fish species. Pelagic fish live near the surface and are usually agile swimmers with streamlined bodies, such as mackerel, herring, sardines, pilchards and certain tuna species. These fish naturally have high levels of free histidine in their flesh. The majority of cases histamine fish poisoning recorded in the UK are linked to the consumption of tuna and mackerel. The pelagic sector represents the largest fish sector in Scotland, by volume of landings (Seafood Scotland, 2011). In the northern waters around Scotland the main pelagic fish species are Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*) and blue whiting (*Micromesistius poutassou*), with smaller populations of sprat (*Sprattus sprattus*) and horse mackerel (*Trachurus trachurus*) (Scottish Government, 2011). A number of

these species have been identified as “at-risk” of containing high histamine levels capable of causing histamine fish poisoning (Table 6). Other pelagic species processed in Scotland include tuna (*Thunnus* spp.), swordfish (*Xiphias gladius*), capelin (*Mallotus villosus*) and silver smelt (*Argentina sillus*).

Table 6. Pelagic fish species landed in Scotland and references for studies that have implicated those species as likely to be “at-risk” of containing high histamine levels capable of causing histamine fish poisoning

Fish species	Studies
Atlantic herring (*Clupea harengus*)	Özogul *et al*., 2002a, b; Özogul, & Özogul, 2005;
Atlantic mackerel (*Scomber scombrus*)	Lokuruka & Regenstein, 2004, 2007; Prester *et al*., 2009
Blue whiting (*Micromesistius poutassou*)	No studies identified
Horse mackerel (*Trachurus trachurus*)	Okuzumi *et al*., 1990; Korashy & Farag, 2005
Sardine (*Sardina pilchardus*)	Visciano *et al*., 2004; Özogul *et al*., 2004; Özogul & Özogul, 2006; Visciano *et al*., 2007; Erkan & Özden, 2008; Prester *et al*., 2009
Sprat (*Sprattus sprattus*)	No studies identified

To date there have been no reported incidents of elevated histidine or histamine in fresh North Atlantic salmon (*Salmo salar*), either wild caught or farmed. Two separate studies have been carried out (de la Hoz *et al*., 2000; Emborg *et al*., 2002) on storing Atlantic salmon (*Salmo salar*) in different packaging atmospheres at 2°C. Both showed that although histamine formed in the product, the rate of accumulation was slow and only low levels (<20 mg/kg) of histamine had accumulated by the time the product was considered spoiled. Although the histamine-forming bacteria *Photobacterium phosphoreum* has been shown to dominate the spoilage microflora of fresh Atlantic salmon (*Salmo salar*) at 2°C, it does not appear to form high levels of histamine (<20 mg/kg), or other biogenic amines, in packaged salmon (Emborg *et al*., 2002). Histamine formation has also been reported to be negligible in coho salmon (*Oncorhynchus kisutch*) stored in ice in a chill room at 2°C for 24 days (Aubourg *et al*., 2007).

High levels of histamine have been reported in cold-smoked Atlantic salmon (*Salmo salar*), but no incidents of histamine food poisoning from this product appear to have been reported in the published literature. A Danish study (Jørgensen *et al*., 2000) of biogenic amine formation in cold-smoked salmon (*Salmo salar*) during chilled storage (5°C) detected histamine above regulatory limits (>200 mg/kg EU limit for Scombridae and Clupeidae fish) at the end of shelf-life (5 to 9 weeks; Table 15). The authors concluded that the “production of biogenic amines in cold-smoked salmon during chill storage is unlikely to result in histamine fish poisoning in humans as indicated by epidemiological data”. In a survey of Norwegian smoked or cured salmon and trout products (Julshamn, 2008), it was reported that 30 out of 35 samples tested for histamine had levels below the general EU level of 100 mg/kg. However, two samples had concentrations between the general and the maximum EU limit for Scombridae and Clupeidae fish of 200 mg/kg, and three samples had histamine content above the maximum level. The highest concentration found was 370 mg/kg. In both surveys no samples reached levels considered to be toxic (<500 mg/kg).

Historically there have been reports of “scombrotoxic” fish poisoning in the UK associated with canned salmon (Bartholomew *et al*., 1987). However, it is not known which species of salmon was implicated. Also, the incriminated fish

contained, in general, very low levels of histamine (<10 mg/kg in 5 of the 6 incidents, 170 mg/kg in 1 of the 6). A histamine food poisoning-like incident has been reported (Gessner *et al*., 1996) following the consumption of smoked sockeye salmon (*Oncorhynchus nerka*). However, again, the implicated product had very low histamine levels (1.9 mg/kg).

A list of all fish species identified as at-risk to histamine formation is shown in Table 7. A wide range of fish products have also been associated with high histamine, as listed in Table 8.

Although histamine-forming bacteria have been found in a number of freshwater fish (Silveira *et al*., 2001), low levels of histidine in these fish mean that it is usually considered unlikely that enough histamine can be formed in such fish to cause histamine fish poisoning. For example, although histamine formation has been detected in rainbow trout (*Oncorhynchus mykiss*) stored on ice, only low levels (<2 mg/kg) have been detected and only towards the end of storage (Rezaei *et al*., 2007). However, Jeya Shakila & Vasundhara (2002) demonstrated that high levels, >200 mg/kg, of histamine could be formed in two Indian freshwater fish species, Catla (*Catla catla*) and Rohu (*Labeo rohita*), stored for 18 hours at 30°C or 5 days at 5°C.

Table 7. Fish species implicated as likely to be "at-risk" of containing high histamine levels capable of causing histamine fish poisoning

Fish species	Studies
Albacore tuna (*Thunnus alalunga*)	Ben-Gigirey *et al*., 1998; Kim *et al*., 1999; Kim *et al*., 2000, 2001b, 2002a, 2002b, 2003a; Economou *et al*., 2007
Atlantic bonito (*Sarda sarda*)	Mbarki *et al*., 2008; Gonzaga *et al*., 2009; Alak *et al*., 2011; Koral & Köse, 2012
Atlantic herring (*Clupea harengus*)	Özogul *et al*., 2002a, b; Özogul, & Özogul, 2005;
Atlantic mackerel (*Scomber scombrus*)	Lokuruka & Regenstein, 2004, 2007; Prester *et al*., 2009
Barracuda (*Sphyraena barracuda*)	Jeyasekaran *et al*., 2004
Barramundi (*Lates calcarifer*)	Bakar *et al*., 2010
Bigeye tuna (*Thunnus obesus*)	Silva *et al*., 1998; Rossi *et al*., 2002; Ruiz-Capillas *et al*., 2005
Blackspot seabream (*Pagellus bogaraveo*)	Fernández-No *et al*., 2011
Blue marlin (*Makaira nigricans*)	Tsai *et al*., 2007b; Chen *et al*. 2010
Bluefish (*Pomatomus salatrix*)	Lorca *et al*., 2001
Broadbill swordfish (*Xiphias gladius*)	Tsai *et al*., 2007b
Bullet tuna (*Auxis rochei rochei*)	Tao *et al*., 2011
Chilean jack mackerel (*Trachurus murphyi*)	Guillén-Velasco *et al*., 2004
Chinese mitten crab (*Eriocheir sinensis*)	Xu *et al*., 2009
Chub mackerel (*Scomber japonicus*)	Mbarki *et al*., 2009; Gonzaga *et al*., 2009
Dophinfish, mahi mahi (*Coryphaena hippurus*)	Kim *et al*., 2002a; Antoine *et al*., 2004; Starkusziewicz *et al*., 2004; Gonzaga *et al*., 2009; Chen *et al*., 2011
Eel (*Anguilla anguilla*)	Özogul *et al*., 2006; Fletcher, 2010
Escolar fish (*Lepidocybium flavobrunneum*)	Kan *et al*., 2000; Feldman *et al*. 2005; Dalgaard *et al*. 2008
European anchovy (*Engraulis encrasicolus*)	Pons-Sanchez-Cascado *et al*., 2003; Visciano *et al*., 2004; Rossano *et al*., 2006; Visciano *et al*., 2007
European anchovy (*Engraulis encrasicolus*)	Pons-Sanchez-Cascado *et al*., 2003; Visciano *et al*., 2004; Chaouqy & Marrachi, 2005; Rossano *et al*., 2006; Visciano *et al*., 2007
Fringescale sardinella, Sardine (*Sardinella fimbriata*)	Jeya Shakila *et al*., 2003a;
Garfish (*Belone belone belone*)	Dalgaard *et al*., 2006
Giant snakehead (*Channa micropeltes*)	Tao *et al*., 2011
Goldstripe sardinella (*Sardinella gibbosa*)	Jeya Shakila *et al*., 2005a; Joshi & Bhoir, 2011
Hardtail scad (*Megalaspis Cordyla*)	Tao *et al*., 2011
Horse mackerel (*Trachurus trachurus*)	Okuzumi *et al*., 1990
Indian anchovy (*Stolephorus indicus*)	Rodtong *et al*., 2005
Indian mackerel (*Rastrelliger kanagurta*)	Jeya Shakila *et al*., 2003a; Joshi & Bhoir, 2011
Jack mackerel (*Trachurus symmetricus*)	Bermejo *et al.,* 2003; 2004

Continued	
Kingfish (*Seriola grandis*)	Foo, 1975
Little mackerel (*Rastrelliger kanagurta*)	Lokuruka & Regenstein, 2004
Milkfish (*Chanos chanos*)	Tsai *et al*. 2007a
Northern bluefin tuna (*Thunnus thynnus*)	Guillén-Velasco *et al*., 2004
Pacific mackerel (*Scomber japonicus*)	Kim *et al*. 2000; Kim *et al*. 2001a; Kim *et al*., 2002a; Kim *et al*., 2009
Pacific saury (*Cololabis saira*)	Kim *et al*., 2009
Rabbitfish (*Siganus oramin*)	Lokuruka & Regenstein, 2004
Sailfish (*Istiophorus platypterus*)	Tsai *et al*., 2004
Sardine (*Sardina pilchardus*)	Gökoğlu *et al*., 2004; Visciano *et al*., 2004; Özogul *et al*., 2004; Özogul & Özogul, 2006; Visciano *et al*., 2007; Erkan & Özden, 2008; Prester *et al*., 2009; Fadhlaoui-Zid *et al*., 2012
Seer fish (*Scomberomorus commersonii*)	Jeya Shakila *et al*., 2005a, b; Mohan *et al*., 2009
Shorthead anchovy (*Stopephorus heterolobus*)	Chotimarkorn, 2011
Short-bodied mackerel (*Rastrelliger brachysoma*)	Tao *et al*., 2011
Silver mullet (*Mugil curema*)	Millán *et al*., 2003; Torres *et al*., 2003
Skipjack tuna (*Katsuwonus pelamis*)	Frank *et al*., 1981; Frank & Yoshinaga, 1987; Silva *et al*., 1998; Rossi *et al*., 2002; Thadhani *et al*., 2002; Lokuruka & Regenstein, 2004; Starkusziewicz *et al*., 2004; Jeya Shakila *et al*., 2005a
Snoek, barracouta or snake mackerel (*Thyrsites atun*)	Auerswald *et al*., 2006
Sockeye salmon (*Oncorhynchus nerka*)	Gessner *et al*., 1996
Spanish mackerel (*Scomberomorus niphonius*)	Edmunds & Eitenmiller, 1975; Middlebrooks *et al*., 1998; Kim *et al*., 2009
Swordfish (*Xiphias gladius*)	Chang *et al*., 2008
Trevally (*Carangoides armatus*)	Jeya Shakila *et al*., 2003a
Yellowfin tuna (*Thunnus albacares*)	Wei *et al*., 1990; Ohuma *et al*., 2001; Du *et al*., 2002; Jeya Shakila *et al*., 2003b; Starkusziewicz *et al*., 2004; Guizani *et al*., 2005; Emborg *et al*., 2005
Yellowtail amberjack, kingfish (*Seriola lalandi*)	Auerswald *et al*., 2006; Kim *et al*., 2009

Many fish sauces from Thailand, Cambodia, Indonesia, Malaysia, Myanmar, Philippines, Vietnam, and Japan utilise histamine-forming fish species and are produced using similar methods of production. High levels of histamine have been detected in these products (Table 8). On examining histamine formation in such products, Brillantes *et al*. (2002) found a good correlation between histamine levels in raw material and final products and concluded that histamine was formed both in the raw material and during fermentation. They speculated that bacterial histidine decarboxylase formed by the growth of histamine-forming bacteria prior to fermentation produced histamine during fermentation.

Table 8. Fish products identified as likely to be "at risk" of containing high histamine levels

Fish product	Studies
Salted herring	Auerswald *et al*., 2006; Vosikis *et al*., 2008
Salted mackerel	Tsai *et al*., 2005a
Marinated sardines (*Sardina pilchardus*)	Gökoglu, 2003; Gokoglu *et al*., 2003; Kilinc & Cakli, 2005
Canned mackerel	Tsai *et al*., 2005
Canned anchovies	Lee *et al*., 2005; Olgunoglu *et al*., 2009
Anchovy paste	Pirazzoli *et al*., 2006
Brined Spanish mackerel	Orawan & Pantip, 2000
Salted mullet roe	Kung *et al*., 2008
Missoltini (salted air dried twaite shad (*Alosa agone*))	Pirani *et al*., 2010
Budu (a Malaysian fermented mixture of anchovies and salt)	Rosma *et al*., 2009
Egyptian salted-fermented Bouri fish (*Mugil cephlus*) (Feseekh)	Mohamed *et al*., 2009
Fermented smoked fish	Petaja *et al*., 2000
Myeolchi-jeot (a Korean salted fermented fish product)	Mah *et al*., 2002
Dried Milkfish (Chanos chanos)	Hsu *et al*., 2009
Fish dumplings	Chen *et al*., 2008; Lee *et al*., 2012
Fish sauce	Poonsap 2000; Brillantes & Samosorn, 2001; Kimura *et al*. 2001; Jiang *et al*. 2010; Zaman *et al*., 2010
Fish-nukazuke (Japanese salted and fermented fish with rice-bran)	Mahendradatta 2003
Indonesian lawa teri (fresh anchovy mixed with citrus juice or vinegar and fried coconut)	Mahendradatta, 2003
Korean smoked and seasoned-dried Pacific saury (*Cololabis saira*)	Cha *et al*. 2001
Salted milkfish (*Chanos chanos*)	Tsai *et al*. 2006, 2007a
Saury paste	Miki *et al*., 2005
Smoked kahawai (a New Zealand product)	Fletcher, 2010
Smoked snoek	Auerswald *et al*., 2006
Smoked yellowfin tuna (*Thunnus albacares*)	Jeya Shakila *et al*., 2003b
Dried tuna	Auerswald *et al*., 2006
Nampla (Thai fish sauce)	Brillantes *et al*., 2002
Canned/salted anchovies	Kim *et al*., 2004b; Pons-Sanchez-Cascado *et al*., 2005b
Dried and salted tuna (*Thunnus thynnus*) roe	Periago *et al*., 2003
Mahyaveh (Iranian fish sauce)	Zarei *et al*., 2012
Dressed fried fish products	Yeh *et al*., 2006
Rihaakuru (cooked fish paste)	Naila *et al*., 2011

9.5 Bacteria responsible

The main bacteria responsible for histamine formation (Table 9) are members of the family Enterobacteriaceae, as well as species of *Clostridia*, *Pseudomonas* and *Lactobacilli* (Lehane & Olley, 1999; Du *et al*., 2002; Gram & Dalgaard, 2002; Köse, 2010). The optimal temperatures for growth for most of these bacteria are in the range 20°C to 30°C (McMeekin *et al*., 1993; Emborg & Dalgaard, 2008a,b), although some histamine-forming bacteria are capable of growing below 10°C. Histamine formation is generally considered to be "more commonly the result of high temperature spoilage than of long term, relatively low temperature spoilage" (Food and Drug Administration, 2011).

Table 9. Histamine-forming bacteria associated with different fish species

Fish species *	Histamine-forming bacteria	Reference
Albacore tuna (*Thunnus alalunga*)	*Hafnia alvei, Photobacterium damsela, Acinetobacter lwoffii, Enterobacter cloacae, Enterobacter aerogenes* (weak <350 mg/kg) *Morganella morganii* (strong)	Kim *et al*., 2001b; 2002b
Albacore tuna (*Thunnus alalunga*)	*Morganella morganii, Klebsiella oxytoca, Staphylococcus hominis, Enterococcus hirae*	Economou *et al*., 2007
Amberjack (*Seriola dumerilii*)	*Enterobacter aerogenes*, *Enterobacter* spp.	Kim *et al*., 2009
Atlantic mackerel (*Scomber scombrus*)	***Citrobacter freundii, Enterobacter agglomerans, Morganella morganii, Proteus mirabilis, Serratia fonticola, Serratia marcescens***	**López-Sabater *et al*., 1996**
Atlantic herring (*Clupea harengus*)	***Klebsiella oxytoca, Hafnia alvei, Proteus vulgaris***	**Özogul & Özogul, 2005**
Blackspot seabream (*Pagellus bogaraveo*)	*Pseudomonas syringae*	Fernández-No *et al*., 2011
Chilean jack mackerel (*Trachurus murphyi*).	*Morganella morganii*	Guillén-Velasco *et al*., 2004
Dolphinfish, mahi mahi (*Coryphaena hippurus*)	*Bacillus subtilis, Enterobacter aerogenes*	Chen *et al*., 2011
Garfish (*Belone belone belone*)	*Photobacterium. phosphoreum*	Dalgaard *et al*., 2006
Horse Mackerel (*Trachurus trachurus*)	***Pseudomonas* spp., *Vibrio* spp., *Photobacterium* spp.**	**Okuzumi *et al*., 1990**
Indian anchovy (*Stolephorus indicus*)	*Morganella morganii, Proteus vulgaris, Enterobacter aerogenes*	Rodtong *et al*., 2005
Mackerel	*Morganella morganii*	Kim *et al*., 2003a
Northern bluefin tuna (*Thunnus thynnus*)	*Serratia liquefaciens*	Guillén-Velasco *et al*., 2004
Pacific mackerel (*Scomber japonicus*)	*Morganella morganii, Proteus vulgaris*	Kim *et al*. 2000; Kim *et al*. 2001a
Pacific mackerel (*Scomber japonicus*)	*Enterobacter aerogenes*, *Enterobacter* spp.	Kim *et al*., 2009
Pacific saury (*Cololabis saira*)	*Enterobacter aerogenes*, *Enterobacter* spp.	Kim *et al*., 2009
Sailfish (*Istiophorus platypterus*)	*Proteus* spp., *Enterobacter* spp., *Klebsiella* spp., *Rahnella* spp., and *Acinetobacter* spp.	Tsai *et al*., 2004
Sardines	*Morganella morganii*	Kim *et al*., 2003a
Sardine (*Sardina pilchardus*)	*Kluyvera intermedia*	Fadhlaoui-Zid *et al*., 2012
Silver mullet (*Mugil curema*)	Enterobacteriaceae, *Vibrio damsella* (highest)	Millán *et al*., 2003
Spanish mackerel (*Scomberomorus niphonius*)	*Enterobacter aerogenes*, *Enterobacter* spp.	Kim *et al*., 2009
Yellowfin tuna (*Thunnus albacares*)	*Morganella morganii, Enterobacter agglomerans, Enterobacter intermedium, Serratia liquefaciens, Proteus vulgaris, Pseudomonas fluorescens*	Du *et al*., 2002
Yellowfin tuna (*Thunnus albacares*)	*Morganella psychrotolerans*, *Photobacterium phosphoreum*	Emborg *et al*., 2005
Tinned tuna	*Raoultella planticola*, *Raoultella ornithinolytica*	Kung *et al*., 2009

* Species landed in Scotland in bold

M. morganii has been cited by many recent studies (López-Sabater *et al*., 1996; Kim *et al*., 2003a; Veciana-Nogués *et al*., 2004; Emborg & Dalgaard, 2008a,b; Fletcher, 2010) as probably "the most important mesophilic bacteria with respect to histamine formation in seafood stored at temperatures above 10°C to 15°C" (Emborg & Dalgaard, 2008a,b). However, this bacterium does not produce toxic concentrations of histamine in seafood chilled to below about 7°C. Other mesophilic bacteria, such as *Clostridium perfringens*, *Hafnia alvei, Raoultella ornithinolytica* and *Raoultella*

planticola, are also significant histamine formers (Kanki *et al*., 2002; Hungerford, 2010).

Although refrigeration has been the main control for histamine formation, a number of psychrophilic and psychrotolerant bacteria (such as *Morganella psychrotolerans* and *Photobacterium phosphoreum*) have been identified as significant histamine formers at low temperatures. *M. psychrotolerans* is able to form toxic concentrations of histamine in seafood at storage temperatures as low at 0°C (Emborg *et al*., 2005, 2006; Emborg & Dalgaard, 2008a,b). Other *Photobacterium* spp., particularly *P. phosphoreum*, and *Vibrio* spp. may also be responsible for histamine formation at lower temperatures (Lehane & Olley, 1999; Kanki *et al*., 2004; Dalgaard *et al*., 2006; Köse, 2010). *Photobacterium damselae* has been identified as a likely causative agent of histamine fish poisoning in frozen-thawed fish (Kanki *et al*., 2007). Psychrophilic *Enterobacter* spp. have been identified as being able to form histamine at temperatures as low as 4°C in Pacific mackerel (*Scomber japonicus*), Pacific saury (*Cololabis saira*) and Spanish mackerel (*Scomberomorus niphonius*) sourced from Korean waters (Kim *et al*., 2009). Histamine concentrations were observed to rise from 4.2 to 79.3 mg/kg in Pacific mackerel, from not detectable to 2.2 mg/kg in Pacific saury and from 10.0 to 32.5 mg/kg in Spanish mackerel, after 3 days of storage at 4°C and thereafter remained constant. At 7°C and 10°C, the concentrations of histamine in all species began to increase after 3 and 2 days, respectively, and increased more significantly throughout the storage period. Maximum concentrations in Pacific mackerel, Pacific saury, Spanish mackerel and amberjack reached up to 4496.4 mg/kg, 2613.3 mg/kg, 997.6 mg/kg and 2410.7 mg/kg at 7°C; and 5553.2 mg/kg, 5649.8 mg/kg, 1147.4 mg/kg and 5898.2 mg/kg at 10°C, respectively, after 7 days.

At least some of the histamine-forming bacteria are halotolerant (salt-tolerant) or halophilic (salt-loving) (Food and Drug Administration, 2011; more details in Section 9.10.8). *Tetragenococcus muriaticus*, a histamine-forming halophilic lactic acid bacterium isolated from fish sauce has been demonstrated to form histamine in the presence of 20% w/v NaCl (Kimura *et al*., 2001). *Chromohalobacter beijerinckii* has been identified as potential source of histamine formation in salted fish (Beutling *et al*., 2009). The species is psychrophilic, and can grow at temperatures between 5°C to 42°C and is highly salt-tolerant, growing between 0.5 and 25% NaCl.

A number of the histamine-forming bacteria, such as *P. phosphoreum*, are facultative anaerobes and can grow in reduced oxygen environments (Food and Drug Administration, 2011). A number of studies (Özogul *et al*., 2002b; Jeya Shakila *et al*., 2005b; Emborg *et al*., 2005) have shown that vacuum packing does not inhibit the growth of histamine-forming bacteria and histamine formation. Although, some modified atmospheres will inhibit histamine formation (more details in Section 9.10.11)

9.5.1 Thermal death characteristics

There appears to be little data on the thermal death characteristics of histamine-forming bacteria. Some D and z values have been published for *H. alvei* and *M. morganii*, as shown in Table 10.

Table 10. Thermal death characteristics of histamine-forming bacteria

	Temperature (°C)	*Hafnia alvei*	*Morganella morganii*
D-value (min) *	54	0.63	
	55	0.36	
	56	0.20	
	57	0.11	
	58	0.06	15.27
	59		8.81
	60		4.79
	61		2.68
	62		1.46
z-value (°C) **		4.14	3.85
Reference		Fletcher, 2010	Osborne & Bremer, 2000

* The decimal reduction time: The time required at a quoted temperature to reduce the numbers of vegetative organisms or spores of a specific microorganism in a particular substrate or suspending medium to 10% of the initial number, 90% reduction, 1 log reduction.

** The number of degrees (°C) required for a tenfold change in the D value of a microorganism in a particular substrate.

9.6 Histamine forming enzyme

Optimum conditions for bacterial histidine decarboxylase enzyme activity are not completely understood, largely because of the many factors that need to be interpreted, including bacterial source, bacterial cell propagation, initial cell concentrations, and initial composition of the microflora. Once the bacterial enzyme histidine decarboxylase has been formed, it can continue to produce histamine in the fish even if the bacteria are not active (Köse, 2010; Food and Drug Administration, 2011). This has been confirmed in experiments using recombinant histidine decarboxylases of the histamine-forming bacteria *P. phosphoreum*, *P. damselae*, *R. planticola*, and *M. morganii* in which the bacteria themselves were absent (Kanki *et al*., 2007). The enzyme can be active at or near refrigeration temperatures (0°C to 4°C). The enzyme is likely to remain stable while in the frozen state and may be reactivated very rapidly after thawing (Kanki *et al*., 2007; Hungerford, 2010). It is widely reported that heat (cooking) destroys the enzyme activity (Hungerford, 2010), but it is unclear what the temperature/time requirements are.

9.7 Rate of histamine formation

Histamine formation can be very fast, as little as 2 to 3 hours at temperatures above 20°C (Clark *et al*., 1999). Data on the rates of histamine formation in different fish species at different temperatures is shown in Table 11. The majority of these studies were laboratory based and many were carried out at extreme temperature abuse conditions.

Table 11. Histamine formation in various at-risk fish species stored at different temperatures

Species *	Temperature (°C)	Storage time (days)	Histamine (mg/kg)	Reference
Albacore tuna (*Thunnus alalunga*)	0	18	0	Kim *et al*., 1999
	25	7	604	
Atlantic herring (*Clupea harengus*)	**2**	**12**	**42**	**Klausen & Lund, 1986**
	10	**9**	**109**	
Atlantic herring (*Clupea harengus*)	**0**	**8**	**90**	**Özogul *et al*., 2002b**
Atlantic herring (*Clupea harengus*)	**0**	**13**	**45**	**Mackie *et al*., 1997**
	10	**2**	**236**	
Atlantic mackerel (*Scomber scombrus*)	**2**	**10**	**43**	**Klausen & Lund, 1986**
	10	**7**	**114**	
Atlantic mackerel (*Scomber scombrus*)	**0**	**13**	**2 (gutted) 39 (ungutted)**	**Lokuruka & Regenstein, 2007**
	11	**2**	**56 (gutted) 43 (ungutted)**	
Atlantic mackerel (*Scomber scombrus*)	**22**	**1**	**1090**	**Prester *et al*., 2009**
Atlantic mackerel (*Scomber scombrus*)	**0**	**11**	**0.6**	**Mackie *et al*., 1997**
	10	**2**	**8.5**	
Atlantic bonito (*Sarda sarda*)	0	7	18	Koral & Köse, 2012
	4	7	114	
Barramundi (*Lates calcarifer*)	0	15	82	Bakar *et al*., 2010
	4	15	274	
Bigeye tuna (*Thunnus obesus*)	4	12	2000	Silva *et al*., 1998
	10	9	5000	
	22	5	1000	
Dophinfish, mahi mahi (*Coryphaena hippurus*)	26	12 h	>50	Starkusziewicz *et al*., 2004
	35	9 h	>50	
Dophinfish, mahi mahi (*Coryphaena hippurus*)	1.7	5	2	Du *et al*., 2001
	7.2	5	6	
	12.8	5	329	
Eel (*Anguilla anguilla*)	0	19	126	Özogul *et al*., 2006
	3	12	179	
European anchovy (*Engraulis encrasicholus*)	4	1	0	Rossano *et al*., 2006
	10	1	11	
	20	1	750	
	30	1	1664	
European anchovy (*Engraulis encrasicholus*)	4	3	523-1465	Visciano *et al*., 2007
	25	1	15-46	
European anchovy (*Engraulis encrasicholus*)	0	Day 1	2.7	Chaouqy & Marrachi, 2005
	0	12	101	

Continued

Species	Temperature (°C)	Storage time (days)	Histamine (mg/kg)	Reference
Garfish (*Belone belone belone*)	0	15	0 (spring)	Dalgaard *et al*., 2006
	0	17	11 (autumn)	
	5	9	149 (spring)	
	5	7	28 (autumn)	
Indian anchovy (*Stolephorus indicus*)	0	15	19	Rodtong *et al*., 2005
	15	32 h	190	
	35	8 h	254	
Chinese mitten crab (*Eriocheir sinensis*)	4	3	91	Xu *et al*., 2009
	20	3 h	63	
Pacific mackerel (*Scomber japonicus*)	0	14	0	Kim *et al*., 2001a
	4	14	574	
Mackerel (probably Pacific)	5	5	>50	Ohashi, 2002
	20	1	>50	
Sardine (*Sardina pilchardus*)	4	4	49	Gökoğlu *et al*., 2004;
	20	16 h	123	
Sardine (*Sardina pilchardus*)	0	12	1000	Ababouch *et al*., 1996
	25	2	1000	
Sardine (*Sardina pilchardus*)	4	3	168-1106	Visciano *et al*., 2007
	25	1	<0.5	
Sardine (*Sardina pilchardus*)	22	1	577	Prester *et al*., 2009
Shorthead anchovy (*Stolephorus heterolobus*)	0	7	22	Chotimarkorn, 2011
	4	7	49	
Spanish mackerel (*Scomberomorus niphonius*)	4	14	1	Edmunds & Eitenmiller, 1975
	10	14	238	
Spanish mackerel (*Scomberomorus niphonius*)	0	16	6	Middlebrooks *et al*., 1988
	15	5	400	
	30	2	250	
Skipjack tuna (*Katsuwonus pelamis*)	21	2	812	Frank *et al*., 1983
	25	1.5	576	
	29	1.5	1390	
	32	1.5	2750	
	38	1	3430	
Skipjack tuna (*Katsuwonus pelamis*)	-1	42	20	Frank & Yoshinaga, 1987
	4	24	73	
	10	12	169	
Skipjack tuna (*Katsuwonus pelamis*)	0	18	3	Rossi *et al*., 2002
	21	2	1600	
Skipjack tuna (*Katsuwonus pelamis*)	4	12	4000	Silva *et al*., 1998
	10	9	8000	
	22	5	3500	

Continued

Species	Temperature (°C)	Storage time (days)	Histamine (mg/kg)	Reference
Silver mullet (*Mugil curema*)	0	4	11	Millán *et al*., 2003
	6	4	18	
	25	4	1289	
Silver mullet (*Mugil curema*)	4	3	115	Torres *et al*., 2003
	10	3	156	
Tuna	0	12	20	Veciana-Nogués *et al*., 1997
	8	5	110	
	20	1.5	924	
Yellowfin tuna (*Thunnus albacares*)	0	5	8	Du *et al*., 2002
	4	5	31	
	10	5	286	
	22	5	5090	
Yellowfin tuna (*Thunnus albacares*)	0	17	6.1	Guizani *et al*., 2005
	8	8	150	
	20	1	1114	

* Species landed in Scotland in bold

9.8 Predictive models

Nomographs have been constructed to predict histamine formation in skipjack tuna and Arrhenius plots were used for such estimations in Atlantic bonito (*Sarda sarda*) and jack mackerel (*Trachurus symmetricus*; Bermejo *et al.,* 2003; 2004). However, Lehane & Olley (2000) claim that these methods involve too many assumptions and are inaccurate in their predictions. Working on jack mackerel (*Trachurus symmetricus*; Bermejo *et al.* (2003; 2004) fitted mathematical models for both bacterial growth and histamine formation. From these studies they concluded that jack mackerel could be stored for 4.5-5.5 days at 5°C, 1-2 days at 15°C and 17 hours to 2 days at 25°C before the quality of fishmeal produced from the mackerel would be affected. Similarly, Frank *et al*. (1983) developed a nomograph for estimating the rate of histamine formation in skipjack tuna (*Katsuwonus pelamis*) on storage at elevated temperatures. This nomograph accurately reflected the results obtained in their earlier spoilage studies. However, their studies indicated that the optimal temperature for histamine formation was 37°C, a finding that some reviewers (Taylor, 1986) note is not in agreement with many other studies. The nomograph was further developed (Frank & Yoshinaga, 1987) into a table (Table 12) to determine the effect of time and temperature on histamine formation in skipjack tuna (*Katsuwonus pelamis*).

Table 12. Effect of storage time and temperature on histamine formation in skipjack tuna (*Katsuwonus pelamis*) (adapted from Frank & Yoshinaga, 1987)

Histamine mg/kg	Days required to produce indicated histamine level at various temperatures						
	-1°C	1.7°C	4.4°C	7.2°C	10°C	12.8°C	15.6°C
5	25	12	2	<1			
10	33	20	10	1			
25	40	27	17	8	1		
50	52	38	28	19	12	6	
100	60	46	36	27	20	14	8
200	65	51	41	32	25	19	13
500	78	64	55	45	38	32	26
1000	86	73	63	54	46	40	34
1500	104	91	81	72	65	58	52

The Seafood Spoilage and Safety Predictor (SSSP) ver. 3.1 (http://sssp.dtuaqua.dk/HTML_Pages/Help/English/Index.htm) program, has been developed to model histamine formation in fish through the cold-chain. The SSSP program includes models to predict histamine formation by both *M. psychrotolerans* and *M. morganii*. This allows prediction of, for example, the effect of delayed chilling (as may happen on vessels with no refrigeration during catching) where fish can be exposed to some time of storage at ambient temperature followed by chilled storage at 0-5°C (Emborg & Dalgaard, 2008a,b). Details and issues with the practical use of this model are discussed in Appendix 10 (Section 18).

9.9 Detection methods

Isolation of histamine-forming bacteria is usually done using Niven's agar, a media containing tryptone, yeast extract, L-histidine dihydrochloride, sodium chloride, calcium carbonate, agar and a pH indicator bromocresol purple, such that purple colonies indicate possible histamine production (Niven *et al*., 1981). However, this medium has been associated with false positives, and a number of improvements have been suggested to improve isolation (Lehane & Olley, 2000).

Sensory evaluation is generally used to screen fish for spoilage odours that develop when the fish is exposed to time/temperature abuse. The US FDA recommends it as "an effective means of detecting fish that have been subjected to a variety of abusive conditions" (Food and Drug Administration, 2011). Visual observation of product during processing may also indicate changes in product texture or appearance, e.g. " honeycombing" in precooked tuna loins intended for canning, that are caused by exposure to the kinds of temperature abuse that can lead to histamine development. The US FDA recommends that fish which demonstrate the trait should be destroyed (Food and Drug Administration, 2011). However, odours of decomposition that are typical of relatively low temperature spoilage may not be present if the fish has undergone high temperature spoilage. This condition makes sensory examination alone an ineffective control for histamine (Food and Drug Administration, 2011).

Many methods (Etienne, 2006; Hungerford, 2010; Mermelstein, 2010; FAO-WHO, 2012) have been developed to detect histamine such as: thin layer chromatographic method, fluorometric method, high-performance liquid chromatography (HPLC), capillary electrophoresis, copper chelation method, oxygen-sensor-based method, enzyme-linked immunosorbent assay (ELISA). Chemical testing is an effective

means of detecting the presence of histamine in fish flesh. However, the validity of such testing is dependent upon the design of the sampling plan with variability both within individual fish and between fish from the same source being large. A review of the different methods for histamine chemical analysis was undertaken as part of the EU funded Seafood Plus program (Etienne, 2006). The EU (and FDA) standard reference method for the quantitative determination of histamine in seafood samples, as set out in Commission Regulation (EC) 2073/2005 is the Association of Official Analytical Chemists' Standard (AOAC) 977.13, in which biogenic amines extracted from the sample are derivitised before being separated using HPLC and measured using a fluorescence detector. Such methods although extremely accurate are not suitable for routine monitoring of samples due to the cost and technical complexity of the equipment required. Alternative "semi-quantitative", rapid, biochemical methods have been developed for use by the industry, which are based either on immuno-assays (ELISA) or measurement of enzymic activity where histamine is the substrate. A comparison of methods can be found in the FAO-WHO expert report on histamine (FAO-WHO, 2012).

The majority of these methods result in a colour change that can be used to indicate the presence of, or approximate concentration of, histamine in the sample. There have been numerous studies in which comparative assessments of different commercially available histamine test kits against the standard method have been undertaken (Rogers & Staruszkiewicz, 2000) and between themselves (Hungerford & Wu, 2012). Whilst these methods will never replace the HPLC methods for accuracy and precision, they do provide methods for use by industry to rapidly screen/assess product for the presence/absence of histamine in a semi-quantitative manner.

9.10 Review of controls

To develop controls for histamine there is a need to understand how it occurs naturally and how it is affected by processing.

9.10.1 Harvest

Harvesting practices, such as long lining and gillnetting, which cause death of the fish before the fish is removed from the water may allow histamine-forming bacteria to grow before the fish is landed on the vessel (Food and Drug Administration, 2011; Fletcher, 2010). This condition may be aggravated when the fish is allowed to remain on the line for a period of time after death especially during summer time when the water temperature around Scottish fishing areas increases to 10-15˚C from the winter range of 5-10˚C (DEFRA, 2010). In addition, the quantity of fish landed in a purse seine or on a long line may exceed a vessel's ability to rapidly chill the product (Köse, 2010).

9.10.2 Post-harvest chilling

Since histamine-forming bacteria are predominately mesophilic, rapid cooling of the fish immediately after catching is recognised as the key control for reducing histamine formation (Köse, 2010; Lehane & Olley, 2000; Starkusziewicz *et al*., 2004; Fletcher, 2010), together with the maintenance of adequate temperature control during the rest of the cold-chain (including retail, catering and the home). Initial cooling has been shown to be important in reducing the rate of histamine formation, even if temperatures rise at a later stage (Mitchell, 1993). For this reason, many countries have specific recommendations for initial cooling time or restrictions on the

time that fish may be stored unrefrigerated before cooling. These depend on fish size, water temperature, and climatic conditions. Since tuna are large fish and have a higher body temperature than other fish, shipboard handling and cooling is particularly critical for this species (Lehane & Olley, 2000; Fletcher, 2010). It is generally recommended that fish is chilled to between 4 and 0°C in less than 12 hours post harvesting. Regulations on chilling and freezing requirements aboard vessels in the EU are cited in EC Regulation No 853/2004. The most commonly applied recommendations in this regard are those of the US Sea Grant Extension Program (Lampila & Tom, 2009). Recommendations include:

- Generally, fish should be placed in ice or in refrigerated seawater or brine at 4.4°C or less within 12 hours of death, or placed in refrigerated seawater or brine at 10°C or less within 9 hours of death;
- Fish exposed to air or water temperatures above 28.3°C, or large tuna (i.e. above 9 kg) that are eviscerated before on-board chilling, should be placed in ice (including packing the belly cavity of large tuna with ice) or in refrigerated seawater or brine at 4.4°C or less within 6 hours of death;
- Large tuna (i.e. above 9 kg) that are not eviscerated before on-board chilling should be chilled to an internal temperature of 10°C or less within 6 hours of death.

Rates of cooling are different for different chilling methods (Köse, 2010). As a consequence of reduced contact area and heat transfer, ice alone will take longer to chill fish than ice slurry, re-circulated refrigerated seawater, or brine does. In addition the quantity of ice or ice slurry and the capacity of refrigerated seawater or brine systems must be suitable for the quantity of catch. However, we have been unable to find data that demonstrates an effect of different chilling methods on histamine formation.

When using ice to chill it is recommended best practice to place a layer of ice in the bottom and a layer at the top (Graham *et al*., 1992). Such fish can then be described as “well iced”. Example times for chilling a layer of “well iced” fish from various starting temperatures are shown in Table 13. Although the initial temperature has some effect on the cooling time, the thickness of the layer of fish has a much greater effect. Icing only on the top of the box will substantially increase the cooling time, as shown in Table 14.

Table 13. Time to chill a layer of fish (iced top and bottom) from various starting temperatures (adapted from Graham *et al*., 1992)

Thickness of layer (cm)	Starting temperature at centre of box (°C)	Time to chill to 2°C at the centre (hours)
7	5	1.5
7.5	10	2
7.5	15	2.75
15	5	6
15	10	9
15	15	>10

Table 14. Time to chill a layer of fish from 10°C to 4°C and 2°C if only iced on the top (adapted from Graham *et al*., 1992)

Thickness of layer (cm)	Starting temperature at centre of box (°C)	Time to chill to 2°C at the centre (hours)
1.3	<1	4
2.5	2	18
5.0	8	>24
7.5	18	>24

Although Jeyasekaran *et al*. (2004) found that delayed icing of barracudas (*Sphyraena barracuda*) had a significant affect on shelf life, a delay of 6 h reduced shelf life by 6 days, it had no significant effect on the growth of histamine-forming bacteria. Similarly Ben-Gigirey *et al*. (1998) found that delayed chilling of albacore tuna (*Thunnus alalunga*) did not result in significant histamine formation.

Tropical fish have been shown to have a predominantly mesophilic flora, while temperate fish have a predominance of psychrophilic microflora (Gram & Huss, 1996). Thus, icing is more effective in suppressing bacterial growth in tropical fish than in temperate fish and, therefore, it should be expected that less biogenic amines are formed in well-iced tropical fish than in temperate fish treated similarly (Lokuruka & Regenstein, 2004). Lokuruka & Regenstein (2004) confirmed this in trials comparing Atlantic mackerel (*Scomber scombrus*) to tropical species (skipjack, *Euthynnus pelamis*; rabbitfish, *Siganus oramin*; and little mackerel, *Rastrelliger kanagurta*). Prior storage in seawater at 19°C of Atlantic mackerel (*Scomber scombrus*) before icing, and leaving it undressed, positively influenced the formation of progressively higher histamine, cadaverine, putrescine, and spermine than the tropical fish over storage time and at ambient temperature (Lokuruka & Regenstein, 2004). Nevertheless, ice storage does inhibit the growth of histamine-forming bacteria in temperate species, such as anchovy (*Engraulis encrasicholus*; Pons-Sanchez-Cascado *et al*., 2005a).

9.10.3 Heading, gutting and skinning

The main bacteria responsible for histamine formation (Table 9) are all commonly present in the salt-water environment. They naturally exist on the gills and in the gut of the live fish (Taylor & Speckhard, 1983; Kim *et al*., 2003a). The most susceptible part of fish to bacterial colonization is the gill, followed by the outer skin and surface slime (Kim *et al*., 2003a). Thus, sanitary evisceration and removal of the gills may reduce, but not eliminate, the number of histamine-forming bacteria (Food and Drug Administration, 2011; Fletcher, 2010). However, Kim *et al*. (2003a) was of the opinion that additional handling of the fish by cutting gills out, which causes bleeding, may exacerbate bacterial contamination on board the vessel as well as in processing facilities. There is some evidence that the gills may be contaminated during handling and subsequently become a source of further cross-contamination (Taylor & Speckhard, 1983).

There is evidence that histamine formation is faster in whole ungutted fish than gutted for some species, such as Atlantic mackerel (*Scomber scombrus*; Lokuruka & Regenstein, 2007, Figure 2), albacore tuna (*Thunnus alalunga*; Kim *et al*., 1999), skipjack tuna (*Katsuwonus pelamis*; Rossi *et al*., 2002), European anchovies (*Engraulis encrasicholus*; Pons-Sanchez-Cascado *et al*., 2003), sardine (*Sardina pilchardus*; Erkan & Özden, 2008, Figure 3), Atlantic bonito (*Sarda sarda*; Koral &

Köse, 2012, Figure 4). Thus delayed gutting may increase histamine formation during subsequent processing and storage (Pons-Sanchez-Cascado *et al*., 2003).

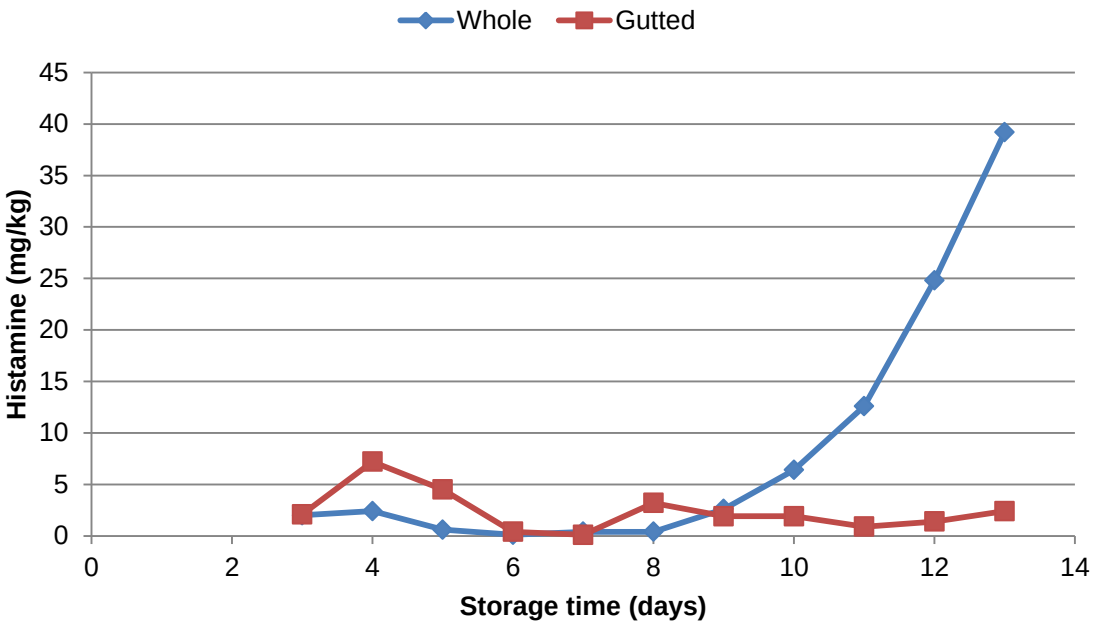

Figure 2. Histamine formation in whole and gutted Atlantic mackerel (*Scomber scombrus*) stored in ice (adapted from Lokuruka & Regenstein, 2007)

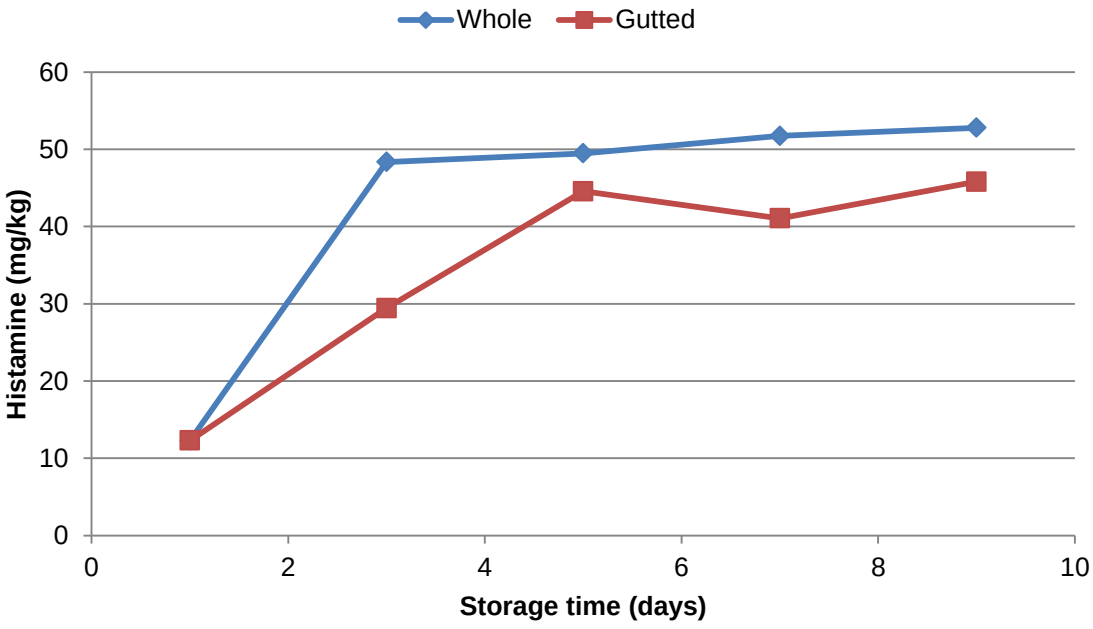

Figure 3. Histamine formation in whole and gutted Sardine (*Sardina pilchardus*) stored in ice (adapted from Erkan & Özden, 2008)

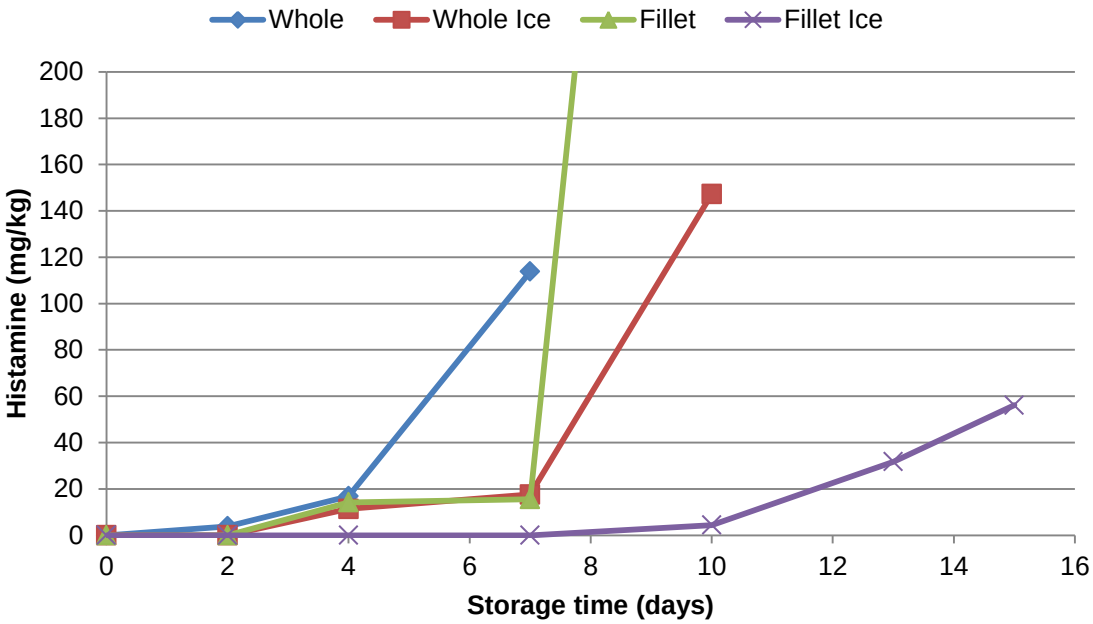

Figure 4. Histamine formation in whole and filleted Atlantic bonito (*Sarda sarda*) stored with or without ice at 4±1°C (adapted from Koral & Köse, 2012)

A number of studies have shown that short time temperature abuse during processing may lead to an increase in histamine formation. Economou *et al*. (2007) studied histamine formation in albacore tuna (*Thunnus alalunga*) loins stored at 0-2°C, 3-4°C and 6-7°C and abused for 2 hours daily at 20°C and 30°C for 7-12 days. Loins abused at 30°C for 2 hours daily, contained 67–382 mg/kg when stored at low temperature (0-2°C), and higher concentrations, 544.5–4156.6 mg/kg, when stored at 6-7°C.

Few studies have surveyed the presence of histamine-forming bacteria in fish processing facilities. In a survey of three US processors, processing a range of at-risk species including mahi mahi (*Coryphaena* spp.), bluefish (*Pomatomus saltatrix)*, blue fin tuna (*Katsuwonus* spp.), and king mackerel (*Scomberomorus* spp.), histamine-forming bacteria were only isolated on four occasions (Gingerich *et al*., 2001). Each of the three facilities was sampled during processing operations in the colder months of January, February, and March, and then again in the warmer months of May, June, or July for a two year period. Histamine-forming *Klebsiella ozaenae* was isolated from a sanitizing solution used to store knives, demonstrating that for a knife dip to be effective it must be changed regularly. The authors concluded that histamine-forming bacteria comprise only a small part of the microbiological flora within fish processing facilities and that cross-contamination from the general environment within these facilities is not an important source of contamination of these particular species. Kim *et al*. (2003a) detected histamine-forming bacteria in exudate from raw fish during processing. Although there was limited handling of the fish, *M. morganii* was detected on the surfaces that came in direct contact with the skin of processed mackerel and sardines. Allen *et al*. (2005) found histamine-forming bacteria on fish contact surfaces and on a knife used to cut fish on board a fishing vessel.

9.10.4 Freezing and thawing

There is very little data on the effect of freezing on histamine formation in at-risk fish species. It is generally considered that freezing will inhibit any growth of histamine-forming bacteria, and in some cases may kill histamine-forming bacteria. However there is little data on any effect of freezing on enzymic activity (bacterial histidine decarboxylase) or histamine.

There is evidence that freezing rate may have an effect on histamine formation. In a comparison of plate and air freezing of whole Indian mackerel (*Rastrelliger kanagurta*), Lakshmisha *et al*. (2008) reported that levels of histamine were higher in fish frozen using the slower of the two methods (Figure 5). Under the conditions used the plate freezing was over twice as fast (90 minutes to freeze from 26°C to -18°C using nominal operating temperature of -40°C) as the air freezing (220 minutes to freeze from 26°C to -18°C using nominal operating temperature of -40°C and 5 ms^{-2}). This data shows little difference in histamine levels during frozen storage, indicating that bacterial histidine decarboxylase is not active during storage at -18°C.

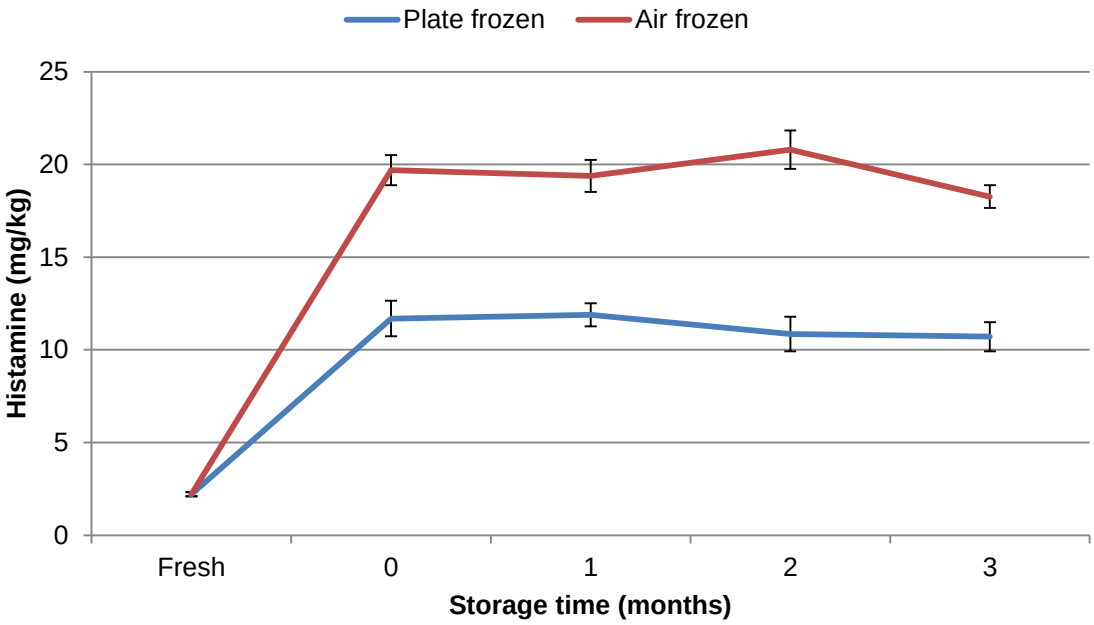

Figure 5. Histamine formation in plate and air frozen whole Indian mackerel (*Rastrelliger kanagurta*) stored at -18°C (adapted from Lakshmisha *et al*., 2008)

A number of studies have noted reductions in the rate of histamine formation in thawed fish compared with fresh. This has been observed in European anchovy (*Engraulis encrasicholus*; Rossano *et al*., 2006), albacore tuna (*Thunnus alalunga*; Economou *et al*., 2007) and garfish (*Belone belone belone*; Dalgaard *et al*., 2006). There is some evidence that the duration of frozen storage has an influence on the rate of histamine formation in the subsequently thawed fish. Rossano *et al*. (2006) observed a sequentially decreasing rate of histamine formation in thawed European anchovy (*Engraulis encrasicholus*) samples (stored for up to 6 days at 4°C) that had previously been stored at -20°C for 3 hours, 7 hours, 24 hours, 72 hours, and 10 days. A 3 hour storage at -20°C had little effect on histamine formation in comparison with unfrozen control samples, but a 7 hour storage significantly reduced the subsequent rate of formation. Dalgaard *et al*. (2006) attributed the different rate

of histamine formation in garfish to the inactivation by freezing below -20°C of *P. phosphoreum*, the major bacteria responsible for histamine formation in this fish species. Thus, the slower rate of histamine formation in the thawed fish was due to slower growing histamine-forming bacteria.

However, other studies report the opposite. Kim *et al*. (2002a) found that histamine accumulated rapidly when thawed fish was stored at 25°C. This may relate to pre-formed bacterial histidine decarboxylase or to the survival of heat-resistant histamine-producing bacteria other than *P. phosphoreum*. Kanki *et al*. (2007) have identified *P. damselae* as a likely causative agent of histamine fish poisoning in frozen-thawed fish. Staruszkiewicz *et al*. (2004) also demonstrated that bacterial histidine decarboxylase activity could be retained in frozen fish and could cause increases in histamine levels on thawing. Tsai *et al*. (2005c) found that although histamine formation stopped when fish were frozen, once samples were thawed histamine accumulated rapid exceeding 500 mg/kg within 36 hours at 25°C.

Overall, there is clear evidence that freezing can reduce histamine formation both by preventing the growth of histamine-forming bacteria and by reducing the activity of pre-formed bacterial histidine decarboxylase. Thus, freezing may be considered a control for the prevention of histamine formation. However, there is little data on optimum processing conditions and factors involved. There is little data on the effect of specific freezing rates, or storage temperatures/times.

While freezing may limit histamine formation it will not necessarily prevent its occurrence in thawed fish, and there is evidence that pre-formed bacterial histidine decarboxylase may reactivate rapidly after thawing. Thus, strict temperature controls during thawing of at-risk fish species are to be recommended to prevent histamine formation during thawing.

9.10.5 Heat processing

Thermal interventions, such as steam and hot water, have been widely evaluated and used in the red meat and poultry industries throughout the world to reduce pathogenic and spoilage bacteria on raw meats, and some studies have also been carried out on fish (Purnell & James, 2012). However, no published studies have been identified on the effect of such treatments on histamine-forming bacteria. Since histamine-forming bacteria do not appear to be heat tolerant it is likely that such interventions could be used to control histamine formation in at-risk fish and seafood products.

9.10.6 Canning (retorting)

There is conflicting opinion on the effect of canning on histamine levels in at-risk fish species. It is widely reported that while canning will kill histamine-forming bacteria and denature the enzyme responsible for formation, it does not destroy histamine that has already formed (Lehane & Olley, 1999; Kim *et al*., 2002; Fletcher, 2010). A number of studies report lower levels of histamine in fish after canning than in the raw material prior to canning (Baygar & Gokoglu, 2004; Jeya Shakila *et al*., 2005a). Since these studies are reporting formed histamine levels, this indicates that canning (and cooking) may reduce levels of histamine after formation. However, a Tunisian study (Selmi *et al*., 2008) reported higher histamine levels in canned tuna and sardine after cooking and canning than before processing (Figure 6), although levels were lower than safety threshold limits (50 mg/kg).

The addition of other food ingredients to canned fish, such as tuna, can change the histamine levels. For example, a survey of Brazilian produced canned tuna found that canned tuna in water and salt had lower histamine levels compared to tuna in oil or with tomato sauce (Silva *et al*., 2011).

It should be noted that not all "canned" fish products are heat treated (retorted), for example anchovies (Lee *et al*., 2005).

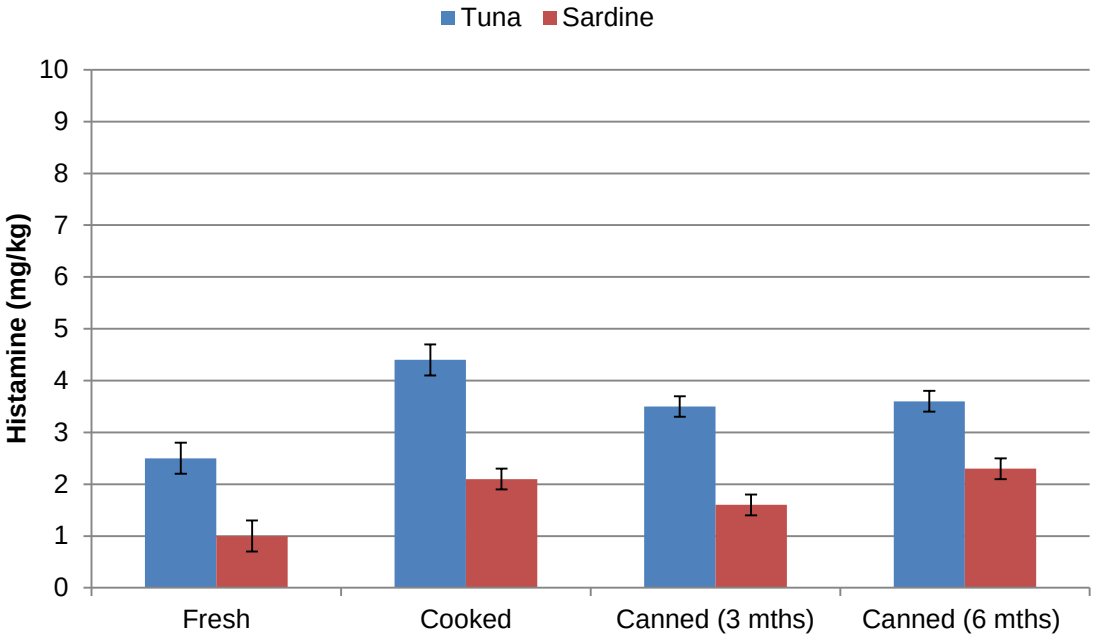

Figure 6. Changes in histamine levels in Northern bluefin tuna (*Thunnus thynnus*; in olive oil) and sardine (*Sardina pilchardus*; in tomato sauce) flesh during the canning process and following 3 and 6 months of storage (adapted from Selmi *et al*., 2008)

A study of canning of three fish species, seer fish (*Scomberomorus commersonii*), goldstripe sardinella (*Sardinella gibbosa*) and skipjack tuna (*Katsuwonus pelamis*), found that canning reduced histamine levels in all three species from 5, 5, and 9 mg/kg in the raw fish to approximately 1, 1, and 2 mg/kg, respectively (Jaya Shakila *et al*., 2005a). Holding the fish prior to canning at 30±2°C for 6 hours increased levels of histamine to 17, 8, and 14 mg/kg in the seer fish (*Scomberomorus commersonii*), goldstripe sardinella (*Sardinella gibbosa*) and skipjack tuna (*Katsuwonus pelamis*), respectively, before canning. These elevated levels were reduced to 2 to 4 mg/kg in the canned fish.

9.10.7 Smoking

Two methods of smoking are usually distinguished, cold and hot smoking, although there are also variations within these types, and a third category, warm smoking, may be used according to the temperature of smoke applied (Köse, 2010). The temperature of the smoke is in the range of:

1. About 12 to 25°C during cold-smoking.
2. About 25 to 45°C in warm-smoking.
3. About 40–100°C in hot-smoking, during which the centre of the product may reach up to 85°C.

Other than its effect on taste and flavour, smoking was original developed as a preservation method. The combined preservative effect of smoking arises from 4 main factors (Horner, 1997) that can be summarized as:

1. Surface drying, providing a physical barrier to bacterial pathogens and preventing aerobic microbial proliferation.
2. Drying, decreasing the water activity, which has inhibition effect on pathogenic and spoilage bacteria (although a certain salt content is required).
3. Deposition of phenolic antioxidant substances, delaying autoxidation (and rancidity).
4. Deposition of antimicrobial substances, such as phenols, formaldehyde and nitrites.

An additional factor, heat (which will kill histamine-forming bacteria and denature the enzyme responsible for formation), can also be added in the case of hot-smoking.

Various pre-treatments such as salting, marinating, or drying may be applied prior to smoking. Various salt contents were suggested for salting fish prior to smoking. Horner (1997) reported that 2-3% salt content in the fish is the maximum salt content required if the product has to be eaten as a main dish rather than a condiment. Zaitsev *et al*. (2004) suggested a salt content of 1.8-2% although some products may contain higher salt contents for such products.

High histamine levels have been measured in both cold and hot-smoked fish products (Lehane & Olley, 1999).

9.10.7.1 Hot smoking

Little data is available on the effect of hot-smoking on histamine, histamine levels or histamine-forming bacteria. Zotos *et al*. (2001) concluded that histamine levels did not exceed 75 mg/kg immediately after hot smoking tuna (Kawakawa, *Euthynnus affinis*), made from raw material that had been held for 2 months at -20°C, processed under a number of different conditions (a: 30 minutes at 30°C, 30 minutes at 50°C and 15 minutes at 75°C; b: 60 minutes at 30°C, 30 minutes at 50°C, and 15 minutes at 75°C). However, they did not test increases in histamine during storage despite storing the product vacuum packed for 3 months at 5°C.

Histamine levels in yellowfin tuna (*Thunnus albacares*) were reported to increase to 12 mg/kg during mild hot-smoking (70°C, 2 hours) whilst numbers of amine-producing bacteria decreased but were not eliminated (Jeya Shakila *et al*., 2003b). Predominant amine-forming bacteria identified were *Micrococcus* spp., *Alcaligenes* spp. and *Corynebacterium* spp. While the temperature within the smoker was monitored, Shakila *et al*., (2003) did not measure actual product temperatures, and it is likely that product temperatures did not reach the levels recommended to inactivate vegetative bacterial hazards or those determined to inactivate *M. morganii* (Osborne & Bremer, 2000) or *H. alvei* (Bremer *et al*., 1998). However, given the low levels of histamine produced during smoking, Shakila *et al*. (2003) suggest that histamine development in smoked yellowfin tuna may be predominantly associated with delays in the hot blanching performed before smoking, or delays in smoking.

Hot-smoking has been shown to eliminate histamine-forming bacteria and denature the enzyme responsible for formation, however it does not destroy any histamine that has already formed (Lehane & Olley, 1999; Kim *et al*., 2002; Fletcher, 2010).

Thus hazard analysis and critical control point (HACCP) plans need to ensure that the fish has not been stored for too long before smoking to prevent histamine-forming before smoking. In addition, hot-smoked products are susceptible to histamine formation if recontamination were to occur due to poor handling during packing.

Fletcher *et al*. (1998) recommended, with hot-smoked products, that processors should rely on the heat process to eliminate histamine-forming bacteria rather than adding high levels of salt to such products to inhibit their growth.

9.10.7.2 Cold smoking

Emborg & Dalgaard (2006) found that cold-smoked tuna linked to outbreaks of histamine fish poisoning in New Zealand had a low (1.3-2.2%) water phase salt content (WPS) compared to other commercially available product (4.1-12.7%). The 2006 report found that although *M. psychrotolerans* (a histamine-forming bacteria) grew at a WPS level of 4.4%, at 6.9% the microflora was dominated by benign lactic acid bacteria and neither *M. psychrotolerans* nor *P. phosphoreum* grew. Therefore it was recommended that a WPS of >5% was used in cold-smoked tuna to prevent histamine formation. However, Emborg & Dalgaard (2006) considered this as quite high for New Zealand smoked products and did note that such a recommendation went against dietary recommendations to reduce salt intake.

High histamine levels have been reported in cold-smoked Atlantic salmon (*Salmo salar*; Jørgensen *et al*., 2000; Julshamn, 2008). In both surveys, no samples reached levels considered to be toxic (<500 mg/kg). A Danish study (Jørgensen *et al*., 2000) of biogenic amine formation in cold-smoked salmon (*Salmo salar*) during chilled storage (5°C) detected histamine above regulatory limits (>200 mg/kg EU limit for Scombridae and Clupeidae fish) at the end of its shelf-life (5 to 9 weeks; Table 15). The authors concluded that the "production of biogenic amines in cold-smoked salmon during chill storage is unlikely to result in histamine fish poisoning in humans as indicated by epidemiological data".

Table 15. Levels of histamine formed in sliced vacuum-packaged cold-smoked salmon stored at 5°C (adapted from Jørgensen *et al*., 2000)

Smokehouse	Lot no	Process	NaCl (% WPS)	$NaNO_2$ (ppm)	Shelf-life (weeks)	Histamine (mg/kg)
A	97-1	Brine injection; ingredients: salt; Drying: 3-4 hours, 26°C, no humidity control; Smoking: 4 hours, 26°C, no humidity control	5.4 ± 0.4	<0.6	4-5	135 ± 73
	97-2		5.0 ± 0.5	<0.6	4.5-5	190 ± 130
	98-1		4.9 ± 0.2	<0.6	4-5	3 ± 3
	98-2		4.1 ± 0.5	<0.6	4.5-5.5	96 ± 20
B	97-3	Brine injection; ingredients: salt, sucrose; Drying: no separate process; Smoking: 4-7 hours, 21-22°C, no humidity control	7.5 ± 0.6	<0.6	8.5-9	19 ± 27
	97-4		5.9 ± 0.4	<0.6	7-8	4 ± 2
	98-3		4.2 ± 0.3	<0.6	3-4	102 ± 15
	98-4		4.2 ± 0.6	<0.6	5.5-6.5	50 ± 41
C	97-5	Dry salting; ingredients: salt, nitrite, sucrose; Drying: 6 - 12 hours, 27°C, 50% relative humidity; Smoking: 6 - 12 hours, 27°C, 65% relative humidity	7.9 ± 1.3	16 ± 7	5-6	108 ± 118
	97-6		5.6 ± 0.5	22 ± 10	4.5-5	240 ± 64
	98-5		3.9 ± 0.5	8 ± 5	4-4.5	10 ± 6
	98-6		4.9 ± 0.7	12 ± 8	5.5-6.5	16 ± 10

In a survey of Norwegian smoked or cured salmon and trout products (Julshamn, 2008) it was reported that 30 out of 35 samples tested for histamine had levels

below the general EU level of 100 mg/kg. However, two samples had concentrations between the general and the maximum EU limit for Scombridae and Clupeidae fish of 200 mg/kg, and three samples had a histamine content above the maximum level. The highest concentration found was 370 mg/kg.

9.10.8 Salting/brining

At least some of the histamine-forming bacteria are reported to be halotolerant or halophilic (Food and Drug Administration, 2011). *Chromohalobacter beijerinckii* has been identified as potential source of histamine formation in salted fish (Beutling *et al*., 2009). The species is psychrophilic, growing from 5 to 42°C and highly salt-tolerant, growing between 0.5 and 25% salt (NaCl). *Tetragenococcus muriaticus*, a histamine-forming halophilic lactic acid bacterium isolated from fish sauce has been demonstrated to form histamine in the presence of 20% w/v salt (Kimura *et al*., 2001).

Kongpun & Suwansakornkul (2000) found that histamine levels in Spanish salted mackerel at fish:salt levels of 1:1, 2:1 and 3:1 by weight, respectively, increased to 1155, 1583 and 1251 mg/kg during the first 6 days as salt levels increased to 15%, but subsequently decreased as salt levels increased to 19%.

Polish salted herrings prepared in high salt (26%) brines did not produce any histamine when stored at 4 or 22°C for 21 days, and those prepared in low salt (16%) brines produced 35 mg/kg during 3 weeks of storage (Fonberg-Broczek *et al*., 2003).

Lower salt levels (≥10%) have been found to be effective in inhibiting the growth of histamine-forming bacteria isolated from Indian anchovy (*Stolephorus indicus*; Rodtong *et al*., 2005).

Anchovies are traditionally preserved by salting and ripening for 2 to 9 months. After ripening, the semi-preserved anchovy is desalted, filleted, and packed in brine or oil in jars or cans without any heating process. These products are treated as a refrigerated product in some countries, for example many European countries, and as ambient products in others, for example in the USA (Lee *et al*., 2005). In a survey of canned anchovy products imported into the USA, Lee *et al*. (2005) detected histamine levels above the FDA guideline of 50 mg/kg in 20% of samples (total n=62). Histamine-forming bacteria were not detected in the products, probably due to high salt contents (12% to 15%), leading the authors to conclude that poor-quality raw fish and improper handling practices could contribute to the formation of histamine in retails products.

High salt levels (20 or 23 g/L in the aqueous phase) in anchovy paste stored for 1 year prevented histamine formation (Pirazzoli *et al*., 2006). As histamine levels did not relate to bacterial numbers, these researchers suggest that the histamine might have been due to the presence of pre-formed bacterial histidine decarboxylase in the anchovies.

9.10.9 Marinating/added ingredients

Ingredients such as lemon, herbs or antimicrobial agents may be added to a number of fish products to either alter product taste/aroma or to delay spoilage. Some of these ingredients may inhibit histamine-forming bacteria, for example, rosemary and sage tea extracts have been shown to significantly reduce histamine accumulation (probably through by inhibiting histamine-forming bacteria) in sardine (*Sardina*

pilchardus) fillets (Özogul *et al*., 2011). However, enzymatic action (bacterial histidine decarboxylase) and thus histamine formation may still continue (Köse, 2010). A decrease in pH occurring either by adding lemon or due to the presence lactic acid bacteria has been reported to enhance histamine formation by Köse (2010), however no explaination was given of why this should be.

Marinated fish products are also produced, usually with the addition of acid (usually vinegar, acetic acid) and salt. There are two main types of marinating (Köse, 2010):

1. Cold or salt marinating
2. Warm marinades prepared from pre-fried, pre-cooked or pre-smoked fish.

Histamine levels have been show to increase during the refrigerated storage (4°C) of marinated fish products such as sardines (Kilinc & Cakli, 2005). Although vinegar marinating of anchovies has been shown to decrease histamine accumulation (Pons-Sánchez-Cascado *et al*., 2005). Several factors are reported contributing histamine development in marinated fish products (Gökoğlu, 2003). They may be summarized (Köse, 2010) as follows:

1. Amino acid decarboxylase activity is higher in an acidic environment (the optimum pH being between 4.0 and 5.5) and bacteria produce decarboxylases, as a part of their defence mechanisms against the acidity.
2. Acidic conditions of marinades make the tissue cathepsins more active resulting in the degradation of some muscle proteins into peptides and amino acids which are the precursors of amine formation in the presence of amino acid decarboxylase-positive microorganisms.

Since marinating may enhance the formation of histamine, it is recommended that such products should be processed and stored at as low a temperature as possible to inhibit the initial growth of histamine-forming bacteria. Since marinating also enhances the action of bacterial histidine decarboxylase, care must be taken to ensure that raw materials have low histamine levels prior to processing.

9.10.10 Drying

Drying after salting is an ancient method of fish preservation. Both mechanical and natural methods are utilised. The low moisture content and resulting low water activity (a_w) of such products directly inhibits the growth of histamine-forming bacteria and thus such products are considered a low risk regarding histamine fish poisoning (Köse, 2010).

9.10.11 Packaging

Modified atmosphere packaging (MAP) has become an increasingly popular method to extend the shelf life of fresh refrigerated foods. MAP is defined as the packaging of a perishable product in an atmosphere that has been modified so that its composition is different than that of air. Gases commonly used in modified atmosphere packing are nitrogen (N_2), oxygen (O_2), and carbon dioxide (CO_2). Nitrogen is used as an inert gas to prevent package collapse in products that absorb CO_2 because of its low solubility in water and fat, but it has no antimicrobial properties. CO_2 is used to replace O_2 in packages to delay oxidative rancidity and inhibit the growth of aerobic microorganisms. Oxygen is generally avoided in gas packaging mixtures used for high-fat fish (which includes most fish species associated with histamine fish poisoning) since it promotes oxidative rancidity.

However, it may be used in low concentration in both high and low-fat fish products to prevent anaerobic conditions and thereby limit the growth of potentially harmful anaerobic microorganisms. Vacuum packaging (VP) is usually treated as an alternative to MAP, but may be considered a form of MAP since the removal of air is essentially a modification of the atmosphere.

Some MAP conditions have been reported to control the formation of histamine in susceptible products. A 40% CO_2:40% O_2:20% N_2 gas mix inhibited histamine formation in bigeye tuna (*Thunnus obesus*), whereas fish packed in a 60:15:25 mix, or air, resulted in product exceeding 100 mg/kg histamine during 33 days storage at 2°C (Ruiz-Capillas & Moral, 2005). Aytac *et al*. (2000) found that MAP in 100% CO_2 inhibited growth and histamine formation of *M. morganii* in mackerel, inhibition was even greater in combination with the addition of 5% salt. Similarly, Alak *et al*. (2011) found that a 100% CO_2 MAP inhibited histamine formation in Atlantic bonito (*Sarda sarda*) fillets (Figure 7). MAP (60% CO_2:40% N_2) decreased histamine formation in Atlantic herring (*Clupea harengus*) compared to air storage at 2°C or in ice (Özogul *et al*., 2002a, 2002b). Although levels of histamine did not exceed the EU limit of 100 mg/kg while the fish remained edible by sensory assessment, the US 50 mg/kg limit was surpassed in edible herring stored in ice (8 days 90 mg/kg), in vacuum packaging (8 days 82 mg/kg), and in MAP (10 days 83 mg/kg). Similar results were recorded for sardines (*Sardina pilchardus*) at 4°C (Özogul *et al*., 2004; Özogul & Özogul, 2006). After 14 days storage at 4°C, the concentration of histamine increased to 200 mg/kg, 130 mg/kg and 100 mg/kg for air, VP, and MAP stored samples, respectively.

Emborg *et al*. (2005) found that although vacuum packaging and MAP in an atmosphere of 60% CO_2:40% N_2 did not prevent the development of *M. psychrotolerans* or *P. phosphoreum* in yellowfin tuna (*Thunnus albacares*), however, MAP storage in an atmosphere of 40% CO_2:60% O_2 did. No histamine was produced in naturally contaminated yellowfin tuna (*Thunnus albacares*) stored under this gas mix for 28 days at 1°C, they therefore recommended this mixture be used instead of vacuum packing for chilled storage of tuna. However, as Fletcher (2010) notes, they did not report whether storing tuna under atmospheres with high oxygen concentrations had any sensory effects. Dalgaard *et al*. (2006) found that MAP did not reduce histamine formation by P*. phosphoreum* in garfish (*Belone belone belone*) stored at 5°C. Lopez-Caballero *et al*. (2002) found that although the growth of *P. phosphoreum* was reduced by mixtures containing 60% CO_2, histamine formation was higher in an atmosphere containing 60% CO_2:15% O_2:25% N_2 than in air. Although Kanki *et al*. (2004) reports that *P. phosphoreum* is resistant to CO_2.

There are conflicting reports on the effectiveness of vacuum packing on controlling histamine formation. Vacuum packing was not found to control histamine formation in seer fish (*Scomberomorus commersonii*) although it did increase shelf-life (Jeya Shakila *et al*., 2005b). Fletcher (2010) speculated that in doing so it might reduce "the margin of safety between spoilage and histamine production". Vacuum packaging has also not been found to control histamine formation in yellowfin tuna (*Thunnus albacares*; Wei *et al*., 1990; Emborg *et al*., 2005) or Atlantic bonito (*Sarda sarda*) fillets (Figure 7; Alak *et al*. (2011). Vacuum packaging was reported to increase histamine formation in herring compared to ice storage (Özogul *et al*., 2002b) although the reverse was true in sardines at 4°C. However, a later study by the same authors (Özogul & Özogul, 2005) reported that vacuum packing inhibits the

growth of the main histamine-forming bacteria (*Klebsiella oxytoca*, *H. alvei*, *Proteus vulgaris* and *Pantoea agglomerans*) on herring.

The use of an oxygen scavenger (oxygen absorbent sachet) to remove oxygen from packs has been reported to significantly decrease histamine formation in seer fish (*Scomberomorus commersonii*) while also increasing shelf-life (Mohan *et al*., 2009).

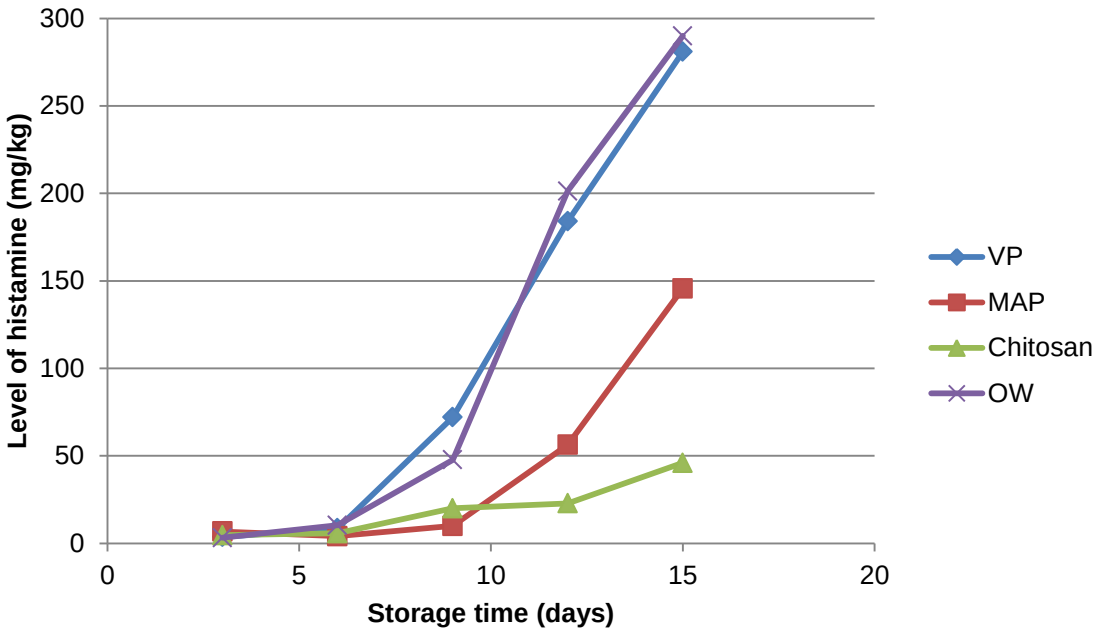

Figure 7. Histamine formation in packaged Atlantic bonito (*Sarda sarda*) fillets stored at 4°C (VP: Vacuum Packaging; MAP: Modified Atmosphere Packaging; OW: Overwrapped packaging) (adapted from Alak et al., 2011)

Chitosan film has been reported to be more effective than MAP (100% CO_2), vacuum packaging or standard over-wrapping with cling film in inhibiting histamine formation in Atlantic bonito (*Sarda sarda*) fillets (Figure 7; Alak *et al*., 2011).

Overall, MAP, vacuum, and active packaging, inhibit or delay histamine formation when compared to air packaging. The degree of control appears to depend on the type of microflora present, storage conditions (particularly temperature), and gas mix used in the case of MAP. It may also be product specific (Naila *et al*., 2010).

9.10.12 Frozen distribution cold chain

No specific studies regarding the impact of frozen distribution on histamine in fish have been identified.

9.10.13 Chilled distribution cold chain

Few specific studies regarding the impact of chilled distribution on histamine in fish have been identified. There is plenty of data on the effect of storage temperature on histamine formation, as shown in Table 11, but these studies have generally been laboratory based and looked at static temperatures rather than a simulated or surveyed chill-chain. It is generally accepted that storage of fish below 4°C is best way to control and reduce histamine development. However, histamine can be formed at temperatures below 4°C. In addition, slight temperature abuse can stimulate histamine production. Storage at 0˚C or below can prevent histamine formation and reduce histamine in fish muscle. Kim *et al*. (1999) found no histamine

in whole or dressed albacore tuna (*Thunnus alalunga*) stored in ice for up to 18 days. Middlebrooks *et al*. (1988) found low levels of histamine in Spanish mackerel (*Scomberomorus niphonius*) during storage at 0˚C for 16 days, with mean levels remaining less than 6 mg/kg. Guizani *et al*. (2005) found that histamine levels dropped in yellowfin tuna (*Thunnus albacares*) stored at 0˚C, from an initial level of 27.8 mg/kg on day one to 6.1 mg/kg at day 17. They attributed this reduction to the presence and growth of histamine decomposing bacteria (see Section 9.10.20). However, other than this study and that of Sato *et al*. (1994), very few studies, and few reviews, have studied the decomposition of histamine during storage.

Whole fish, gutted whole fish or fillets are often distributed in fish boxes with ice. The ice is intended to do two things, firstly to chill the fish to 0°C and secondly to maintain it at that temperature. Many studies using ice to store fish have not regularly changed that ice throughout storage. Daily change of ice has been show to have the potential of increasing storage-life and minimising the formation of histamine, and other amines, in fish such as Atlantic mackerel (*Scomber scombrus*; Figure 8, Lokuruka & Regenstein, 2005).

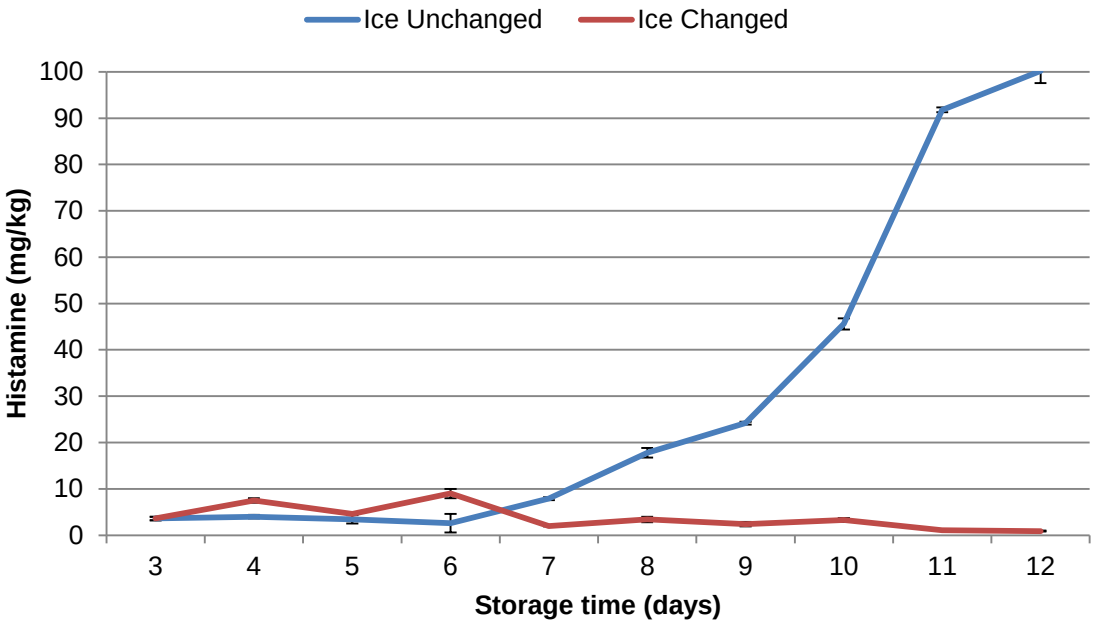

Figure 8. The effect of regularly changing ice on histamine formation in ungutted whole Atlantic mackerel (*Scomber scombrus*) stored in ice (adapted from Lokuruka & Regenstein, 2005)

At present most fish supply chains operate a conventional First In First Out (FIFO) approach. A Time Temperature Integrator (TTI) based chill chain management system such as SMAS (Safety Monitoring and Assurance System) could offer advantages compared to FIFO. SMAS (Tsironi *et al*., 2008) is an integrated chill chain management system that "leads to an optimized handling of products in terms of both safety and quality". It is based on kinetic models of spoilage and pathogen food microorganisms, data on intrinsic product's characteristics and the time–temperature history, using the information from the TTI response at designated points of the chill chain. TTIs are simple and potentially inexpensive devices that are capable of reporting a visual and straightforward summary of the time-temperature exposure history of a food product. The TTI monitors both time and temperature

during a given period and show the cumulative effect of temperature fluctuations during the history of the product. SMAS was developed for chilled meat products but its applicability has been demonstrated for fish products (Tsironi *et al*., 2008). However, to reliably apply such a simulation, dynamic kinetic models of histamine formation in fish by the relevant psychrotolerant bacteria such as *M. psychrotolerans* and *Photobacterium* spp. are necessary. Current models, such as the SSSP model, are not designed to easily model dynamic changing temperatures and conditions.

9.10.14 Retail cold chain

No specific studies regarding the impact of retail practice and display conditions on histamine formation in fish have been identified, nor have any specific surveys of fish temperatures during retail display. The display of "wet" fish on ice should not constitute a hazard, providing the fish is well iced and the effects of direct radiant heat on the fish are avoided. However, packaged fish in standard retail display cabinets may be subjected to temperatures that allow histamine formation. General surveys of retail display cabinets have reported wide ranges in temperatures that could result in histamine formation (Evans *et al*., 2007; James & James, 2013).

Data on the effect of different storage temperatures on histamine formation has been discussed in Section 8.7. This data can potentially be used to assess the risk of histamine formation and generation during retail display.

9.10.15 Domestic cold chain

It can be assumed that once purchased by the consumer, fish will be held in a domestic refrigerator or freezer before consumption. Chilled fish will not be held in ice and will be subject to the operating temperature of the refrigerator. In general, recommended refrigerator temperatures throughout the world are below 8°C, with many countries (including the UK) recommending a temperature below 5°C. However, numerous surveys show that actual mean temperatures in consumers refrigerators range between 5°C and 7°C, with 50–70% of domestic refrigerators operating at temperatures above 5°C (James *et al*., 2008; James & James, 2013). Since even the recommended temperatures are higher than the 2°C that is usually recommended for the storing fish to prevent histamine formation, it must be concluded that there is a danger of histamine formation during the domestic storage of at-risk fish species.

Few specific studies regarding the impact of domestic storage on histamine in fish have been identified. Data on the effect of different storage temperatures on histamine formation has been discussed in Section 8.7. This data can potentially be used to assess the risk of histamine formation and generation during domestic storage. Those studies that have sought to replicate domestic storage conditions have generally assumed fridge temperatures to be around 4°C.

9.10.16 Chemical interventions

Chemical washing interventions, such as chlorine or organic acids, have been widely evaluated and used in the red meat and poultry industries throughout the world to reduce pathogenic and spoilage bacteria on raw meats. Some studies have also been carried out on fish (see below). In the EU such substances have been prohibited in the past, however EC Regulations 853/2004 and 1333/2008 do allow for the use of approved substances as "processing aids". The BIOHAZ Panel of EFSA recently revised the joint AFC/BIOHAZ guidance on the submission of data for the evaluation of the efficacy of substances for the removal of microbial surface

contamination of foods of animal origin. The guidance (EFSA, 2010) is intended to provide guidelines for dossiers for applications for the authorisation of substances as processing aids for treating foods of animal origin. However, few published studies have been identified on specific effects of such treatments on histamine-forming bacteria on fish (see below).

9.10.16.1 Electrolysed water/ice

Electrolysed water is generated by electrolysis of a dilute salt (NaCl) solution in an electrolysis chamber where anode and cathode electrodes are separated by a membrane. On the anode side, the chlorine ions form acidic electrolysed water (HOCl). Acidic electrolysed water has a strong bactericidal effect on most known pathogenic bacteria, due to its low pH (2.6), high oxidation–reduction potential (ORP) (about 1100 mV) and the presence of hypochloric acid. Since hypochloric acid is the active agent of all chlorine based disinfectants, acidic electrolysed water is discussed in the chlorine section. The sodium ions are drawn to the cathode to form alkaline electrolysed water (NaOH). Alkaline electrolysed water has a pH of approximately 11.4 and ORP of -795 mV and the presence of sodium hydroxide.

The efficacy of electrolysed water and ice treatments in reducing histamine-forming bacteria on food contact surfaces and fish skin (yellowfin tuna, *Thunnus albacares*) has been evaluated by Phuvasate & Su (2009). Soaking ceramic tile and stainless steel in electrolysed water (50 ppm chlorine) for 5 min inactivated inoculated bacteria (*Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *M. morganii* and *Proteus hauseri*) on the surface (>0.92 to >5.4 $\log_{10}$ cfu cm^{-2} reductions). A treatment of electrolysed ice (100 ppm chlorine) for 24 hours reduced inoculated *E. aerogenes* and *M. morganii* on tuna skin by 2.4 and 3.5 $\log_{10}$ cfu cm^{-2}, respectively. Conversely, McCarthy & Burkhardt (2012) did not find electrolysed water (50 ppm chlorine) to be effective against *M. morganii* on fish surfaces, although a 5 min treatment did reduce levels by 1-2.5 $\log_{10}$ cfu cm^{-2} on conveyor belt surfaces.

9.10.16.2 Chitosan

Treatments with chitosan have been shown to inhibit a range of histamine-forming bacteria and to prolong the storage life of fish. Chang *et al*. (2010) found that minimal inhibitive concentrations (MICs) of chitosan against histamine-forming bacteria were as follows: 800 ppm for *Enterobacter aerogenes*, 1,000 ppm for *M. morganii* and *P. vulgaris*, and >2,000 ppm for *R. ornithinolytica*. Soaking marlin (exact species not quoted) flesh in solutions of chitosan (0.2 and 1%) for 1 hour followed by storage at 4°C reduced histamine formation and therefore prolonged the safe storage period (based on the USFDA guideline criteria of 50 mg/kg) from 9 to 15 days.

9.10.17 Irradiation

Ionising radiation involves the application of electromagnetic waves or electrons to foods. Radiation sources are either gamma rays from cobalt-60, electron beams or X-rays, and the amount of radiation absorbed by a food is measured in kGy (1Gy = 1 J/kg). Irradiation has been shown to both reduce the number of histamine-forming bacteria and degrade biogenic amines, including histamine (Naila *et al*., 2010). There is evidence that irradiation can control histamine formation in at-risk fish and seafood products. For example, irradiation has been shown to inhibit growth of *M. morganii* and histamine formation in mackerel fillets, a dose of 2.0 kGy being

more effective than 0.5 (Aytac *et al*., 2000) and to reduce histamine content in Atlantic bonito (*Sarda sarda*) in a dose-dependent fashion (Mbarki *et al*., 2008). Irradiation of vacuum-packed chub mackerel (*Scomber japonicus*) with a low dose of 1.5 kGy doubled shelf-life (from 7 to 14 days) and reduced histamine formation during that time (Mbarki *et al*., 2009). When blue jack mackerel (*Trachurus picturatus*) was stored for 7 days in ice, histamine levels exceeded 100 mg/kg within 7 days while in fish that had been irradiated with 3 kGy histamine only reached 54 mg/kg after 23 days (Mendes *et al*., 2000). Histamine levels approached 100 mg/kg in Atlantic horse mackerel (*Trachurus trachurus*) during 23 days' ice storage, while no histamine was detected in fish that had been irradiated at 1 kGy during this time (Mendes *et al*., 2005).

9.10.18 High pressure treatment

High pressure is a non-thermal food preservation method. The history of high pressure treatment and its effects on microorganisms and biological systems has been reviewed by Hoover *et al*. (1989) and Hoover (1993). The engineering problems in the design of such equipment are discussed in detail by Mertens & Deplace (1993). The pressures used in most studies are very high, from 101 MNm^{-2} (1000 atm) to 608 to 1013 MNm^{-2} (6000-10000 atm). The antimicrobial action of high pressure on microorganisms is not fully understood. High pressure has many effects on microorganisms, such as membrane damage, protein modification or ionic equilibrium changes, and one or more of these may account for its action (Hoover *et al*., 1989; Patterson, 2005). High pressure treated foods are commercially available in the United States (for example, guacamole, oysters), Japan (for example, fruit jam), and Spain (for example, cooked and vacuum packed ham) (Patterson 2005).

It has been reported that histamine-forming bacteria and bacterial histidine decarboxylase activity in yellowfin tuna and mahi mahi fish can be reduced by applying high pressure treatments between 300 and 400 MPa without affecting the quality of the fish (Bolton *et al*., 2009).

9.10.19 Additives

A number of food additives / ingredients have been shown to reduce the formation of histamine and other biogenic amines in prepared fish products.

Glycine was found to be more effective in reducing the formation of histamine and other biogenic amines in myeolchi-jeot (a Korean salted and fermented anchovy (*Engraulis japonicus*) product) than other additives (salt, sucrose, glucose, D-sorbitol, lactic acid, citric acid and sorbic acid) (Mah & Hwang, 2009a). In culture, glycine reduced histamine formation by 93% with similar reductions when 5% glycine was included in the myeolchi-jeot during the ripening process. The glycine acted by inhibiting the growth and histamine formation by histamine-forming bacteria. According to the authors, the mechanism by which glycine inhibits formation of biogenic amines is largely unknown. They propose two possible mechanisms:

1. It inhibits the growth of biogenic amine producers in the manner described above and/or,
2. It may somehow suppress the biosynthesis of amino acid decarboxylases or other substances required for biogenic amine formation.

The authors proposed that glycine would be useful in inhibiting histamine formation in other fermented fish products.

A number of spices (turmeric, pepper, cardamom, cinnamon and clove) have been shown to inhibit biogenic amine formation in Indian mackerel (*Rastrelliger kanagurta*) during refrigerated storage (Jeya Shakila *et al*., 1996); the effect was greater in the turmeric, pepper and cardamom treatments. No histamine was detected in turmeric and pepper treated samples after up to 6 days storage at 5°C, whereas histamine was detected in the control samples after 4 days. Histamine formation in cardamom treated samples was slightly greater than in turmeric and pepper samples, but significantly less than in cinnamon and clove treated samples.

Garlic was found to be more effective than other spices (ginger, green onion, red pepper, clove cinnamon) at reducing the formation of histamine and other biogenic amines in myeolchi-jeot (Mah *et al*., 2009). However, histamine formation was only reduced by 12% in culture, and 9% when garlic was incorporated at 5% during the fermentation process. The garlic reduced histamine by inhibiting the growth of histamine-forming bacteria.

Nuka, a Japanese by-product of rice polishing, may be useful in reducing histamine in fermented fish products such as fish sauces (Kuda & Miyawaki, 2010). The spice *Garcinia cambogia* has been shown to inhibit bacterial histidine decarboxylation in homogenised skipjack (*Katsuwonus pelamis*) samples (Thadhani *et al*., 2002); this was attributed to its effect on pH (lowered to 3.6). *Tamarindus indica* (tamarind) and fruits of *Avverhoea bilimbi* (bilin) did not prevent histamine formation (Thadhani *et al*., 2002).

Overall, while studies show that certain additives may assist in reducing histamine formation, none of those identified so far, appear to be a reliable method of control by themselves. Also, in general, most proposed additives would appear to only be suitable for controlling histamine formation in prepared seafood products rather than raw fish.

9.10.20 Protective bacterial cultures

Some bacteria produce diamine oxidases that are capable of degrading histamine. Such bacteria can be naturally present on fish and may naturally inhibit histamine accumulation during storage (see Section 9.10.13). Research by Zaman *et al*. (2010) suggests that the ability of bacteria to degrade amines is strain specific rather than species specific.

Enes Dapkevicius *et al*. (2000) proposed two such lactic acid bacteria (*Lactobacillus sakei* and *Lb. curvatus*) as suitable starter cultures for the production of fish silage. It is possible that such bacteria could also be used in other fermented seafood products to inactivate or at least prevent the accumulation of histamine in seafood.

Staphylococcus xylosus has been proposed as a protective organism that could be used as a starter culture to prevent histamine formation in Korean fermented foods (such as myeolchi-jeot, (*Engraulis japonicus*) product) as well as other products (Mah & Hwang, 2009b). *S. xylosus* was able to degrade both histamine and tyramine and also produced a bacteriocin-like inhibitory substance that had antimicrobial activity against *Staphylococcus licheniformis* strains, which are histamine producers themselves.

Lactic acid-producing bacteria that do not actively inhibit histamine-producing bacteria or degrade histamine themselves may assist in preventing the development of histamine in fermented products by dominating the microflora (Petaja *et al*., 2000).

Overall, while studies show that certain bacterial cultures may assist in controlling histamine formation in fermented seafood products, they do not appear to have been evaluated for control in raw fish.

9.10.21 Overall

The concentration of histamine and other biogenic amines can vary substantially between fish from a single catch (Emborg, 2008). The potential for histamine-formation is dependent on a number of intrinsic and extrinsic factors. Firstly, there needs to be presence of histidine and histamine-forming bacteria. Subsequent growth of histamine-forming bacteria and histamine formation will depend on:

1. Species of the fish.
2. Harvest practice.
3. Rate of post-harvest chilling.
4. Storage times and temperature.
5. Storage atmosphere.
6. Handling procedures.
7. Salt (NaCl).
8. pH.

It is clear from the literature that there are four key stages from catch-to-fork where the fish may be subjected to elevated temperatures with the potential to cause a rise in histamine levels:

1. Initial cooling of the fish immediately after harvest.
2. Thawing of previously frozen fish.
3. Retail display.
4. Domestic storage.

A number of studies have also cited post-harvesting contamination as a primary source of histamine-forming bacteria (Lehane & Olley, 2000).

Currently, time and temperature are the only hurdles generally used by the industry to reduce the risk of histamine formation. Other options for reducing the growth of histamine-producing bacteria may be the incorporation of anti-microbial agents in the ice or saltwater/ice slurry used to chill fish. Such interventions have been adopted for other foods of animal origin, such as red meat and poultry.

10. Appendix 2: Other histamine reviews

Since 2000, there have been at least eleven published reviews on aspects of histamine fish poisoning associated with fish (Lehane & Olley, 2000; Mavromatis & Quantick, 2002; Kim *et al*., 2003b; Kim *et al*., 2004a; Dalgaard *et al*., 2008; Emborg, 2008; Al-Bulushi *et al*., 2009; Fletcher, 2010; Hungerford, 2010; Köse, 2010; Naila *et al*., 2010). Five of these reviews were in turn recently reviewed by Fletcher (2010). The following is a summary of the key conclusions of each of these reviews:

Lehane & Olley's (2000) published review is derived from a larger review written for, and available from, the National Office of Animal and Plant Health, Agriculture, Fisheries and Forestry – Australia, Canberra (Lehane & Olley, 1999). Fletcher (2010) notes that of particular value is their summary of knowledge "on one of the more vexing issues of histamine fish poisoning" – why fish that cause histamine fish poisoning invariably contain high levels of histamine but when histamine is fed to people at similar levels in volunteer studies histamine fish poisoning does not result. The reviewers hypothesised that cis-urocanic acid, a different breakdown product of histidine and a recognised mast cell degranulator might play a role in augmenting the exogenous histamine. More recent studies and reviews (Rossi *et al*., 2002; Al-Bulushi *et al*., 2009; Hungerford, 2010) and FDA guidance (FDA, 2011) point to evidence of the role of other biogenic amines, putrescine and cadaverine, as potentiators of histamine toxicity.

Mavromatis & Quantick (2002) reviewing the mechanism of histamine fish poisoning state "There is strong evidence that the primary scombrotoxin is a mast cell degranulator that causes the release of indigenous histamine and other biologically active substances, which in turn are responsible for the gastrointestinal symptoms. Therefore, the toxic role of dietary histamine as posed by indigenous histamine is slight."

Kim *et al*. (2003b; 2004a) concluded that histamine is mostly formed by a few prolific enteric bacteria such as *M. morganella.* They recommend proper chilling and freezing of fish, starting at the point of harvesting, as the most important controls.

Dalgaard *et al*. (2008) focused on the work carried out in the European BIOCOM project, which ran from 2004 to 2007. Their recommendations included:

1. More investigations of outbreaks of histamine fish poisoning incidents that examine the microbiology of the organisms involved, particularly with regard to their psychrotolerance. Although hundreds of outbreaks are reported each year they could only find 15 incidents that formally reported on the microbiology involved. Five of these were their own from which they drew quite different conclusions to those of other studies.
2. When possible investigations are needed to examine the actual remains of meals that caused the outbreaks. They noted that there are considerable discrepancies in the literature as to the amounts of histamine required to cause histamine fish poisoning, but also noted that while fish from the same batch may be examined, actual meal remains were seldom examined. However, they note that the levels of histamine vary quite dramatically from different positions within a fish let alone between fish. They recommended that outbreak studies should also evaluate the correlation between histamine and other biogenic amines in agreement with the recommendation of Al-Bulushi *et al*. (2009).

BIOCOM results suggested that hazard is directly proportional to histamine levels while others have suggested that other amines are required as potentiators.

3. Development of mathematical models for growth and histamine formation by important bacterial species other than the model for *M. psychrotolerans* that was developed under BIOCOM. They have a model for *M. morganii* but this only accounted for temperature, not atmosphere, salt and pH, as included in their model for *M. psychrotolerans*. Section 9.5 of the literature review shows that there are many other important histamine-producing bacteria whose growth and histamine formation could be mathematically modelled.
4. More studies on the potential of modified atmosphere packaging (MAP) to control histamine fish poisoning. The BIOCOM project suggested combinations of high CO_2 and high O_2 might be effective in achieving this.
5. Obtaining more quantitative data on the occurrence of strong histamine-producing bacteria in marine and seafood processing environments in order to determine how these organisms come to contaminate fish or whether they are naturally present.
6. More studies on the oral toxicity of histamine, with or without other biogenic amines, to elucidate the mechanism of action of the intoxication.

Emborg's (2008) report was produced as part of the EU integrated project SEAFOODplus (506359) and identified what they saw as the gaps in available information on amines in seafood. The following gaps in information are identified in the report:

1. Factors that influence the formation of toxic concentration of histamine and biogenic amines in chilled seafood stored below 7-10°C. It is known that toxic concentration of histamine and biogenic amines can be formed in correctly chilled seafood at 0-4°C. Important information related to psychrotolerant *M. morganii* and *P. phosphoreum* and their histamine and biogenic amine formation is lacking and this markedly reduce our ability to assess and manage consumers' exposure to histamine and biogenic amines in seafoods. In this area the following information is particularly lacking:

    - Techniques for simple and reliable differentiation and identification of psychrotolerant and mesophilic variants of *M. morganii*.
    - Enumeration methods that allow occurrence and concentrations of psychrotolerant *M. morganii* in seafoods to be determined
    - Rates of growth and biogenic amine formation by psychrotolerant *M. morganii*-like bacteria as a function of temperature, atmosphere, pH and NaCl.
    - Combinations of temperature, atmosphere, pH and NaCl that prevent growth of psychrotolerant *M. morganii* and *P. phosphoreum*.
    - Seafood processing or storage conditions that inactivate psychrotolerant *M. morganii* and *P. phosphoreum*.

- Variability in histamine formation between isolates of psychrotolerant *M. morganii* and isolates of *P. phosphoreum* as a function of seafood product characteristics.

2. Knowledge about which species of bacteria cause histamine food poisoning. Isolation and identification from leftovers from outbreaks is necessary for a better understanding of how histamine food poisoning can be avoided. This knowledge could lead to more targeted and directed investigations attempting to avoid and reduce the occurrence and growth of these bacteria.
3. Quantitative distribution of histamine and other biogenic amines in various seafoods.
4. Rapid and inexpensive methods for determination of histamine together with concentrations of other biogenic amines.
5. Techniques that allow the formation of histamine and other biogenic amines in seafood to be predicted at different temperatures.

Al-Bulushi *et al*. (2009) reviewed biogenic amines in fish. Their review was based on the view that other amines, putrescine and cadaverine, are required to potentiate histamine fish poisoning. They concluded that levels of cadaverine and putrescine need to be considered in any histamine toxicity assessment and that nitrosamine levels should also be closely monitored. Current FDA guidance (FDA, 2011) also mentions the possible importance of cadaverine and putrescine in histamine food poisoning.

Fletcher (2010) focussed on research of relevance to histamine fish poisoning in New Zealand, particularly regarding the processing of kahawai (*Arripis trutta*). His recommendations include:

1. Agencies investigating food-borne outbreaks of histamine fish poisoning should carry out more detailed investigations: where possible remains of the actual implicated product should be tested and laboratories commissioned to determine the species of fish involved, to isolated and identify the bacteria involved and to determine the levels of histamine and other biogenic amines present. Results of such investigations would provide a clearer picture of the factors contributing to outbreaks of histamine fish poisoning in New Zealand and help guide the development of methods to prevent such outbreaks.
2. The list of imported fish species that might be of food safety concern in New Zealand should be revised to include species recently implicated in outbreaks overseas. This will provide a warning to those handling such species to take extra care to prevent histamine fish poisoning from them.
3. Analytical methods to quantify histamine and other biogenic amines should be reviewed and implemented so that analyses of samples from New Zealand outbreaks can contribute to the understanding of the aetiology of histamine fish poisoning.
4. Currently unpublished work on growth and histamine production by individual strains of histamine-forming bacteria should be published in an international peer-reviewed journal to make the knowledge obtained available to international researchers and to provide information to those developing mathematical models and software packages to predict histamine formation.

5. The contribution of psychrotrophic bacteria to histamine production in fresh fish in the New Zealand environment/context should be evaluated to determine whether these contribute to the hazard and whether different handling and control guidelines are required to protect against the hazard.
6. Histamine production in kahawai (*Arripis trutta*) from the point of capture, including time spent in gill nets should be investigated to determine the extent to which this presents a hazard and whether new controls are needed for this aspect.
7. Methods should be developed and validated to evaluate the quality of at-risk fish species so that fish that has been stored for too long can be identified and not be used for hot smoking.
8. Factors that allow fish to develop problematic levels of histamine whilst appearing to be safe to consume from a sensory perspective should be studied to understand whether other methods of identifying hazardous fish need to be applied.

Hungerford (2010) is a general review that focuses on histamine food poisoning, its symptoms, mechanisms of toxicity, origins, and methods of detection. The review concludes that contamination of fish with histamine is primarily due to mishandling. Although the role of histamine as a seafood ‘‘toxin’’ in histamine fish poisoning is not fully understood, detection of histamine and the enforcement of action levels are useful for control purposes. Hypothesized mechanisms for histamine fish poisoning remain unproven, and improved prevention of histamine fish poisoning can result from investigations of these mechanisms to gain a better understanding of the origins of histamine fish poisoning. Many methods for detecting histamine have been described. However, refinement and international validation of laboratory and field-testing methods should be pursued to improve the protection of public health amidst globalization of the seafood supply.

Köse (2010) reviews the health risks, including histamine, associated with traditional fish products and suggests preventative measures and monitoring issues associated with each preserving methods used for such products, i.e. salting/brining, fermentation, marinating, smoking and drying.

Naila *et al*. (2010) review methods for controlling biogenic amines in all foods. They conclude that the main existing method for control is refrigeration. However, they point out that refrigeration on its own is not sufficient since some bacteria that form biogenic amines can grow under refrigerated conditions; it is also “not always a feasible option for artesian fishers”. They review a number of emerging control measures including high hydrostatic pressure, irradiation, packaging, and the use of food additives or preservatives. They conclude that amine oxidising bacteria and enzymes (see Section 9.10.20) are the best options for artisan fishers.

Overall these reviews come to similar conclusions regarding the cause of histamine fish poisoning and its control. All conclude that the main method for control is refrigeration, and that rapid chilling of fish immediately after death is the most important element in any strategy for control. Recommended specific controls measures and practices quoted in many of these reviews are mainly based on Chapter 7 of the US FDA *Fish and Fishery Products Hazards and Control Guidance* (FDA, 2011).

11. Appendix 3: Scottish pelagic fish industry: An overview

The Scottish pelagic sector is an important component of the fishing industry of Scotland, being responsible for 46% of the quantity and 24% of the value of seafood landed in 2010 (MMO, 2011). Whilst there have been reviews of many sectors of the Scottish Seafood Industry, there were no currently available details or overall description of the pelagic sector supply chains from capture to consumer. It was therefore necessary to map out the sector and its component supply chains in order that the survey of the histamine controls could be conducted.

This section of the report therefore provides an overview of the sector with information both gathered as part of the project survey activity and from published sources in order to identify the nature and extent of the pelagic supply chains for Scottish products.

11.1 Fishing Activity

The primary pelagic species caught by the Scottish fishing fleet are mackerel (*Scomber scombrus*) and herring (*Clupea harengus*).

Herring and mackerel are sourced by Scottish vessels fishing the North Sea (ICES area IVa & IVb) and the West of Scotland Fisheries (ICES area VIa, North East Atlantic), areas shown in Figure 9.

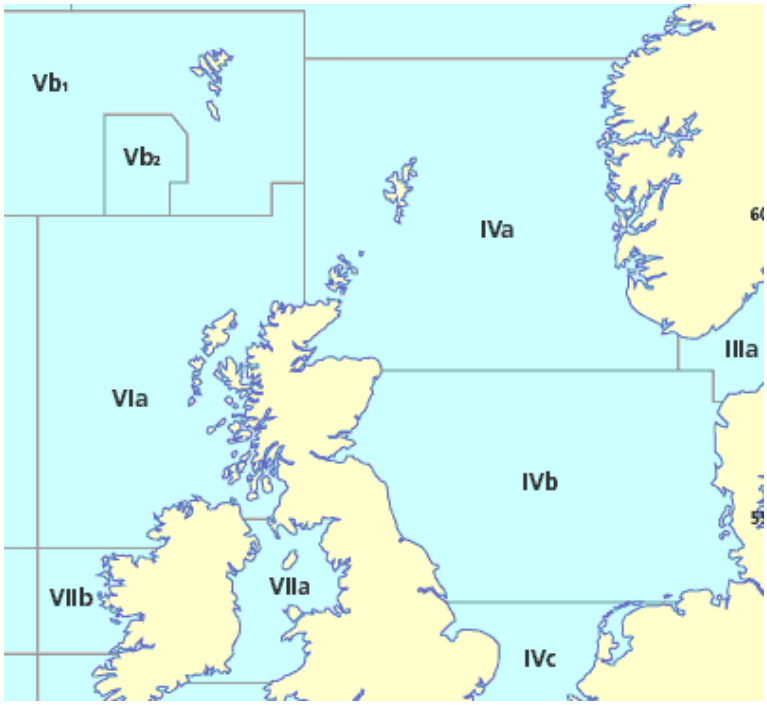

Figure 9. ICES Fishing areas for Scottish vessels (http://www.scotland.gov.uk/Publications/2006/10/30105313/5)

Separate quotas are negotiated each year for the fishing areas and the individual species, by the contracting nations (European Union, Iceland, Norway, Russia and Denmark (with respect to Faeroe & Greenland)) of the North East Atlantic Fisheries Commission (NEAFC). Their recommendations are then put to the European Fisheries Commission who then determines total allowable catches (TACs) for each member state. The responsibility for the implementation of both the quotas and the

common fishery policy (CFP) in general in Scotland falls to the Scottish Government and its enforcement to the Marine Scotland Directorate.

11.2 The Scottish Pelagic Fishing Fleet

A range of vessels are involved in the capture of pelagic fish species from Scottish waters and these can be placed into the following categories:

V1: Purpose built large (>20m) vessels specifically designed and built to catch and handle large volumes of pelagic fish. The vessels generally use a pelagic (mid-water) trawl although some also have the capability for seine-netting of shoals. The fish are brought aboard and immediately placed into refrigerated seawater tanks. The Scottish fleet consists of 26 modern, purpose built, specialist pelagic trawlers. All vessels are members of the Scottish Pelagic Fishermen Association (SFPA, 2012) and details of the vessels are provided in the Annex 4 (Section 12).

In addition, landings at the main ports of Peterhead, Lerwick and to a lesser extent Fraserburgh are undertaken by large pelagic trawlers vessels of similar design registered in other European nations (Napier, 2011). Some of these vessels are designed for different fishing techniques, for example Norwegian, auto-jiggers/ long-liners fish for mackerel around Shetland during the summer months landing their catch at Lerwick within 24 hours of capture rather than in their home ports. These vessels whilst using automated long-lines, are also equipped with refrigerated sea water (RSW) tanks for the rapid cooling and storage of the mackerel, although the normal fishing areas are within 4 hours of the landing in Lerwick and as such the fish temperature may not have had time to be reduced to <2°C at landing. The combination of line-caught and RSW storage is thought to produce some of the highest quality mackerel available.

V2: Intermediary vessels: Are not specifically designed or used solely for targeting pelagic species; they are employed to utilise any available quota, provided that they can modify their fishing gear to target pelagic species.

They are generally mid-sized trawlers (>10 m) that can adapt their fishing gear to mid-water trawls in order to target pelagic species. This will involve investment in different nets and as such is only undertaken by those vessels that have a history (and customers) for seasonal pelagic fishing. Typical of these vessels are those involved in the Mallaig sprat fishery and Loch Fyne herring fisheries.

The Mallaig sprat fishery is based on 4 small trawlers (<20 m); the Corsalisa (OB956), Margaret Ann (OB198), Ocean Hunter (SY503) and Rebecca Jeneen (OB38), all of which belong to the West Of Scotland FPO. They operate as inshore pelagic trawlers, with the fishing taking place at night. On hauling the nets, the fish are transferred and stored in a refrigerated fish hold (not RSW). Landing at the dockside in Mallaig takes place the following morning with the fish being transferred from the hold into fish bins with ice, before being transported directly to the processor by refrigerated transport. This fishery is limited by quota to 500 tonnes p.a.

A small summer herring fishery has been recently re-established operating out of Tarbert on Loch Fyne, that uses pair trawlers (>10 m) who fish at night

before returning to Tarbert to land the fish each morning. This fishery is limited by quota to 50 tonnes p.a.

V3: A wide range of smaller inshore vessels that target a mixed fishery throughout the year that can switch depending on season (usually summer) and quota availability to targeting hand-line caught mackerel. These vessels (<10 m) fish the inshore waters using typically a line of baited hooks. Their catch is limited by quota to 300 tonnes p.a. (or 0.001% of total mackerel landings in 2010) for Scottish waters. The vessels will typically stream a weighted line of hooks, the length and number of hooks being dependant on the size of the vessel. Larger vessels may incorporate a winch and automated de-hooking device that delivers the fish into a clean fish box. Fish are stored on ice, either within an insulated fish hold or on deck depending on the vessel until they are landed, usually within 6 hours of capture.

The vessels that are licensed for the mackerel fishery in the North Sea areas IVa and IVb for 2012 are listed in Appendix 4 (Section 12).

For all inshore vessels fishing for mackerel in the West of Scotland area VIa there is currently a monthly total maximum catch limit of 1.5 tonnes (MMO, 2012a).

11.3 Scottish pelagic landings

The primary data source used in this section of the report is the UK Sea Fisheries Statistics published by the Marine Management Organization (MMO, 2011). Although the MMO publish monthly statistics for fish landed in the UK, other data sources take much longer to be published. The most recent year for which all data is available to provide an overall picture of the Scottish pelagic fishery is 2011.

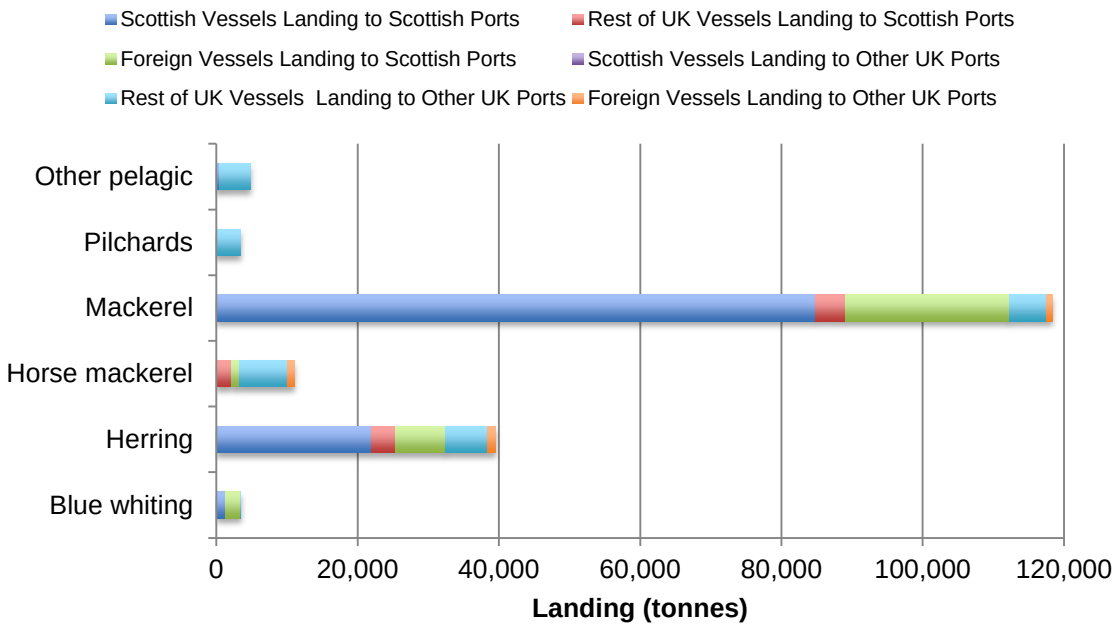

Figure 10. Quantity of pelagic fish landed at both Scottish and other UK ports by UK vessels and by foreign registered vessels (tonnes) within the UK in 2011 (adapted from MMO, 2011)

As the data in Figure 10 shows, 82% of herring and 95% of mackerel landings in the UK are landed in Scotland. Approximately 58% of all herring and mackerel landed in Scotland are landed by Scottish vessels.

These proportions drop to only 68%, for both species, when landings by foreign vessels into UK ports are included. However, what the available data does not show is that due to the industrial nature of the pelagic industry, and the off-loading facilities at the Scottish ports, the majority of pelagic fish landed by foreign vessels are landed at the Scottish ports. In 2011, the Scottish ports were responsible for handling approximately 99.3% of blue whiting (*Micromesistius poutassou*), 82% of herring, 95% of mackerel and 30% of horse mackerel (*Trachurus trachurus*) landed in the UK.

Table 16. Origin of vessels landing pelagic fish (all species) in Shetland in 2010 (adapted from Napier, 2011)

Country of Vessels	Tonnes	%	£ million
Shetland	14,567	20%	11.8
Rest of Scotland	23,311	31%	13.1
Sweden	3,586	5%	2.0
Norway	6,575	9%	3.7
Eire	6,575	9%	3.7
Denmark	11,954	16%	6.7
Northern Ireland	7,770	10%	4.4
Total	74,339		45.4

Within the Scottish pelagic fisheries sector the business interactions between vessels and primary processors is complex and is dependant on a range of factors that include; location of fish shoals, available quota, weather conditions, available prices, business and contractual links etc. Consequently, landings at any particular site come from a range of vessels, as demonstrated by the range of vessels landing just at one port (Shetland) in 2010 (Table 16).

The pelagic fisheries are both highly seasonal and variable, due to the shoaling nature of the fish species being largely dictated by their breeding, migration and feeding cycles in the various fishing areas.

Mackerel and herring are landed in varying amounts throughout the year in all months, but as shown by the landing data in Figure 11, the greatest mackerel fishing activity takes place in January and September, whilst the greatest herring activity takes place during February and the summer months (June to September).

Other pelagic species, such as blue whiting and horse mackerel, are targeted at other times of year, depending on the availability of a quota in order to maintain continuous commercial operations within the fishing and primary processing sectors.

Additionally there is a small sprat (*Sprattus sprattus*) fishery based at Mallaig (500 tonnes p.a.) in which landings occur during November/December.

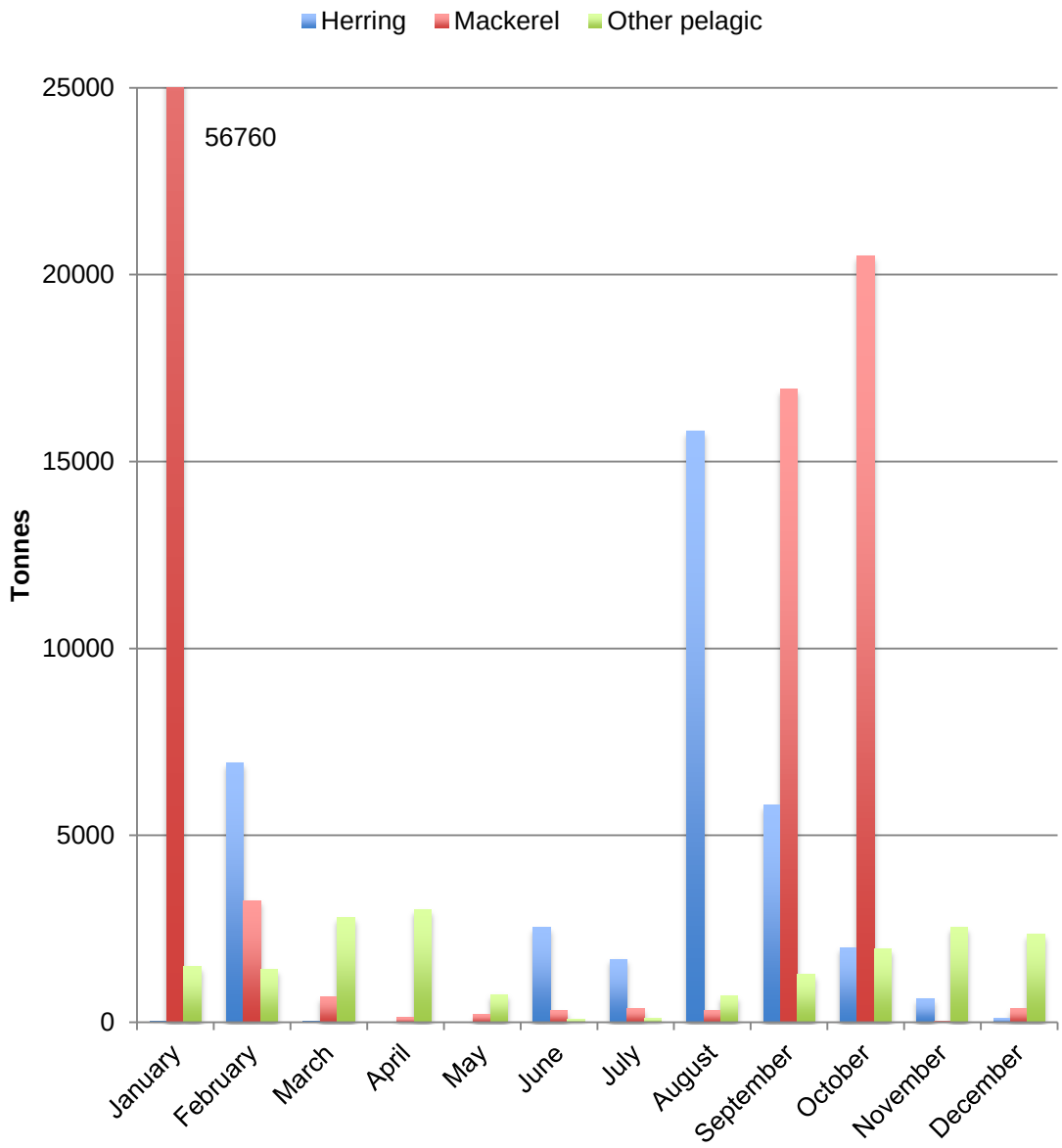

Figure 11. Pelagic landings by UK vessels Scottish ports throughout the year, 2010 (adapted from MMO, 2011)

Of the twenty Scottish ports registered as fish landing sites, 99.7% by volume of all pelagic landings by Scottish vessels in 2011 took place at Peterhead, Lerwick and Fraserburgh (Figure 12). The remaining ports landed less than 100 tonnes each.

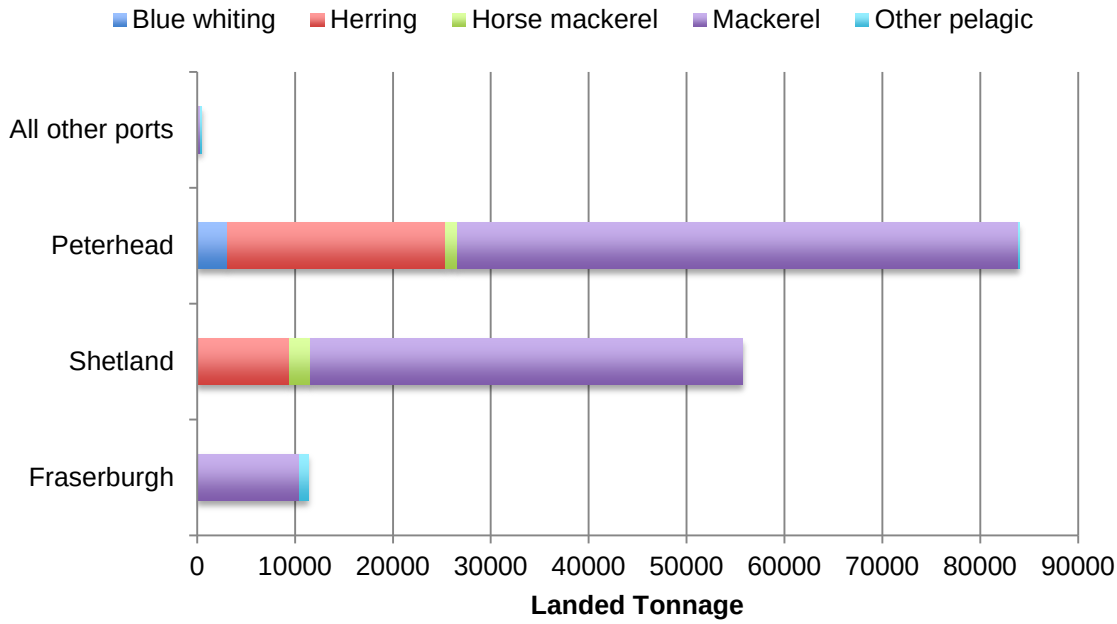

Figure 12. Distribution of pelagic landings by species at Scottish ports by UK vessels, 2011 (adapted from MMO, 2011)

Unlike other fishery products, the majority of pelagic fish are not landed at the fish auction/market but are transferred directly from a contracted fishing vessel to the (consigned) processing factory, with the processing company being responsible for maintaining and submitting landing records to the authorities.

These primary processing facilities (Table 17) have been specifically designed for the efficient and high speed handling of large volumes of fish. Integrated off-loading facilities are installed that allow for pumping of fish from refrigerated seawater tanks aboard the vessel at the dockside, directly into the factory holding tanks/processing line through pipes. Due to the large investment such systems require, only the larger processing factories have such facilities and therefore it is these factories that are responsible for the majority of pelagic landings and processing.

At other ports, the pelagic landings form only a very small proportion of the seafood landings by weight and are from the smaller inshore vessels that use fish boxes and ice to store and transfer fish rather than RSW tanks.

Table 17. Location of major pelagic processors with integrated off-loading facilities

Location	Company	Associated organisations
Peterhead	Lunar	Lunar Group FPO
	Denholm	Klondyke FPO
	Fresh Catch	Various vessels
	NorSea	Various vessels
Fraserburgh	Lunar	Lunar Group FPO
Lerwick	Shetland Catch	Shetland FPO

11.3.1 Other Scottish pelagic fisheries

The Mallaig sprat fishery is a very limited seasonal fishery with only four vessels that landed a total of 500 tonnes during the three week season in December 2010 (MMO, 2011). The size of the fishery is tightly controlled by quota to ensure sustainability of this inshore fishery.

Landing at the dockside in Mallaig takes place in the morning immediately after a night's fishing with the fish being transferred into fish bins with ice, before being transported directly to the processor located in Peterhead, by refrigerated transport. It is normal practice for a single primary processor to contract for the entire catch from this fishery.

The inshore pelagic fishery within Scotland is focused on the "hand lining" for mackerel during the summer season (May to September), however the total catch is limited by quota to around 300 tonnes a year. Of these smaller landings by far the biggest at 50 tonnes p.a., is at Scalloway & Islands (Figure 13). Here, as with all these ports where individual landings are small, the mackerel are landed at the approved landing sites and auctioned at the fish market to local wholesalers and fish merchants for distribution or directly to the secondary processors or local retail and food service providers. The line caught mackerel has a premium market price due to its sustainable method of capture and higher quality.

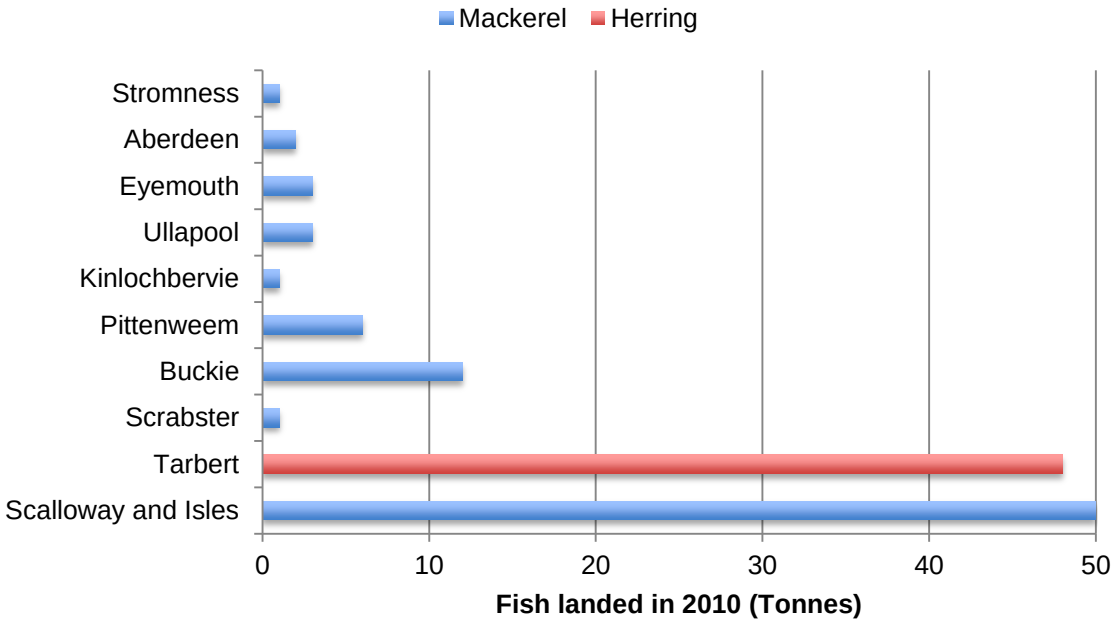

Figure 13. Pelagic landings (2010) at ports for the inshore pelagic fisheries (adapted from Scottish Government, 2011)

The landings of herring at Tarbert, (Argyll & Bute) are from the small fishery located in Loch Fyne and as with the inshore line caught mackerel; the fish are either auctioned at landing or consigned to a processor, and transported on ice to processors and or local retail/food service providers.

11.4 The markets for pelagic fish

The primary processors freeze the majority of pelagic fish that are landed. Some of this fish is processed before freezing, much is frozen as whole unprocessed (ungutted) fish. Sixty one percent of mackerel, 74% herring, and 99% blue whiting

that enter the UK are currently exported as frozen product. Although data for exports does not include horse mackerel, the industry reports that it also follows the same trend in that the majority, if not all, is exported.

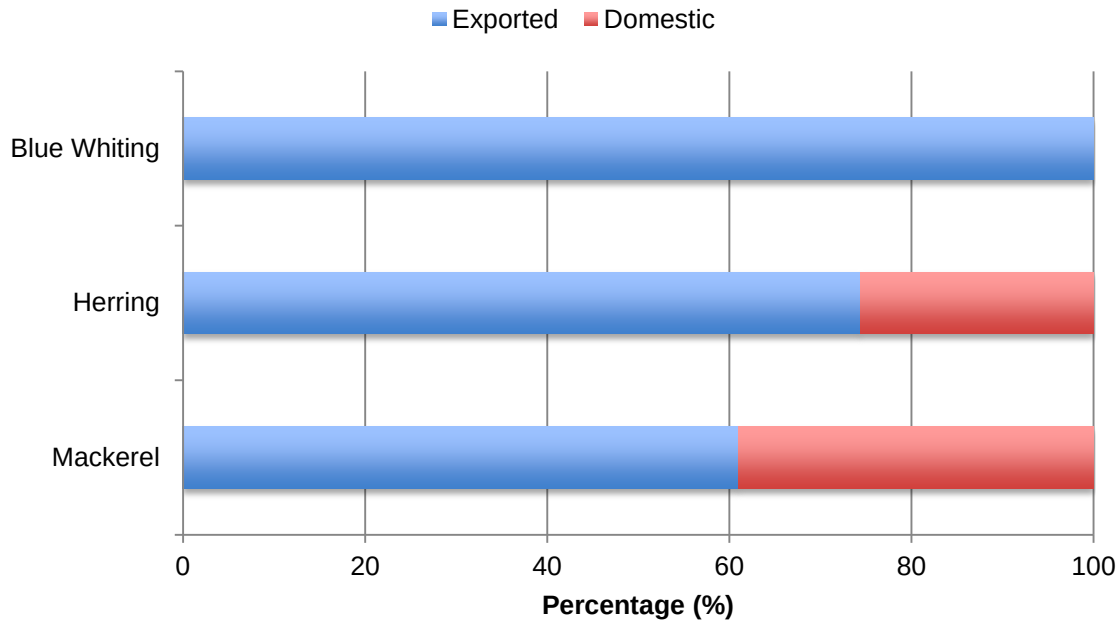

Figure 14. Proportions of pelagic fish landed or imported into the UK market that is exported or retained (adapted from MMO, 2011)

Overall the exported pelagic fish listed in Figure 14, accounted for 44% of total UK fish exports by weight and £164 million (18.2%) of the total value, during 2010. These data emphasize the fact that compared with other fishery sectors, the pelagic sector is a relatively high volume, low value product industry.

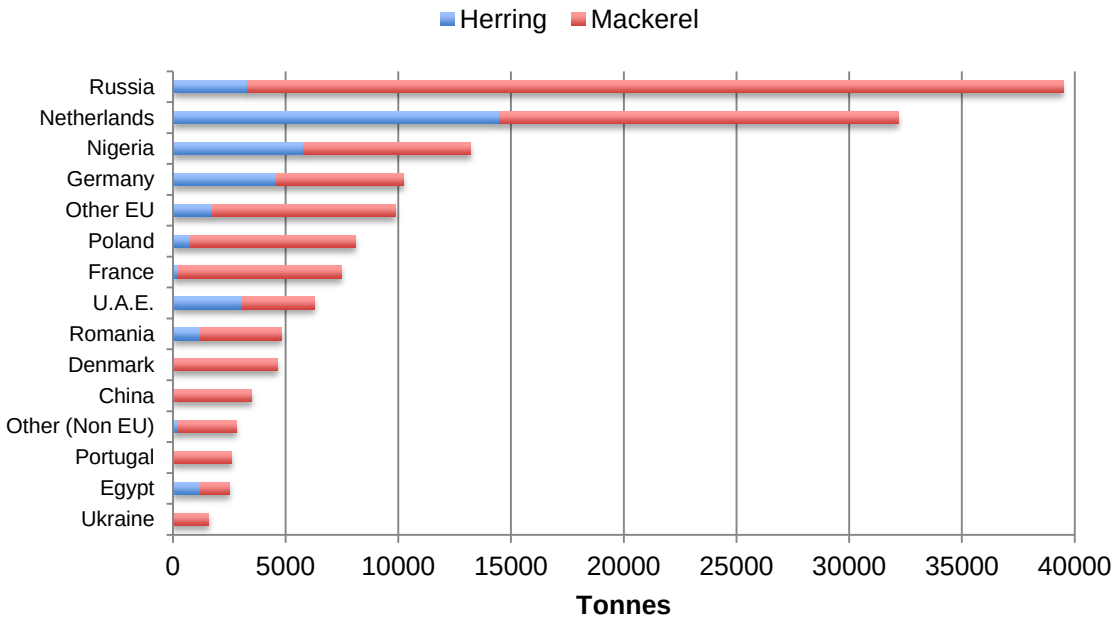

Figure 15. Exports (tonnes) of major pelagic species in 2010 by country (adapted from MMO, 2011)

The major export markets for frozen pelagic fish (whole or fillets) are shown in Figure 15, with Russia, The Netherlands and Nigeria being the biggest markets. Unfortunately, the published data does not include data for blue whiting (*Micromesistius poutassou*). However, DEFRA country reports (DEFRA, 2011) indicate that 27,000 tonnes of blue whiting were exported to Russia and 38,000 tonnes to China in the period between 2009 and 2010, which representing 33% and 47% respectively of the total blue whiting, exported during the same period.

11.5 Processors

According to the Scottish Local Authorities (Food Standards Agency (UK), 2012) there were a total of 302 food business operators (FBOs) approved to handle fishery products in 2011/12. These however include all fish species and all sectors of the shore-based industry from primary processing to fishmongers. As has been previously mentioned, the pelagic sector is a specialised sector and the number of these companies that handle pelagic species is only a small proportion of the overall number. For clarity, the pelagic processing sector can be best described under the following categories:

11.5.1 Primary Processors (PP)

The primary processors can be subdivided into two categories;

PP1. Specialist pelagic processors, who source fish primarily from the larger pelagic vessels and are located adjacent to the quayside and have the facilities to off-load fish from the vessel's RSW holds directly into the factory.

PP2. Other primary processors, who source fish from either smaller inshore vessels or from the large pelagic vessels via an intermediary (PP1) company, will place the fish in covered fish bins for the transfer to the factory, either by forklift truck (for distances typically <500m) or by refrigerated transport for longer distances. Often this category of processor will also undertake some secondary processing within the same processing facility.

In both types of processor, upon arrival at the factory the whole fish are stored either on ice or in iced water until processing. This involves some or all of the following steps.

- Mechanical size grading
- De-heading & gutting
- Filleting
- Packing
- Freezing

Because of the need to be close to the point of landing, the primary processors are located in the main ports of Peterhead (4), Lerwick (1) or Fraserburgh (1).

11.5.2 Secondary Processors (SP)

These companies further process pelagic fish to a final product. Where they are not responsible for the primary processing, the fish are normally supplied to them frozen by a primary processor. The use of frozen raw materials by the industry maintains product quality and safety and just as importantly it permits a constant supply of raw materials from a highly seasonal fishery.

In Scotland, the following products are produced from pelagic species:

- Kippers - cold smoked herring.
- Smoked mackerel - hot smoked with various flavourings.
- Canned mackerel and herring products (1 company).
- Marinated/pickled herring products (2 companies).

Unlike the primary processors that need to be near to source of raw materials, the distribution of the secondary producers within Scotland is not restricted to the major ports and they are found throughout Scotland. Since the raw materials can be delivered by refrigerated / frozen transport, other factors become more important to the business, e.g. proximity to markets and transport links, atmospheric conditions for traditional smoking, company history etc. In addition, some secondary processors sourcing pelagic fish from Scotland have their operations in England.

Secondary processors can be placed into two categories;

A. Large processors often with multiple processing lines and fully integrated supply and distribution chains for both raw materials and final product. These processors are generally those who have the major retailers as customers and supply a wide range of fishery products sourced from throughout the UK, if not the world, and, as such, pelagic fish form only a small proportion of their product range. There are, however, two Scottish based secondary processors that meet these criteria yet have specialised in the production of pelagic products, namely International Fish Canners and NorSea Smokehouses in Fraserburgh and Aberdeen.

B. Smaller “artisanal processors” producing low volume, high quality, “gourmet” products such as traditional “Scottish kippers” or marinated herring, often for local consumption and/or mail order retail.

It should be noted that these categories are not exclusive, as there are some companies where operations cut across more than one category, i.e. a single company that includes primary processing (PP2), secondary processing (SP.A & B) and fish wholesale within its activities.

11.6 Fish merchants/wholesalers

The companies range in size and facilities from small (1-2 persons) with a refrigerated van up to larger companies with their own ice making facilities, packaging area, chill stores, and fleet of refrigerated road transport.

What they all have in common is that they purchase (normally fresh) fish from a range of suppliers (fish markets, vessels and/or primary processors) and distribute it as soon as possible to a range of customers. There is no processing involved in their operations except maybe the re-icing and packaging of bulk product into expanded polystyrene containers for transport and delivery to customers.

In most fish merchants/wholesaler operations there are no, or limited, storage facilities, since each day’s product is sold and dispatched on the same day. The product temperature change is limited by it being kept on ice, and moved using refrigerated/insulated transport.

11.7 The retail sector

The majority of pelagic fish sold in the UK market is via the major retail chains (Tesco, Asda-Walmart, Sainsbury's, Morrison's, M&S, Waitrose) that sell pelagic products from a chilled fish counter and/or refrigerated display cabinets. A spot survey of available pelagic products in the major retailers was conducted by the authors during March 2012. This identified the Scottish pelagic sector as being a major supplier of pelagic products to the main UK retailers (Table 18, Table 19, and

Table 20). Whilst it was not possible to determine the origin of the counter products, supplied in bulk for display on fish counters, both the discussions with counter staff and the labelled packed products indicated that these products were sourced from Scottish suppliers.

Table 18. The availability of fresh counter service Scottish pelagic fish from fish counters at major UK retailers (in East Yorkshire, March 2012)

Retailer	Fresh Fish Counter	Mackerel		Herring	
		Fresh	Smoked	Fresh	Smoked
Tesco	✓	✓	✓	✓	✓
Asda	✓	✓	✓	✓	✓
Morrison's	✓	✓	✓	✓	✓
Waitrose	✓	×	×	×	×
Sainsbury's	✓	×	×	×	×

Table 19. The Producers of packed pelagic products for the major UK retailers

Producer code	Supplier Name	Product	Tesco	Asda	M&S	Morrison's	Waitrose	Sainsbury's
AA036	NorSea; Aberdeen	Kipper	✓	✓			✓	
		Smoked mackerel various toppings	✓	✓				
BB003	NorSea; Fraserburgh	Smoked mackerel various toppings	✓			✓		
BB008	Int. Fish Canners	Tinned mackerel (own label)						
BB011	McCrae's; Fraserburgh	Kipper		✓	✓			✓
		Rollmop				✓		
		Smoked mackerel		✓	✓			
		Rollmop		✓				
WB111	McCrae's; Edinburgh	Smoked mackerel					✓	
IB002	Daniel's Sweet herring	Rollmop	✓					
VY104	F Barraclough Ltd (Bradford)	Frozen mackerel fillets		✓				

Table 20. Branded pelagic products originating from Scotland, available from UK retailers

Producer code	Supplier	Brand	Product	
BB 008	Int Fish Canners	John West	Canned	Sild [1] in Sunflower Oil
			Canned	Sild in Tomato Sauce
			Canned	Skippers [2] in Sunflower Oil
			Canned	Skippers in Tomato Sauce
BB011	McCrae's; Fraserburgh	Young's	Frozen	Kipper Fillets With Butter

[1] Sild and Brisling are common names originating from old Norse to describe processed young and therefore smaller herring

[2] "Skippers" is a brand name for Brisling products

Whilst it is acknowledged that this information does not include details of all suppliers to all retailers, in what is an ever-changing market place, it does provide an overview as to who the main processors are within the pelagic supply chain for UK consumers.

The major retailers take a rigorous approach to controlling product safety throughout their supply chains and as such include detailed specifications as to the controls and

conditions that suppliers need to comply with in order to maintain access to the markets.

The major supermarkets account for the majority of pelagic sales to UK consumers. Other smaller supply chains exist that retail smaller volumes of product via local fishmongers/food service operations, farm/smoked salmon shops, and websites for postal/courier delivery. Products that are retailed in this way are for the most case smoked products from Category B secondary processors although small retailers may also obtain product from Category A secondary processors via fish merchants/wholesalers.

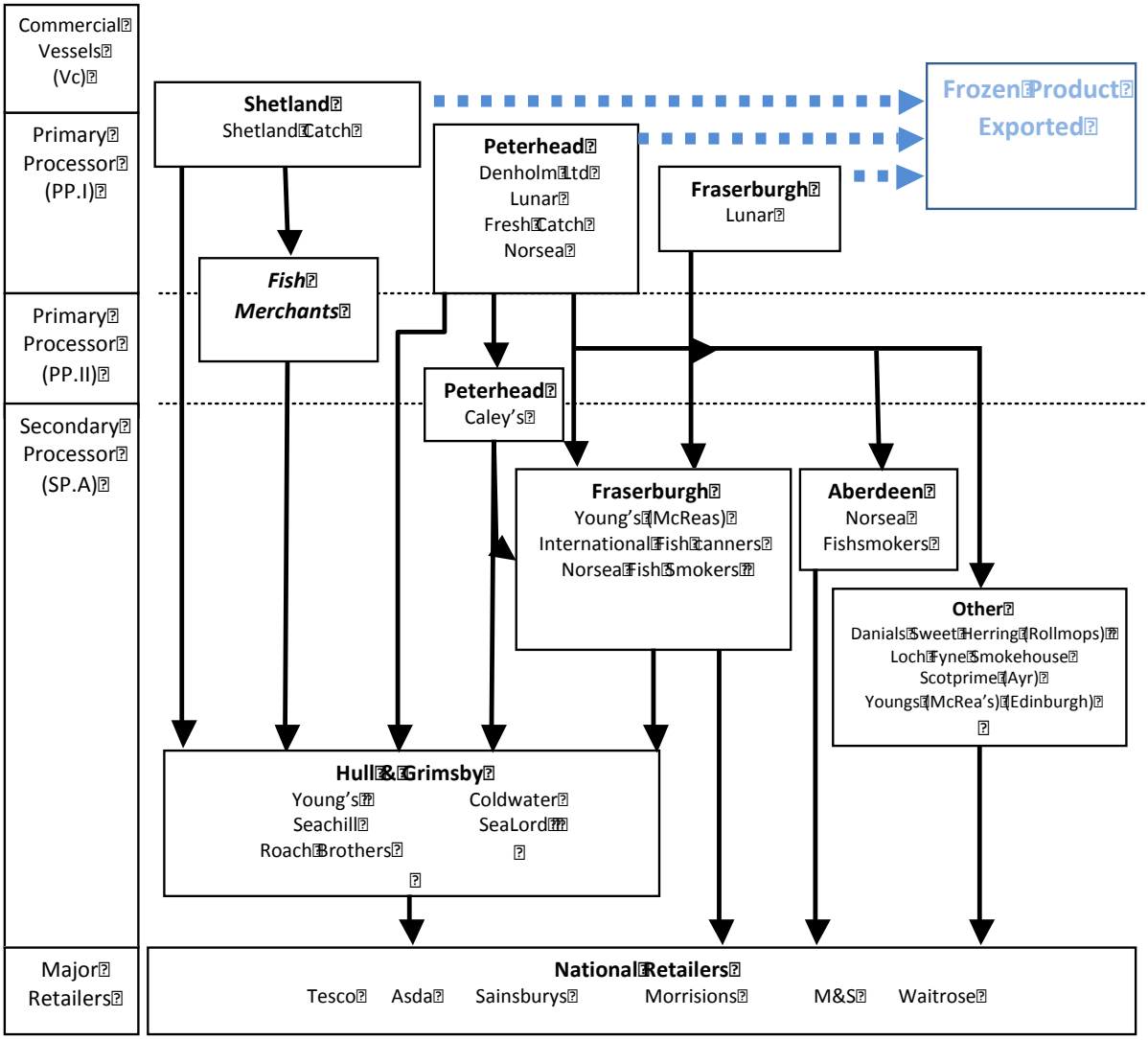

Figure 16. Flow diagram illustrating the "commercial" supply chain for pelagic fish in the UK from Scottish fisheries

From this review, it can be seen that the pelagic fishery sector in Scotland is composed of a relatively small number of specialist vessels and processing

companies that are integrated into a close network of suppliers, processors, and customers.

The “commercial” supply chains within Scotland (Figure 16) consists of only five primary processors, and nine secondary processors, with a further two secondary processors that supply the retail chains identified in the Humberside region of England.

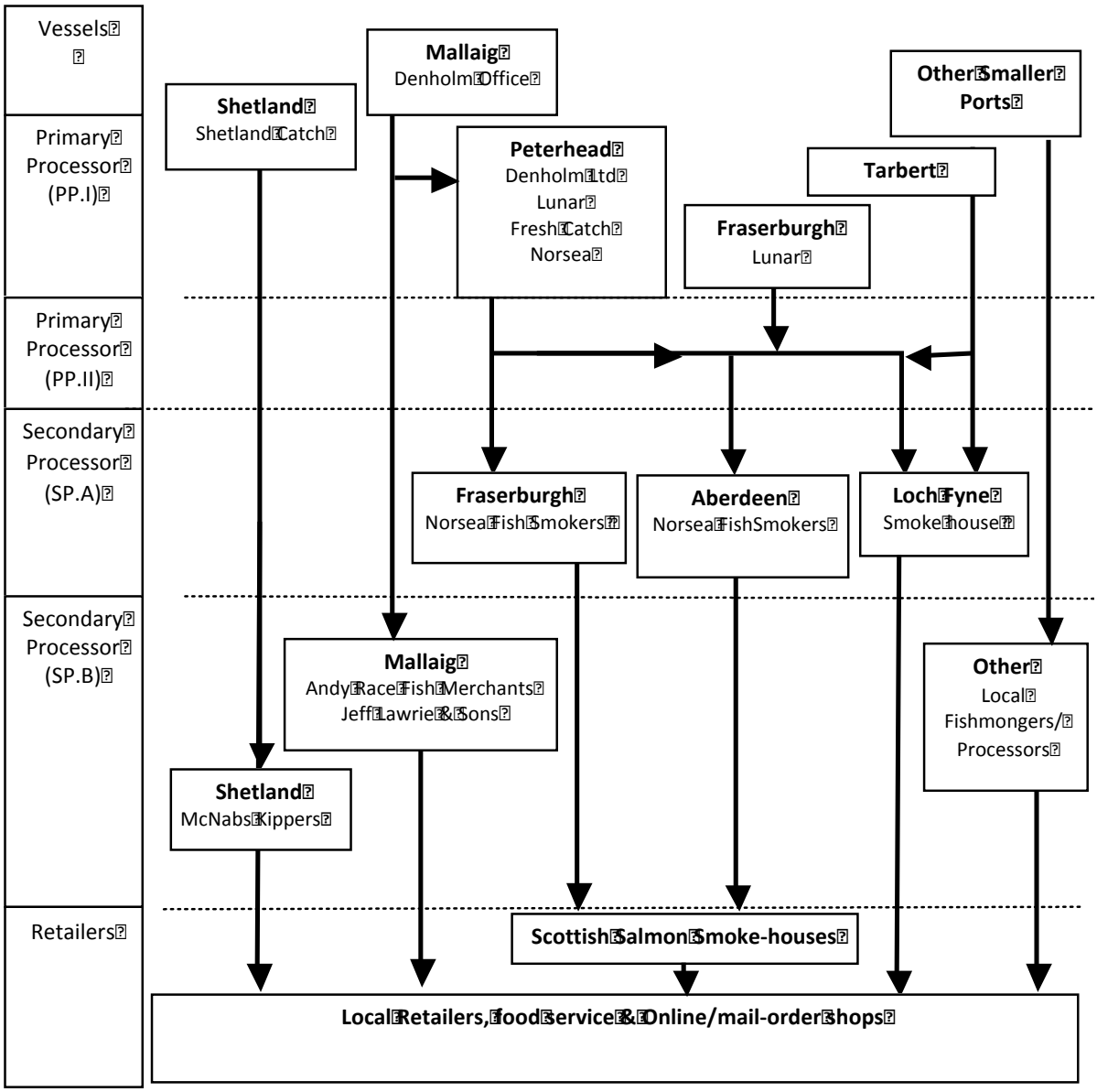

Figure 17. Flow diagram illustrating the supply chain for pelagic fish in the UK from Scottish fisheries from smaller (independent) processors

The situation for the “artisanal” supply chain (Figure 17) is slightly more complicated in that both raw materials and final product enter this supply chain from the commercial supply chain in addition to locally landed inshore mackerel when it is in season.

12. Appendix 4: List of Scottish Pelagic Fishing Vessels

The main Scottish pelagic fleet consists of 26 modern, purpose built, specialist pelagic trawlers. All vessels are members of the Scottish Pelagic Fishermen Association. Details of the individual vessels are provided below:

Fish Producer Organisation	Vessel Name	PLN	Home Port	Country	Overall Length (m)	Year Built
Interfish	Altaire	LK429	Northmavine	Scotland	76	2004
Klondyke	Challenge	FR226	Fraserburgh	Scotland	65	2004
	Chris Andra	FR228	Fraserburgh	Scotland	71	2006
	Taits	FR227	Fraserburgh	Scotland	71	2000
Lunar Group	Kings Cross	FR380	Peterhead	Scotland	70	
	Lunar Bow	PD265	Peterhead	Scotland	69	2008
	Pathway	PD165	Peterhead	Scotland	67	2003
North East Of Scotland Fishermen's Organisation	Ocean Quest	FR375	Fraserburgh	Scotland	21	1982
North Sea Fishermen's Organisation Ltd	Enterprise	PD147	Peterhead	Scotland	45	1995
Scottish Fishermen's Organisation	Charisma	BF296	Macduff	Scotland	14	1981
	Christina S	FR224	Fraserburgh	Scotland	72	2007
	Forever Grateful	FR249	Fraserburgh	Scotland	64	2001
	Ocean Venture II	PD340	Peterhead	Scotland	31	1992
	Prowess	CY720	Stornoway	Scotland	60	1988
	Quantus	PD379	Peterhead	Scotland	65	2008
	Resolute	BF50	Gardenstown	Scotland	64	2003
	Sunbeam	FR487	Fraserburgh	Scotland	56	1999
	Unity	FR165	Fraserburgh	Scotland	45	1989
Shetland FPO Ltd	Adenia II	LK193	Whalsay And Skerries	Scotland	62	2003
	Antares	LK419	Whalsay And Skerries	Scotland	73	1996
	Serene	LK297	Lerwick	Scotland	72	2008
	Zephyr	LK394	Whalsay And Skerries	Scotland	73	1996
Non-Sector	Research	LK78	Whalsay And Skerries	Scotland	9	1969
Northern Ireland FPO Ltd	Havilah	N200	Kilkeel	NI	49	1989
Anglo-North Irish FPO Ltd	Stefanie-M	N265	Kilkeel	NI	49	1987
	Voyager	N905	Londonderry	NI	75	2010

Data from SFPA members list (SFPA, 2012) and the registered vessel lists maintained by the Marine Management Organisation (MMO, 2012b). *PLN = Port & Landing Number.*

13. Appendix 5: Pelagic Landing Data 2010

Port	Mackerel	Herring	Blue Whiting	Horse mackerel	Sardines	Sprats	Tuna	Other Pelagic	Total	%
Peterhead	50,970	19,906	4,937	330				55	76,198	58.9%
Lerwick	36,468	7,541		835				8	44,853	34.6%
Fraserburgh	7,584	6		2					7,592	5.86%
Mallaig		13				537			550	0.42%
Scalloway and isles	50								50	0.04%
Tarbert		48							48	0.04%
Scrabster	1							33	34	0.03%
Buckie	12								12	0.01%
Pittenweem	6								6	0.00%
Kinlochbervie	1							4	5	0.00%
Ullapool	3								3	0.00%
Eyemouth	3								3	0.00%
Aberdeen	2								2	0.00%
Stromness	1								1	0.00%
Troon									-	0.00%
Oban									-	0.00%
Lochinver									-	0.00%
Kirkcudbright									-	0.00%
Cullivoe									-	0.00%
Campbeltown									-	0.00%
OTHERS SCOTLAND (a)	79	52							131	0.10%
Total Scotland	95,179	27,566	4,937	1,167		537	-	101	129,487	

Data from: Scottish Government (2011)

14. Appendix 6: Survey of catching practice

14.1 Objectives

The primary aim of the survey was to obtain information from companies in order to assess the level of understanding and control of histamine within the catching sector. The survey however had an important secondary role in that it publicised the project and provided the contact information necessary to allow further discussions in order to get more detailed information.

14.2 Industry practice

The Scottish pelagic fishing sector is described in Appendix 3 (Section 11). The mapping exercise identified two main categories of catching, defined by the vessels used, method of catching, season and classification of fishery, i.e.:

1. Purpose built pelagic trawlers
2. Inshore line caught mackerel fishery

14.3 Purpose built pelagic trawlers

The modern, purpose built, pelagic trawlers have very high standards of cleanliness and procedures for ensuring that fish are caught and handled without causing damage or contamination during handling and storage. The nature of the vessel design and equipment used is such that the fish are transferred from net to RSW tanks and from vessel to factory without being manually handled or exposed to any sources of potential contamination. In addition the RSW tanks have been shown to maintain the product temperature below 2°C for the maximum 72 hour storage period before landing.

Temperature records for the vessel “Kings Cross” were obtained for seven different fishing days during 2010 and 2012. The temperatures measured in the vessel’s RSW tanks after loading with fish are shown in Figure 18 to Figure 24. The sensors used measured the water temperature in the tanks, the exact position of the sensors in the tanks, and details of the tanks, were not supplied. Temperatures were recorded every two hours.

The maximum water temperature measured after the fish were immersed in the tank approached 10°C although it was generally in the 7 to 8°C temperature range. On 6 of the 7 occasions the water temperature was reduced below 0°C within 7 hours of loading. In the seventh case on 31.01.2012 it required 10 hours.

Data was also provided on the amount of fish being loaded into a tank on each specific day (Table 21). There was a clear relationship between the amount of fish handled and the cooling rate measured in a RSW tank. On 31.01.2012 all the tanks on the vessel were loaded to approximately 70% of their total capacity with the largest amount 140 tonnes being placed in tank 4.1 which cooled slowest. On all other occasions, the overall amount of fish being handled was less and at least five of the RSW tanks were empty.

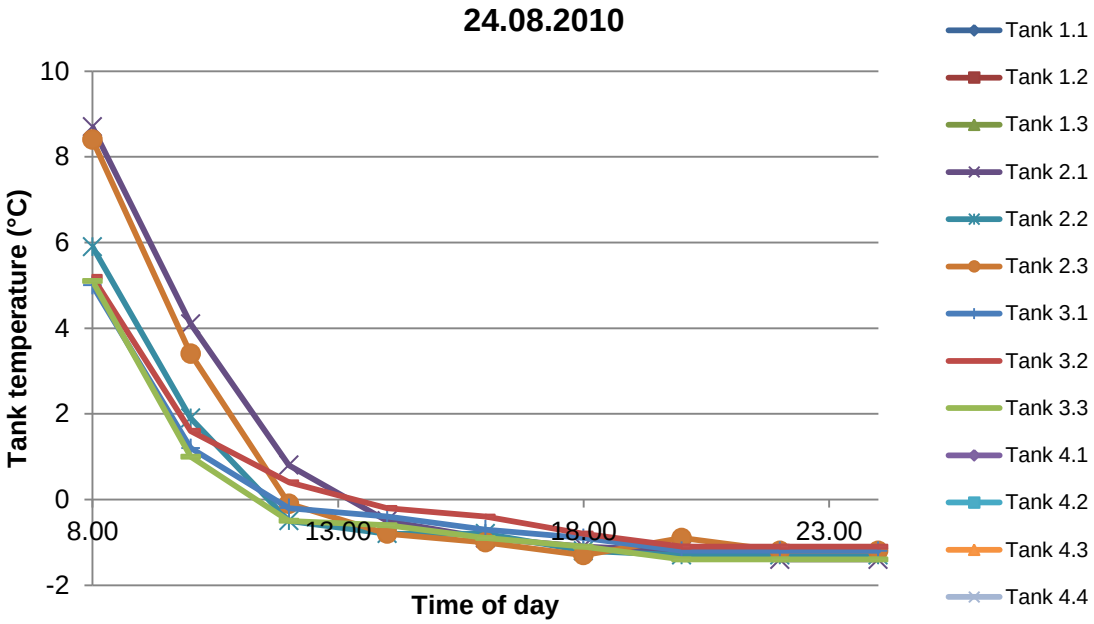

Figure 18. Measured temperatures in RSW tanks on 24.08.2010; target species: herring (company supplied data)

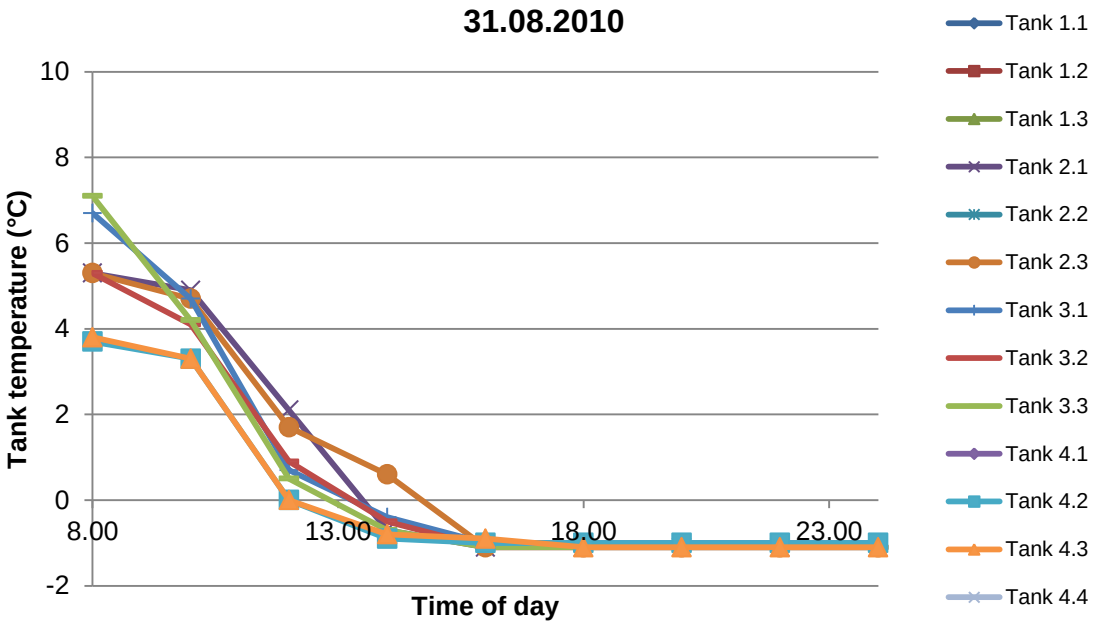

Figure 19. Measured temperatures in RSW tanks on 31.08.2010; target species: herring (company supplied data)

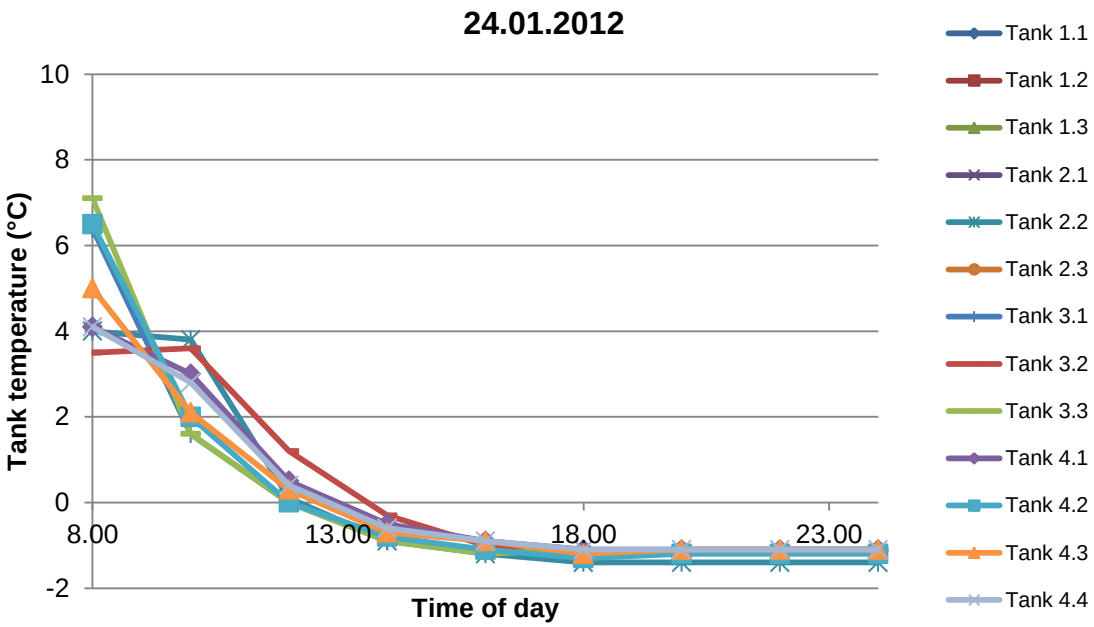

Figure 20. Measured temperatures in RSW tanks on 24.01.2012; target species: mackerel (company supplied data)

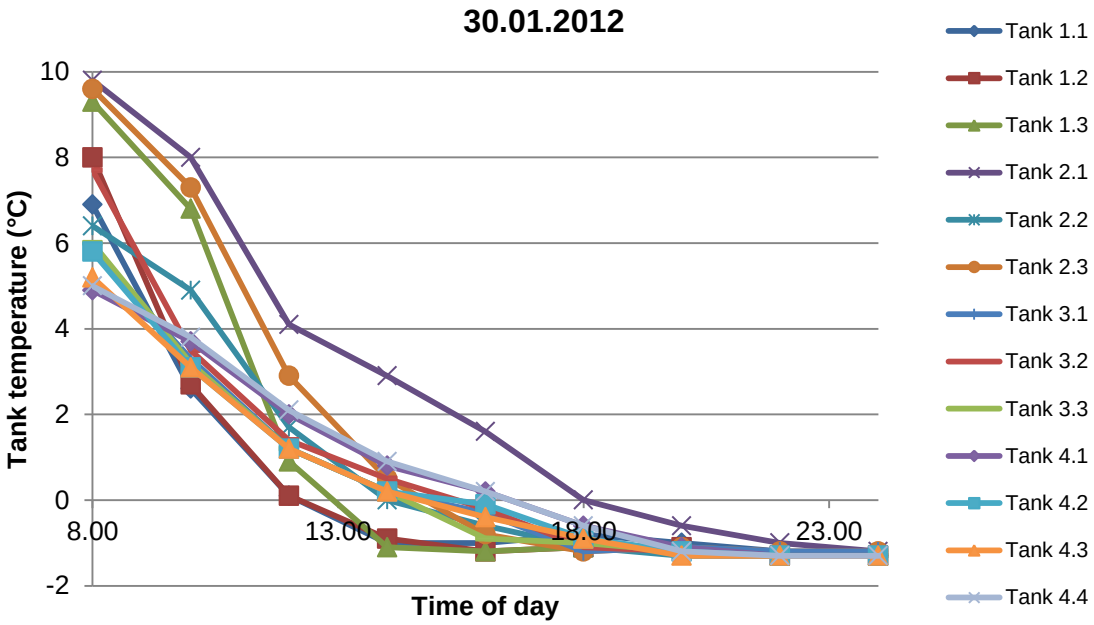

Figure 21. Measured temperatures in RSW tanks on 30.01.2012; target species: mackerel (company supplied data)

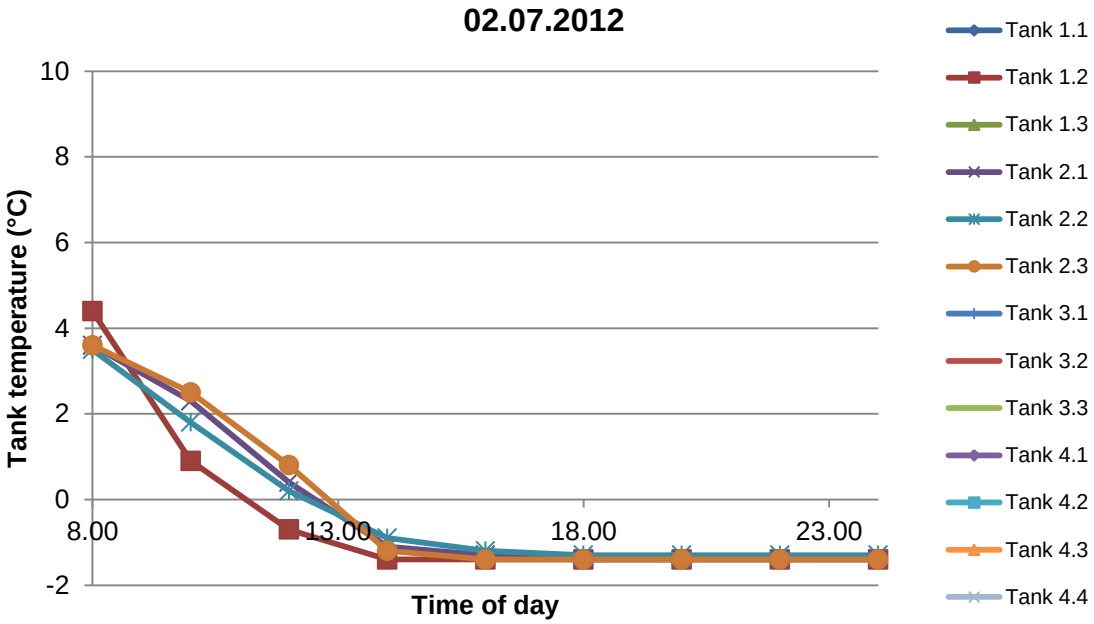

Figure 22. Measured temperatures in RSW tanks on 02.07.2012; target species: herring (company supplied data)

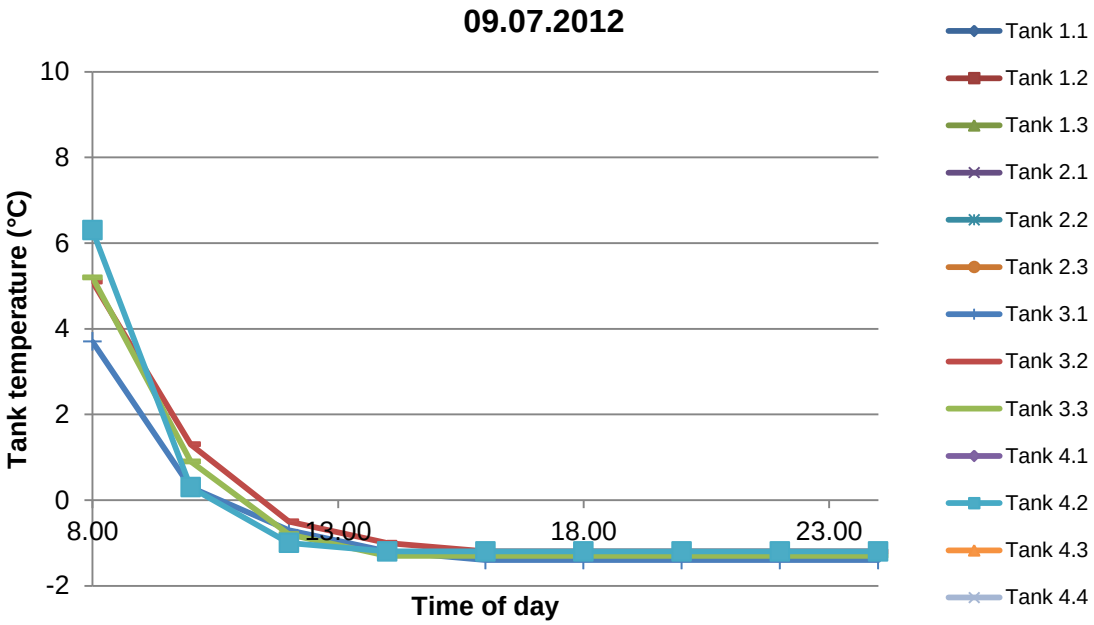

Figure 23. Measured temperatures in RSW tanks on 09.07.2012; target species: herring (company supplied data)

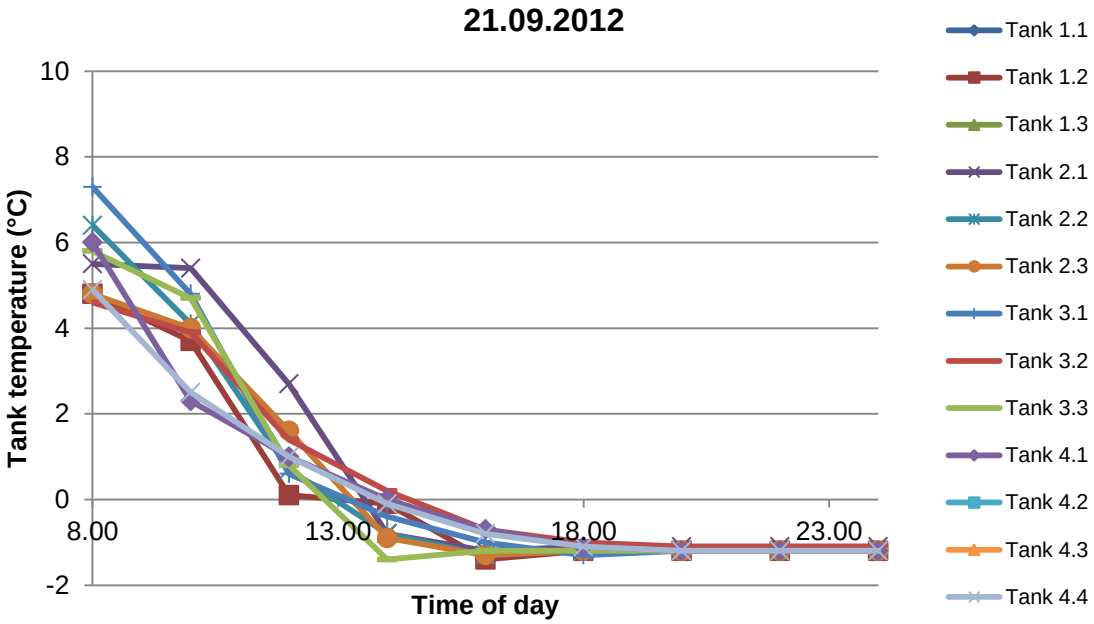

Figure 24. Measured temperatures in RSW tanks on 21.09.2012; target species: mackerel (company supplied data)

Table 21. Amounts of fish loaded into each RSW tank on each specific day of operation

Date/Species	Amounts	Tank number												
		1.1	1.2	1.3	2.1	2.2	2.3	3.1	3.2	3.3	4.1	4.2	4.3	4.4
24.08.2010 Herring	Fish %	0	0	0	0	70	0	70	70	70	70	70	70	70
	Fish (T)	0	0	0	0	82	0	77	120	77	140	65	65	140
	Water (T)					35.1		33.0	51.4	33.0	60.0	27.9	27.9	60.0
31.08.2010 Herring	Fish %	0	0	0	0	70	0	70	70	70	70	70	70	70
	Fish (T)	0	0	0	0	82	0	77	120	77	140	65	65	140
	Water (T)					35.1		33.0	51.4	33.0	60.0	27.9	27.9	60.0
24.01.2012 Mackerel	Fish %	0	0	0	0	70	0	70	70	70	70	70	70	70
	Fish (T)	0	0	0	0	82	0	77	120	77	140	65	65	140
	Water (T)					35.1		33.0	51.4	33.0	60.0	27.9	27.9	60.0
30.01.2012 Mackerel	Fish %	0	0	0	0	70	0	70	70	70	70	70	70	70
	Fish (T)	0	0	0	0	82	0	77	120	77	140	65	65	140
	Water (T)					35.1		33.0	51.4	33.0	60.0	27.9	27.9	60.0
02.07.2012 Herring	Fish %	0	0	0	0	70	0	70	70	70	70	70	70	70
	Fish (T)	0	0	0	0	82	0	77	120	77	140	65	65	140
	Water (T)					35.1		33.0	51.4	33.0	60.0	27.9	27.9	60.0
09.07.2012 Herring	Fish %	0	0	0	0	70	0	70	70	70	70	70	70	70
	Fish (T)	0	0	0	0	82	0	77	120	77	140	65	65	140
	Water (T)					35.1		33.0	51.4	33.0	60.0	27.9	27.9	60.0
21.09.2012 Mackerel	Fish %	0	0	0	0	70	0	70	70	70	70	70	70	70
	Fish (T)	0	0	0	0	82	0	77	120	77	140	65	65	140
	Water (T)					35.1		33.0	51.4	33.0	60.0	27.9	27.9	60.0

It is clear from the data supplied that the cooling rate of the fish after harvest will be determined by the relationship between the amount of fish being caught and the heat

extraction capability of the boat and the specific RSW tanks. In this particular boat then the cooling time to 0°C was extended to approaching 10 hours when the tanks were 70% full. At less than 50% capacity the cooling times were 7 hours or less. It is therefore likely that, at 100% capacity, cooling times could be in excess of the 12 hours recommended as the maximum in FDA guidance (2011, Appendix 11).

Whilst the smaller multi-use vessels (V2), may have compromises in their operations due to the design and availability of their equipment for storage and handling of a range of fish species and fishing gear, the fact that the fisheries are usually nocturnal during the winter months mean that the likelihood of histamine formation is likely to be very low.

14.4 Inshore line caught mackerel fishery

The inshore line caught mackerel fishery is undertaken by a range of inshore vessels. The most common method of capture being hooked lines that are winched in and the fish de-hooked into fish boxes with ice. As such, there is the potential for a large variation in temperature profiles for individual fish. This is dependant on an additional number of variables that may include: the amount of ice used, ambient air temperature, position of fish in box, the time between capture and landing, storage conditions available on vessel (insulated hold/active refrigeration/deck storage) etc.

In discussions with major fish processing companies a general impression was obtained that the industry felt that inshore line caught mackerel from Scottish waters was a potentially a significant source of fish with high histamine levels. Studies were therefore carried out at Peterhead and Shetland to assess operational practices and monitor temperatures of mackerel from these sources. To aid the work, a Grimsby processing company agreed to raise a special order for mackerel that could be monitored to provide real data.

14.4.1 The inshore summer mackerel fishery at Peterhead

The inshore summer mackerel fishery in the Peterhead region is limited to licensed vessels that are normally engaged in other types of fishing such as shellfish and crustacean for the rest of the year. The initial quota allocation is seven boxes (350 kg) per vessel per week.

Figure 25. Main harbouring facilities at Peterhead for under 10m vessels, access via a ramp to floating dock, to cope with the high tidal range (authors own photo)

Fishing activity and therefore landings at Peterhead fish market take place throughout the day and are limited only by the state of the tide within the port. Most fishermen arrange their fishing activity so that landings can take place at high tide to make it easier to land the fish from the small vessels, since there is both a large tidal range and high harbour walls at Peterhead (Figure 25).

Initial discussions with fishermen indicated that the fishing activity takes place within a maximum of 1-2 hours from the port and in many cases is much closer, with shoals of mackerel often found in Peterhead bay itself. The fishermen were aware of the need to quickly chill the caught mackerel in order to obtain the best quality, however their priority was for best price at market rather than concerns of product safety.

Figure 26. Line caught mackerel landed at Peterhead harbour (authors own photo)

Observation of the vessels indicated that a wide range of fish boxes were used to store the caught fish from the standard stacking fish boxes (Figure 26**Error! Reference source not found.**) to small insulated fish tubs with lids. Ice was observed being transferred to vessels leaving port, the ice being supplied by one of two ice plants located in Peterhead harbour.

All mackerel are landed directly into the market, unless the catch has been consigned by a specific processor in which case the fish may be landed and directly transferred to the factory chill store, by forklift truck.

A visit to the market on 26/07/12 (market starts at 7:00am) showed that 28 boxes of mackerel were on the market from seven vessels, all the boxes being clearly labelled with vessel name and port of landing number (PLN). Whilst this was a typical number of boxes for a Thursday, the numbers tend to be higher during the start of the week due to the new weekly quota allowance.

The boxed mackerel could have been on the market from any point during the proceeding 20 hours and whilst the ambient temperature of the market was maintained at between 5-10°C, it was apparent that only some boxes had adequate ice cover. Mackerel surface temperatures, measured using a handheld infrared (IR) thermometer (Fluke 572 CF, Fluke UK Ltd, Norwich, UK), from the un-iced boxes were in the range from 2.5 to 3.5°C (n=5).

Visual assessment of mackerel quality on the market was extremely good. However points of concern were:

1. Due to the wide range of vessel sizes, equipment and fish boxes being used there are likely to range of standards for chilling and maintaining the temperature of the product.
2. Landings took place throughout the day so product maybe on the market for prolonged periods (up to 20 hours) at which point ice may have melted at the point of sale. Although temperatures measured at the time were adequate, some fish were being kept in un-iced boxes.
3. Transport of mackerel from the market without re-icing has potential for elevated temperatures and onset of histamine formation.

14.4.1.1 Assessment of mackerel processing

14.4.1.1.1 Product supply

A vessel (Figure 27) had been contracted to supply high quality line caught mackerel for the trial. This vessel and several others are known to supply line caught mackerel that have been well chilled in ice slurry and are trusted to provide best quality product. However, with a weekly quota limited to seven boxes (350 kg) per vessel per week, the catch from a single vessel was not sufficient to fulfil the planned order in a day. The amount of fish supplied by the vessel was approximately 150 kg. Discussion with the skipper indicated that fishing activity had occurred during the early morning and the vessel had returned to port around 8:00am. The mackerel, stored in a single covered fish bin, had been off-loaded from the vessel before either the vessel or method of transfer could be assessed (11:35). The bin was transported to the factory by forklift along the dockside (4 minutes) and placed in the reception chill store.

Figure 27. Fishing vessel (authors own photo)

14.4.1.1.2 Factory operations

The fish bin was placed in the reception chill store upon arrival at the factory. Inspection of the fish bin, by the company, reported that the product temperatures were -1°C at the surface and -0.9°C in the centre/core and that the mackerel was still in rigor (Figure 28). Studies carried out for this project have shown significant temperature stratification in un-agitated immersion bins (see Section 17, Appendix 9).

Figure 28. Checking raw material (authors own photo)

The reception chill store temperatures were recorded at the beginning of morning and afternoon shift using a hand held probe. However, due to product transfer the doors that open directly onto courtyard were left open for periods of time (>5 minutes). Although this would not normally be an issue due to the product being well iced and the Peterhead climate, because of the warm sunny weather (22°C) there

was the potential for temperature fluctuations to occur. The overall process is defined in Table 22.

Table 22. Processing activity and temperature monitoring

Time	Activity	Temperature (°C)			
		Ambient	Surface fish	Centre/core fish	Fillet
07:00	Capture				
11:35	Landing	20			
11:45	Chill store	5-10	-1	-0.9	Brine -1
12:05	Fillets (immediately post filleting)	15	1.3		0.7
	Fillet (1st fillet after10 min)	15			3.5
12:35	Lunch (boxed fillets transferred to chill store remaining fish left in ice slurry)	5-10 Chill store	3.2		
13:05	Filleting resumed	15			
13:30	Co Mark temperature probes	15			
	Mackerel in ice			0.8	
	Boxed Fillets			Rising to 3.5	
13:46	Chill store				
13:46	Boxed product placed on racks and transferred from chill store to Lunar freezing, (fork lift 2-3 minutes)	22			
13:52	Product placed in pre cooled blast freezer	-30			

Figure 29. Filleting system (authors own photo)

Due to the small quantity of fish being processed, a filleting table was set up (Figure 29) and two filleters taken from the filleting line processed the order. The filleting line was at the time processing white fish, but can be used to hand cut mackerel if quantity so demands. Mackerel and ice slurry were transferred to the filleting bin. Because fish were still in rigor (not atypical for inshore caught fish) filleting was problematic resulting in additional trimming being required to remove pericardial membrane and thus reducing both yield and filleting rate. Fillets were transferred to plastic lined cardboard boxes, which when full were weighed (10 kg), strapped and transferred to the chill store. The filleting operations were interrupted by the (30 min) lunch break during which the boxed fillets were transferred to the chill store and the remaining whole fish were left in the filleting tub with the ice slurry.

Samples of fillets were taken and a rapid test for histamine conducted, the result indicated no detectable values greater than 5 mg/kg. The company have previously been using the Neogen Alert qualitative test kit that indicates either the absence or presence of histamine with a 50 mg/kg indicator level and had ordered the Veratox test kit (quantitative to 2.5 mg/kg) but were awaiting its arrival. The Veratox assay was therefore conducted by a neighbouring processor and the result phoned through.

We were told that normal filleting practice results in the completion of processing records which detail:

- Species, vessel, quantity, destination (internal processing), filleting code (internal use), batch number, plus the raw material temperature and Torry score at the start of processing (the Torry score is a fish quality assessment).
- The batch code, which has the structure of year number/sequential day/vessel PLN, e.g. 2/205/F10, that combined with the species provides both internal & external traceability. The date refers to is the date of reception which in the case of pelagic species is the same as date of production.

However, the records were not seen to be completed during the time the mackerel were processed for this work. A review of the records for the preceding five days indicated that no other line caught mackerel had been filleted during that time, although the automated pelagic filleting plant was in operation for the summer season herring (sourced from the commercial trawlers).

Packed fillets were transferred to the chill store and placed on racks in preparation for transfer to a separate freezer operator. This was achieved by fork lift truck and took four minutes to travel the 200 m between the factories.

The separate freezer operator operated a large blast freezer facility and cold store (in addition to primary processing of both white fish and pelagic species). The product was placed in the pre-chilled blast freezer (-30°C) until other product orders could be loaded at which time it would be blast frozen at -40°C.

Points of concern were:

- No observed weighing of fresh mackerel being supplied.
- No observed landing of fish, only told that it had originated from the identified vessel.
- No records for the order kept at time of processing (it being seen as a “special order”).
- Histamine analysis not currently available on site, but they are up-grading their method to the more sensitive rapid test kit.
- Temperature monitoring and control in the processing facility was problematic especially on warm summer days.
- Only limited manual checking of chill store temperatures.
- Once packed, mackerel fillets were not immediately transferred to the chill store.
- Despite issues with temperature monitoring and control the fish was generally kept at a temperature below 4°C, except for boxed fillets. But in all cases the time of exposure to ambient temperatures was minimised and in total accounted

for less than 15 minutes at temperature greater than 10°C with the fillet temperatures only reaching a maximum of 3.5°C. Whether this is significant with regards to product safety/shelf-life has yet to be determined.

14.4.2 The inshore summer mackerel processing in Shetland

The fishery is limited by season (May-Sept) and quota restrictions, which in 2012 limited the total catch to 110 tonnes. The quota is distributed to the 60 (approx.) licensed vessels by the Shetland Fishermen's association (SFA), which has limited the catch to 360 kg per week per vessel. The boats are <10 m vessels that are used during the rest of the year for other forms of fishing (Figure 30), most being involved with creel fishing. During the mackerel season, the boats are equipped with jigging rigs. Fishing activity takes place mainly during the evening and early morning with landings timed for the Lerwick (& Scalloway) fish markets at 8:00am with the fish on that evening's ferry to Aberdeen. The fishing locations vary with season and weather but are a maximum of a 4 hour voyage from the point of landing.

Figure 30. Typical fishing boat (authors own photo)

The vessels use ice (crushed tube ice) produced by a company (LDH, who also operated the fish markets) on the dockside and available for collection by vessel or containers for road transport. Whilst some vessels use cut down plastic barrels in which to store the caught fish in ice slurry (ice and seawater) there is a trend for vessels to use insulated and lidded fish bins.

14.4.2.1 Landings

A large proportion of the landings take place at small landing sites/jetties along the coast of the southern main island. The fish are transferred to standard fish boxes and transported by road to the Lerwick market in vans or covered trailers a maximum of 60 minutes drive away. Fishermen at any particular landing site will often collaborate and bulk purchase ice from the ice plant. The ice is transported and stored at the landing site in a large insulated fish bin for communal use. However, this is mainly used for icing the fish at sea and not always used to ice fish during the transport to market. The remaining landings are either directly onto the markets or into the wholesalers (contracted catches).

14.4.2.2 The market

Both Lerwick and Scalloway fish markets are operated by LDH as electronic (Dutch) auctions. Under a program funded by a whitefish landing levy, Shetland Seafood

Quality Control (SSQC) undertake a program of monitoring whitefish quality however no such program currently exists for the much smaller (and less valuable) line caught mackerel fishery.

Fish are placed on the market by the fishermen, who have responsibility for ensuring that the fish are de-iced and officially weighed in and each box labelled (weight, date of catch vessel, size/grade), before re-icing (fishermen often collaborate with ice supply as with landings), presenting the fish and placing on the market. Boxes on the market are of two standard weights, 45 and 31 kg, depending on species. However, with the mackerel some boxes are sold on the basis of actual weight where the catch is insufficient to complete a whole box.

Figure 31. Iced fish at market (authors own photo)

Observation of the Lerwick fish market indicated that all mackerel were well iced (Figure 31) and boxes were clearly labelled with the vessel identity, which is also indicated on the auction clock so ensuring traceability to individual vessels. Despite the summer season and relatively high outside temperatures the market was kept at chill temperature (3.0±1°C) and product temperatures (n=5) were approximately 0.3°C (surface) and 1.7°C (centre/core).

14.4.2.3 Processors/packaging agents

Under normal operating conditions, mackerel bought at the market by the wholesaler is palletised and labelled in the same iced boxes and transferred directly from the market floor into refrigerated containers for transport on that night's ferry to mainland UK (Aberdeen), and subsequent deliveries throughout the UK. Where packaging or reweighing into smaller order is required, the iced fish is transferred to a processing facility by truck (10 minutes drive) and the order is made up to specification. The facility has its own ice (flake) making capacity and chill storage facility. Whilst there is the facility to fillet whitefish to meet orders, mackerel are only repacked whole.

14.4.2.4 Chilled transportation

During the survey trip an investigation into the fish temperature history during transportation was arranged. Tiny-Tag data loggers (Gemini Data Loggers (UK) Ltd, Chichester, UK) were used to record the ambient temperature conditions and centre/core product temperatures of mackerel packed on ice in both standard (open) fish boxes and polystyrene boxes during storage at the market, overnight ferry from Lerwick to Aberdeen, and road transport from Aberdeen to Grimsby (where the fish

was processed). The results of this investigation showed that although the ambient temperatures were seen to peak during product transfer and transport on the mainland, the product remained at a temperature "approaching that of melting ice" (as required in EU regulation 853/2004), i.e. ≈0°C, throughout distribution (Figure 32).

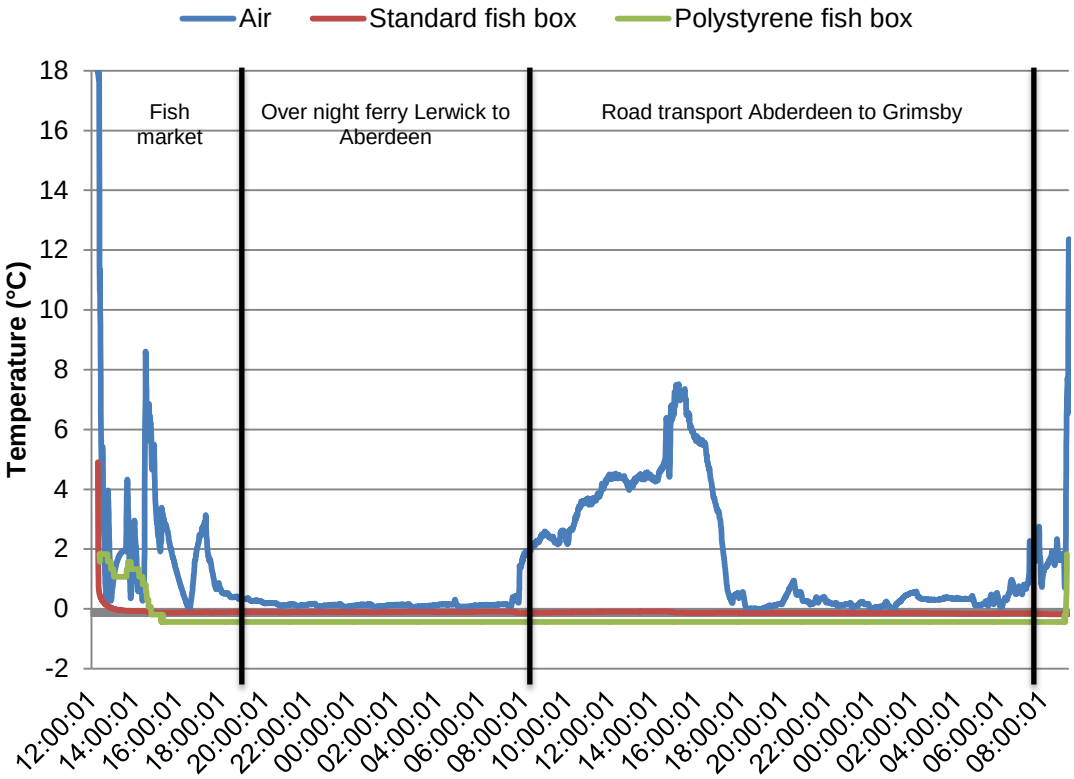

Figure 32. Temperatures during transportation of mackerel packed on ice in both standard (open) fish boxes and polystyrene fish boxes from Lerwick, via Aberdeen, to Grimsby (transported during July 2012)

14.4.3 General points

As part of the inshore fishery survey the following points were raised with the industry:

Time of day for catching and landing, assuming they are all day boats.

Inshore vessels fish mainly during evening and sometimes during early morning with landings taking place either directly to the markets (Lerwick & Scalloway) or at smaller jetties in the south of Shetland (Cunningburgh, Suma), and transported to the markets either in vans or covered trailers a short journey taking a maximum of 60 minutes.

Where do the vessels get their ice?

Ice is obtained from the LDH ice plants located at both Lerwick and Scalloway harbours.

Is the same amount of ice used despite weather conditions or time of year?

Although the fishermen are aware of the need to increase the amount of ice depending on the ambient conditions, the fishing activity was restricted by quota to the summer months June-September in 2012. The end date of the season is dependent on both the behaviour of the fish and the availability of quota and the possible of transferring quota from under-performing fisheries elsewhere in Scotland.

Do they record catch times?

Catch times are not currently recorded, although with fishing activity limited by quota allocation to 360 kg per vessel per week, vessels are only fishing for short periods during each trip and are normally only fishing for a maximum of 4-5 hours with a maximum of an hour travel to landing.

Are temperature checks carried out of product and storage areas?

Although there is no evidence of actual temperature monitoring on the boats, the fishermen are aware of the need to ice the fish upon capture and the landings seen demonstrated that the amount of ice used in the ice slurry should be more than sufficient to chill the fish to acceptable temperatures (although temperature data was not available to confirm this).

Does each vessel carry a fully calibrated probe?

No, since temperature control is seen by fishermen as good practice with sufficient icing to ensure best quality and therefore market price, rather than something that needs to be accurately monitored and recorded. Inshore fishermen are not in the habit of keeping records of fishing activity, apart from determining the quantities landed and the price received, which are both recorded by the auction.

Do the boats have refrigerated seawater tanks?

Vessels are all <10 m and due to the line-caught mackerel fishery being a short season with limited quota, the vessels that are normally engaged in shellfish and crustacean fishing are adapted for fishing with jigged lines to target mackerel. Due to this, the range of sizes and equipment is varied, but none are large enough to have RSW tanks and all rely on the storage of the caught mackerel in a slurry of ice and seawater. The containers used for this can also vary and include plastic half barrels for the smaller boats with limited deck space to covered insulated fish bins for the larger boats normally used as creelers (crab/lobster pots).

Landings at the market involve the de-icing and weighing of the fish in clean fish boxes, before re-icing and placing on the market. The fishermen are responsible for the ice used in re-icing and as such either bring ice at the time of landing or working with other fishermen arrange for a fish bin full of ice to be available at the market, this is clearly labelled and the lid secured form use by others by means of a padlock & chain.

What hygiene practices do the vessels work to?

Standard good hygienic practice for the industry; conversations with fishermen demonstrated that they know of the required hygienic standards and the vessels observed were kept to a good standard

Is there full fish traceability from each vessel?

Traceability is possible at the market with boxes labelled with boat name and PLN. Times of capture and or landing (arrival at the market) are not currently recorded.

14.4.3.1 Fish market controls

Labelling of boat name

The fishermen transfer fish to the market and each box is weighed and labelled with details of the vessel. It is then re-iced as part of the requirements for entering the product into the auction.

What time is the fish landed at the market and what are the storage and icing conditions?

The time of placing of fish on the market is dependant on the vessel, the location of fishing activity and whether the catch is landed at the market or transported by vehicle from a different landing site. Fishing takes place late evening and early morning with the fish being landed before 7:00am for the electronic auction that starts at 8:00am. With the fishing restricted by quota to 360 kg per vessel per week, the maximum number of boxes per vessel is 12. However, the number landed on any one night is usually much lower.

Is the temperature recorded at the point of landing?

Currently a project called the Whitefish Initiative funds the independent inspection by SSQC of all fish landed at Lerwick & Scalloway (http://www.ssqc.co.uk/WhiteFishQualityImprovementInitiative.aspx). The funding is from a levy on whitefish landings and is heavily subsidized by the Shetland Island Council. Currently this scheme is not extended to the much smaller and seasonal line-caught mackerel fishery, and as such there are no official quality checks or temperature measurement at the point of landing or reception.

Does the market own a fully calibrated probe?

SSQC the independent inspectors have a temperature probe in use on the market which is currently (due to funding issues) not used to assess temperature of mackerel upon arrival

Do the Shetland Quality Control inspectors check the landings for icing, temperature and histamine?

Histamine checking at the point of landing is seen as being a market requirement rather than a true critical control (as defined in a HACCP system) since histamine levels are unlikely to be measureable so soon after capture and any temperature abuse will only become evident after a lag phase of 2-3 days. The SSQC do not currently have facilities to undertake histamine analysis. Their HPLC is currently not operational and is routinely used for other analysis. This method, although the official method for histamine determination, is also the most expensive. The utilization of rapid test kits were discussed, but for the previously stated reasons this was seen as an unnecessary monitoring point, at this time. This would be possible if required, however SSQC would require funding to undertake these tests and there is currently no system in place for using levy or auction fees to undertake analysis. The usefulness of testing for histamine at such an early stage in the supply chain is also questioned due to the relatively short time periods between capture and landing it is unlikely that even with temperature abuse sufficient to have a significant effect on shelf life that measurable quantities of histamine would be detected.

How are boxes cleaned?

LDH the auctioneers, ice plant operators etc also operate the box washing facilities. Where fishermen are using insulated fish bins, the bins remain on the vessel (or trailer) as fish de-iced and weighed at arrival on the market. The fish is then re-iced and presented by the fishermen. It is assumed that a similar system operates with vessels that use other types of fish box and, as such, the fishermen are responsible for the cleaning and maintenance of the boxes used on the vessels.

14.5 Overall conclusions

All the pelagic fisheries aim to control histamine by:

1. Rapid reduction to, and maintenance of fish temperature between 0-2°C.
2. Landing of fish as soon as possible after capture (this is usually within 24 hours of capture, but range from 2 hours for inshore vessels to 72 hours for large pelagic trawlers).
3. Prevention of damage to the whole fish during handling to reduce and prevent cross-contamination with histamine-forming bacteria. Since histamine-forming bacteria may be naturally present in the gut, gills and skin of fish, hygienic handling of fish is an important control.

14.5.1 Purpose built pelagic trawlers

The modern, purpose built, pelagic trawlers have high standards of cleanliness and procedures for ensuring that fish are caught and handled without causing damage or contamination. However, based on the data supplied to this study there is evidence that cooling rates may not be as rapid as recommended when handling large capacities.

Whilst the smaller multi-use vessels (V2), may have compromises in their operations due to the design and availability of their equipment for storage and handling of a range of fish species and fishing gear, the fact that the fisheries are usually nocturnal during the winter months mean that the likelihood of histamine formation is likely to be very low.

14.5.2 Inshore line caught mackerel fishery

In discussions with major fish processing companies a general impression was obtained that the industry felt that inshore line caught mackerel from Scottish waters was potentially a significant problem source of fish with high histamine levels, particularly during hot summers. The inshore fishing of pelagic fishes clearly is, or can be, very variable and has the potential to produce significant occasions of temperature abuse.

Despite these concerns, the surveys of operations at Peterhead and Shetland carried out in this study showed that although there were some issues of concern; temperatures control was effective and no significantly elevated temperatures were observed. The high quality image and economic value of line-caught mackerel appears to apply considerable pressure on the fishermen and associated processors to maintain the cold chain.

15. Appendix 7: Survey of on land processing and distribution

15.1 Objectives

The primary aim of the survey was to obtain information from companies in order to assess the level of understanding and control of histamine across the processing sector. The survey however had an important secondary role in that it publicised the project and provided the contact information necessary to allow further discussions in order to get more detailed information.

15.2 The survey

The survey was produced in a range of formats to facilitate its completion by a wide range of respondents, from differing circumstances, these included;

- A one page introduction to the project with links to the survey, available both as a webpage and as down-loadable document. http://tinyurl.com/Histamine-Intro.
- A document based survey that could be either printed out and returned by post or completed on the computer and returned by email. http://tinyurl.com/Histamine-paper-survey.
- An online Survey, using the Survey Monkey web based survey design and collection service, that allow respondents to quickly and easily complete the survey on-line, the survey questions were adjusted for individual sectors, e.g.
    - Smokehouses and Secondary Processors: https://www.surveymonkey.com/s/Histamine-Processor.
    - All other sectors: www.surveymonkey.com/s/FSA_Histamine_Survey.

The survey was designed to be a first point of contact with the project, in that the information it requested would provide the details necessary to identify those companies that should and could be contacted for a follow up investigation via visits or phone calls, whilst at the same time collecting the relevant information from other companies.

15.2.1 Identification of relevant companies

Scottish companies that are approved to handle fishery products were obtained from the list of 302 companies, published by the FSA (Food Standards Agency (UK), 2012). Those that were potentially involved with handling pelagic fish species were targeted, using a range of methods including:

- Identification of suppliers throughout the supply chain in discussions with companies.
- Via membership of producer/trade organisations e.g. Scottish Pelagic Fishermen's Association (SFPA), Seafood Scotland, etc.
- Information provided by regional Environmental Health Officers (EHOs).
- Reference to a company's online publicity and product information.
- A survey carried out by the authors of pelagic fishery products available at major UK retailers.

- Direct contact with company either via phone, email or meetings.

15.2.2 Contacting companies

Seafood Scotland supported this project by contacting all their members with involvement with pelagic species (151 companies) and provided an introduction to the project and links to the survey website. They also provided a list of those companies contacted to the researchers.

Other companies that were not members of Seafood Scotland were contacted directly by the researchers, initially by email and then in subsequent phone calls.

15.2.3 Survey responses

Despite follow up phone calls and reposting of emails, the initial response to the survey was poor, with only six companies completing the online or paper based survey (Table 23). However, this figure includes a response by SPFA on behalf of all their 28 member vessels. Follow up contact with some of the non-responding companies indicated that whilst they have pelagic fish on their Seafood Scotland data-base entry, they in fact only retail such product and as such did not employ specific controls for histamine. Others who were contacted made it clear that they were not interested in, or too busy to participate in the survey.

Table 23. Companies that completed the initial survey

Company	Location	Category
1	Peterhead	PP.I
2	Peterhead	PP.II
3	Fraserburgh	SP.A
4	Inverawe, Argyll	SP.B
5	Linlithgow	R
Scottish Pelagic Fishermen's Association	Fraserburgh	IA (28 Vessels)

Table 24. Companies surveyed via interview and visits

Company	Location	Category
6	Peterhead	PP.I
7	Lerwick	PP.I
8	Peterhead	PP.II
9	Lerwick	FM
10	Fraserburgh	SP.A
11	Stromness	SP.B
12	Mallaig	SP.B
13	Newton Stewart	SP.B

Because of the relatively low response from industry, the focus of the survey was shifted to a greater reliance of obtaining the required information directly via phone calls and meetings with the companies and with the survey forms completed by the interviewer (Table 24). In addition, some non-Scottish companies that both source product from Scottish suppliers and are key players in the supply of pelagic fishery products to the UK retailers were also included in the survey (Table 25).

Table 25. Other companies within the supply chains that were surveyed via interview and visits

Company	Location	Category
14	Grimsby	SP.A
15	Hull	SP.A

15.3 Survey findings

The survey identified two distinct supply chains of Scottish pelagic fish and fish products to the UK markets. The first supply chain consists of companies that are supplying the major retailers (SP.A) and as such have a tight control of product quality and storage conditions throughout the supply chain. The second involves secondary processing (principally of smoked product) by smaller companies (SP.B) that sell via local retail outlets or online/mail order.

15.3.1 Landing sites

The majority of pelagic fish are landed directly into the factory responsible for primary processing (Section 15.3.2).

In the smaller fisheries, such as Mallaig or Loch Fyne (Tarbert), fish are landed, immediately re-iced and loaded on transport for immediate delivery to the primary processors. Adding an additional stage in the supply chain does pose an additional risk, unless strict procedures for hygiene conditions and temperature control are implemented within the transport. The primary processor has this responsibility and undertakes the checks at reception for both product temperature and histamine monitoring.

The landings of small amounts of summer line-caught mackerel, that occur at various ports throughout Scotland (Figure 13) are either sold on the fish market to local fish merchants with other fish species, or direct to local processors (e.g. smokehouses (SP.B) that undertake some primary processing). The product that enters the market only goes through the standard market handling and icing procedures. The only checks are based on product visual quality and sometimes temperature monitoring at the point of landing, i.e. there are no additional checks for histamine or temperature at landing for these products.

15.3.2 Primary processors (PP.I & PP.II)

The Scottish pelagic processing sector is a highly industrialised, integrated and centralised industry, with just five specialist factories dominating the sector. These factories have been specifically designed and built to handle and process the large volumes of pelagic fish delivered from the pelagic fishing fleet, in such a way that temperatures are controlled throughout the process and that the product is frozen as quickly as possible. Both categories of primary processor (I & II) operate the same levels of histamine control and such their responses are combined in this section.

15.3.2.1 HACCP and histamine

All companies who responded have HACCP plans that include histamine as a potential hazard, and whilst the assessment of potential severity varies depending on individual companies experience or customer requirements, the assessment of likelihood is predominantly low.

The sources of information on which these assessments were based, cited by the primary processors, were predominantly based on previous experience and records, rather than scientific or industry advisory publications.

The HACCP plans that were reviewed, during visits to the companies, all had similar controls for histamine, i.e. monitoring of raw material at the point of reception, with continued temperature control up to the point at which the product was frozen being the main control measure. However, due to the relatively low risk assessment of histamine as a hazard these controls were not classed as a critical control point (CCP), rather a control that required only formal level of verification, i.e. regular monitoring and recording of histamine levels.

15.3.2.2 Histamine monitoring

On reception of fish from the vessels, the primary processors are provided (sometimes only on request) with full details of date and time of capture, and temperature records from the RSW tanks, for each haul of the nets, from the vessels. This allows the processor to determine whether the temperature of the fish is acceptable when the fish temperature is greater than 2°C due to a short period between capture and landing (<4 hours) that occasionally occurs when fish are found close to shore.

Samples of fish are then taken from either the processor's holding tanks or directly from the grading line for further quality checks, including histamine analysis. A typical sample consists of three fish taken at random, that are gutted, de-headed and de-tailed. The remaining muscle is cut into three sections, combined with sections from the other sample fish and minced. The resulting tissue sample is then tested according to the methodology of the assay being used.

The routine analysis of histamine at product reception is conducted by either rapid test kits, such as that produced by the Neogen range (Neogen Corporation, US) (see Section 16, Appendix 8 for details), or (in one company) the fluorometric chemical method (AOAC Official Method 977.13, the EU standard reference method as set out in Commission Regulation (EC) 2073/2005). As discussed in Section 9.9, although extremely accurate the fluorometric chemical method is not particularly suitable for routine monitoring of samples due to the cost and technical complexity of the equipment required.

A review of the records from two of the companies plus verbal confirmation from the others indicates that over the last three to four years that there have been no instances of histamine values being observed at greater than the 2.5 mg/kg detection limit at this point in the supply chain.

The industry, whilst concerned about the cost of the testing, for what is a negative result from a trusted supplier (V1); continue with the monitoring to meet both the regulatory and customer requirements.

The type of testing varied between processors:

- A quantitative assay method.
- Kit that determination histamine levels to 2.5 mg/kg.
- Or, in two out of the five companies, a qualitative assay with a positive detection limit of 50 mg/kg. It was stated by the companies that this was to reduce the cost of monitoring whilst meeting the requirement for testing.

15.4 Secondary processors (SP.A)

This category of processors, due to the requirements of their primary customers (the major retailers), have high levels of controls and monitoring not only for histamine, but for all aspects of hygiene and product quality within their operations.

15.4.1 HACCP and histamine

The risk assessments conducted for histamine by this sector resulted in a range of control levels being used by companies, dependant on their specific suppliers and species. Whilst imported warm water species such as tuna were seen to have a high likelihood of histamine being present and as such required a CCP at the point of reception; cold water pelagic species were perceived to have a lower likelihood. This resulted in the level of control being reduced in the HACCP plan to formal verification at reception (histamine monitoring) and temperature control throughout processing. These processors however were seen to take histamine as a hazard very seriously, mainly due to the product specifications set and concerns for product safety by their main customers, the major retailers.

15.4.2 Histamine monitoring

At factory reception all product is checked against the specifications the supplier is to meet, with reference to histamine in pelagic species. This involves recording temperature of product, and for specific species monitoring each batch/consignment for histamine using a rapid test kit.

The test kits used for monitoring were, where used, quantitative rapid test kits such as the Neogen Veratox kit or its equivalent with validation of the results periodically undertaken by certified laboratories on a commercial basis.

One processor, however, contracted out all histamine analysis to a commercial laboratory, an arrangement that was specific to the individual company's operations in that all raw materials were frozen and samples were taken on reception, whilst the sampled consignment was kept in frozen storage until results confirmed product safety and the consignment could then be released for thawing and smoking.

A review of records in two major companies showed that product at reception would be immediately rejected for not being at the correct temperature (<-18°C for frozen, or <4°C for fresh). Histamine testing records for the previous year (2011) showed that for mackerel, 16% of consignments tested had measurable histamine levels. However, with one exception, the histamine values were below 2.5 mg/kg. The exception, fresh line-caught from Shetland, had a value of 60 mg/kg and was therefore rejected.

Due to the requirements of the major retailers these companies also undertake regular shelf-life and end of life testing of the final product in order to demonstrate that the product remained safe with respect to both microbial and histamine levels beyond the use-by date displayed on the packaging.

15.5 Secondary processors (SP.B)

This category of processor being the more "traditional" or "artisanal processor", did not necessarily have the same level of detailed documented controls in operation as other processors. However, they did operate to a standard of hygiene and with the necessary facilities and controls to meet the standards as required for a food business as enforced by the local EHOs.

15.5.1 HACCP and histamine

The controls for histamine in these smaller processors are limited to relying on the assurances of suppliers, if raw materials are frozen, and visual quality for fresh, raw materials rather than any active measurement of histamine. The HACCP plans were completed to meet the legal requirements rather than a detailed statement of risk assessment and preventative measures, and as such lacked referenced sources of information on which risk assessments had been based, relying instead on personal experience and knowledge of the product.

All companies surveyed were found to rate histamine as a low severity risk to product safety, whilst only a few of the more commercially orientated secondary processors would place the risk as slightly higher at medium to address the concerns of their customers. The likelihood of histamine occurring in Scottish mackerel or herring was generally set at a low value within their raw materials, products and processes in their operations.

A potential area of concern for some processors who receive frozen raw materials from the main primary processors, is the procedure by which the material is thawed in an uncontrolled temperature and environment. Whilst this was not observed during any of the detailed company visits, the lack of temperature controlled facilities observed at some premises (who were visited but were not willing to fully participate in this study) suggested that thawing would take place at ambient temperature, with potentially elevated surface temperatures resulting in conditions for histamine formation.

15.5.1.1 Histamine monitoring

All companies in this sector surveyed relied heavily on the visual inspection of fish quality to determine the acceptability of product for further processing. Testing for histamine either by the use of test kits or external analysis was not typically undertaken. For the companies visited, records were kept of where raw materials were sourced and temperature monitoring and quality assessments at reception were kept, therefore providing product traceability but not necessarily safety of raw materials or final product.

15.6 Incidents of high histamine values in product

The responses to the survey indicated that the industry claim that there were no significant incidents of high histamine levels in respondents' product during the last three years. This assertion is very dependant on their interpretation of significant as being one that has involved the enforcement authorities. Within the supply chains there have been one or two instances where elevated levels of histamine have been detected and evidenced in the histamine monitoring records that have resulted in product being either rejected or retested.

One such incident was identified in the records of a secondary processor (SP.A) that was traced back through the supply chain to a consignment that had included fish from outside the normal controlled supply chains. This was a large shoal of mackerel entering Peterhead bay one hot summer day (2011) where many (licensed) vessels were involved in the localised fishing activity. It would appear that during the frantic fishing activity that temperature control measures were not always implemented. This was based on the belief that fish landed within 1-2 hours of capture did not necessarily need icing. Indeed, the monitoring records of the primary processor for that day did not show any histamine levels in the product although fish

temperatures were above 5°C at the point of reception. It was only later in the supply chain for the frozen mackerel fillets, further monitoring within a secondary processor (SP.A) indicated a histamine level of 11 mg/kg. Whilst this value was within acceptable limits, the measurements indicated that histamine limits may be exceeded by the end of the product shelf life, and therefore appropriate corrective actions were taken by the secondary processor. Another incident involved fresh line caught mackerel from Shetland and although it was not possible to trace all the records, it is likely that the histamine level of greater 60 mg/kg was due to inadequate temperature control within the supply chain, probably due to lack of ice aboard the vessel during high summer temperatures.

These incidents indicate that, although the controlled supply chain can occasionally break down, there are, in general, sufficient controls throughout the “commercial” supply chain to at least reduce the risk to the consumer.

15.7 Summary of survey findings

The key points identified from the on-line survey, subsequent visits and discussions with stakeholders in the Scottish pelagic seafood industry are:

1. The general perception of the industry is that the necessity for histamine control, although important for product safety, is undertaken primarily to meet customer requirements because their industrial experience suggests the likelihood of histamine occurring in their product is low to non-existent.
2. The consensus of the industry is that the risk assessment for histamine as part of their HACCP plans results in a level of control that does not require a Critical Control Point in order to ensure product safety.
3. HACCP plans in all sectors have only a single risk assessment for histamine, whereas the likelihood of its occurrence is largely dependant on the product temperature history, i.e. source of raw materials and the nature of the product (frozen or fresh).
4. Evidence to support the risk assessment for histamine in their HACCP plans, is largely based on experience and advice from enforcement authorities. Only the largest secondary processors relate their assessment to published information.
5. The rapid test kits used by processors are not always the most suitable for the concentrations of histamine likely to be present in the pelagic fish at the point where monitoring takes place in the specific supply chain(s).
6. The supply chains for the major retail markets have generally adequate control for histamine with monitoring of histamine levels and temperature at all the key points within the chain. There is, however, a small risk of uncontrolled raw materials entering the supply chain at the point of landing.
7. Gaps in the control of histamine were identified at the following points in the supply chain.
    a. The summer hand-line mackerel fishery does not always have adequate temperature control from point of capture to landing.
    b. Landing sites and fish markets do not differentiate between pelagic and other species with respect to the checks and monitoring conducted at the point of landing (Shetland).

c. Artisanal secondary processors (SP.B) are unlikely to test for histamine in either raw materials or final product.
d. Artisanal secondary processors (SP.B) do not always have adequate control of product temperature during thawing/tempering of raw materials, resulting in a potential increase in the likelihood of histamine formation.

16. Appendix 8: Histamine analysis methods

The Scottish pelagic processing sector uses a range of methods in order to monitor histamine concentrations in the raw material at the point of reception, the final product, and during shelf life trials. The survey carried out in this project indicated that those companies that employ monitoring used the analysis methods described below (Table 26).

Table 26. Histamine analysis methods used by companies surveyed (details supplied by manufacturer)

Manufacturer:	Neogen	Neogen	Neogen
Assay name:	Verotox	Alert	Reveal
Website	http://www.neogen.com/foodsafety/FS_Product_List.asp?Test_Kit_Cat=207a		
Kit Details	Veratox for Histamine — 9505 Quantitative microwell assay with a range of 2.5-50 mg/kg up to 38 samples.	Alert for Histamine — 9515 Qualitative microwell test that visually screens at 50 mg/kg, up to 20 samples.	Reveal for Histamine — 9501 Simple lateral flow test that screens at 50 mg/kg. 25 samples per kit.
Method:	Water extraction (see details below) For a minimum three gutted fish, cut three cross sectional (2.5cm) slices from behind dorsal fin to post vent for each fish (and remove bones). Homogenise tissue in a blender. Mix 10 g of homogenised sample with 190 ml distilled water in plastic sample bottle Shake well for 15 to 20 seconds Wait 5 minutes for sample to settle & repeat. Finally shake bottle well for 15 to 20 seconds and allow sample to settle for 30 seconds before pouring into a filter syringe (plastic syringe containing glass wool). Insert plunger and apply gentle pressure until sample collection tube is at least 1/3 full. If necessary dilute sample before conducting analysis. Extraction time 20 minutes.		
	CD-Elisa assay	CD-Elisa assay	Elisa assay
	Microwell	Microwell	Dip stick
	20 min	20 min	5 min
Measurement	Optical density reader used to determine colour density for control standards that for calibration curve from which sample histamine concentrations can be determined.	Visual comparison of colour (blue) between sample & control.	Visual inspection of test strips but recommended use of specialist reader to minimise operator variability & link in with PC
Results	Quantitative 2-50 mg/kg	Qualitative Positive at 50 mg/kg	Qualitative Positive at 50 mg/kg
Time	Extraction + 20 minutes	Extraction + 20 minutes	Extraction + 5 minutes
Assays per kit	38	20	25
Indicative cost per assay *	£5.57	£6.35	£5.40
Notes	Test conducted in batches of 12. (6 standard & 6 test solutions)	Requires minimum of 2 assays for each test i.e. Control & sample	Requires water extraction, but no filtration.
Additional requirements **	Starter kit -9529(£650[1]) 12-channel pipettor, 100 µL pipettor, 1000 pipette tips, tip rack, timer, 250 ml graduated cylinder, wash bottle, (2) 1 L bottles, 15 ml graduated tubes (500), demonstration video, lab station, well holder, test tube rack (13 mm), and reagent boats.	Starter kit- 9528 (£245[1]) 100 µL pipettor, pipette tips, tip rack, test tube rack (13 mm), timer, well holder, digital scale and demonstration video	

	Extraction kit — 9510 38 extraction bottles, filter syringes and sample collection tubes.	Extraction kit — 9520 Contains 20 extraction bottles, filter syringes and sample collection tubes.	
	Microwell Reader (£1950[1])		Accuscan reader -optional (£1080[1])
Supplier details	Neogen Europe ltd., The Dairy School, Auchincruive, Ayr, KA6 5HW, Scotland, UK Tel. International: + 44 (0) 1292 525 610**,** Fax International: + 44 (0) 1292 525 601		

* The indicative costs include the cost of the extraction & test kits and are provided for comparative purposes only. The manufacturer should be contacted directly for exact quote.

** The starter kits include the necessary pipettes, test tube racks, timer etc to enable analysis to start, further ongoing consumable cost will be required to replace disposable items.

17. Appendix 9: Experimental investigations

The industrial survey and literature review revealed a lack of published experimental data for the cooling, warming and thawing rates for herring and Atlantic mackerel under industrial processing conditions. The rates of temperature change are important to determine the periods at which products remain at the histamine-forming elevated temperatures. Trials were therefore carried out with an industrial company to obtain some data on thawing rates in a commercial operation. In addition, a short series of practical trials were conducted to determine example cooling and warming rates for herring and Atlantic mackerel under a range of conditions.

17.1 Experimental investigation of industrial thawing of Atlantic mackerel and herring

17.1.1 Introduction

In general, fish thawing is a very difficult and often overlooked operation. In freezing, the temperature of surface of the fish is quickly reduced to one where growth of microorganisms is severely limited and then stopped. In addition, the thermal conductivity of the frozen layer formed at the surface is far higher than the unfrozen fish. So as freezing proceeds it is progressively easier to extract the required heat from the centre/core of the fish. In thawing, the situation is reversed and as the surface temperature rises, microbial growth of both spoilage and pathogenic organisms can recommence. As the surface temperature further rises the rate of growth increases. In addition, as soon as the surface regions thaw a layer of poorly conducting material is formed which progressively lowers the rate heat can flow into the centre/core of the frozen fish. From a fish quality view it is undesirable to let the temperature of the fish rise above that of melting ice (0°C) during the thawing operation. High temperatures during thawing can increase drip, resulting in yield losses, and surface desiccation, in the later producing undesirable colour and appearance changes to the fish.

When thawing large amounts of fish that are contained in cartons the problem grows. There are problems in distributing the thawing medium (usually air) in terms of temperature and velocity evenly over tonnes of product in a room. Doing this and at the same time maintaining low surface fish temperatures is difficult to achieve and will result in very long and uneconomic thawing systems.

Cartons of frozen mackerel and herring are often the raw materials used in cold smoked fish operations. This material has to be thawed prior to processing and there is the potential for histamine levels to rise during thawing. If this happens then the smoking process will not remove the histamine formed.

Initial practical trials were carried out to provide an indication of the relationship between fish temperature and time during the thawing of mackerel cartons in a commercial operation.

17.1.2 Materials and methods

A trial was carried on a commercial thawing system used by a small smoked fish processor. The thawing system consisting of a room, approximately 5 x 7 m with a ceiling height of 2.5 m, was constructed of aluminium insulated panels with a solid concrete floor.

A one end of the room air was circulated over a heating coil and the resulting warm air blown into the room. Water in the heating coil was heated via a heat exchanger, which was fed with hot water from an external boiler.

The raw material being thawed consisted of blocks of frozen Atlantic mackerel and herring sourced from the commercial pelagic fisheries and primary processor in Shetland. The blocks had been packed in polythene lined cardboard boxes and had dimensions and weights as given in Table 27.

Table 27. Dimensions and weights of frozen raw materials

	Dimensions (cm) w-l-d	Weight (kg)
Whole Herring	36 x 39 x 6	8
Whole Mackerel	39 x 59 x 12	20
Mackerel Fillets	39 x 59 x 12	20

The process steps carried out by the company prior to, during and post the thawing operation are shown in Table 28. During the trial an opportunity was taken to measure the ambient temperatures in the areas used and they are also shown on the table.

Air temperatures were measured with Tiny-Tag temperature data loggers (Gemini Data Loggers (UK) Ltd, Chichester, UK) in the thawing room at the locations indicated on the plan (Figure 33). Three loggers were set up at ceiling height, their location being dependant on light fittings (1 & 2) and the process control temperature sensor (3). Temperature probes were also placed on the air on (intake) and air off (outflow) of the heater unit.

Centre/core and sub-surface fish block temperatures were measured during thawing (process step 8) using single point metal shrouded T-type (copper-constantan) probes (1.2 mm diameter, 100 mm length; RS Components Ltd, UK) linked to Comark Diligence data loggers (EV2014, Comark Instruments, Norwich, UK). The centre/core temperature probes were placed so that they were located approximately in the centre of both the frozen block and an individual fish. The sub-surface probes were inserted almost horizontally into the frozen block so that the tip was approximately 5mm below the surface. The data loggers, placed on the shelf next to the fish block, also recorded the temperature of their immediate surroundings, i.e. air temperature. The blocks were located towards the centre of the rack with one on the top shelf and the other two shelves down and (after a delay) the rack was placed in the centre of the defrost room overnight.

The airflow within the defrost room was measured using a digital anemometer (AV-2, Airflow Instruments, High Wycombe, UK) at the same locations the temperature measurements were made, with readings taken at approximately 10 cm from the floor and ceiling and at the mid point, whilst the racked product was in the defrost room.

In addition, surface temperatures of the top layers of fish (worst case scenario) stored in containers (plastic fish boxes) during the various stages of processing (wet side) were measured using a handheld infrared (IR) thermometer (Fluke 572 CF, Fluke UK Ltd, Norwich, UK),

Table 28. Process steps and time-temperature values observed during investigation of industrial thawing of herring and mackerel

Step		Time (approx.)	Air temperature (°C)
1	Raw materials stored in local cold store and the required amount for the following days production delivered each morning to factory (7:30 to 8:30 am).	-	-
2	Delivery frozen raw materials by van and pallets unloaded by forklift.	½ h	16.0
3	Frozen product storage in factory yard	5 h max	22.0
4	Transfer to production area (wet side)		16.2
5	Product unboxed and placed on racks (still in plastic)	½ h	16.2
6	Delay	1 h	16.2
7	Racks transferred to “defrost” room		
8	Product thaw (product thawed overnight for following days processing)	8-16 h	
9	Racks moved to production area (wet side)		16.2
10	Thawed product removed from polythene wrapping and transferred to fish crates	1-2 h	16.2
11	Fish crates transferred to work in progress chill store		2.6 to 5
12	Fish crates moved to filleting area of production area (wet side) for further processing		16.2

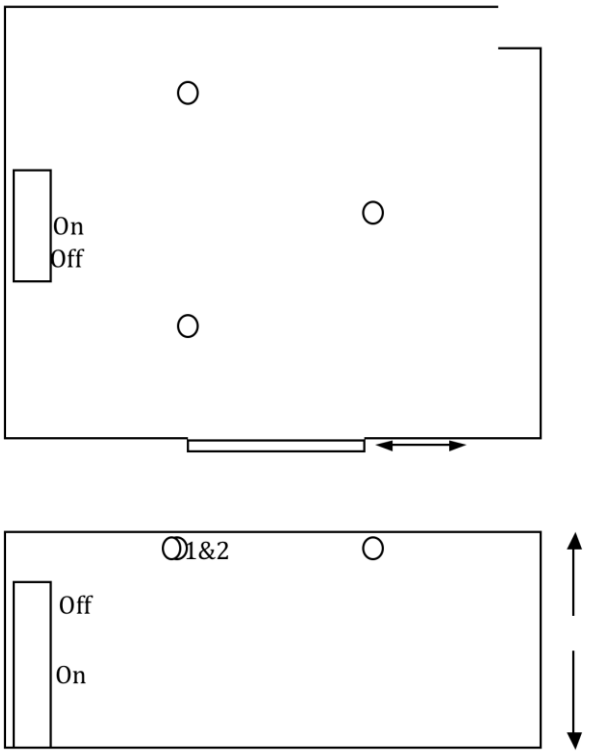

Figure 33. Position of air temperature sensors in thawing room

17.1.3 Results and discussion

17.1.3.1 Air Temperatures

The air temperatures measured (Figure 34) indicate that the heater unit control unit is a simple on/off switching system that results in a cycling of the "air off" temperature between 25°C and 30°C with air temperatures around the product of up to 25°C. The sudden decrease in air temperatures observed at 19:00 was caused by the opening of the door during transfer of frozen product to the defrost room. The data indicates that with the inrush of cooler air and additional frozen product substantially reduced the air temperature within the room and it takes nearly an hour for the air temperatures to re-establish equilibrium.

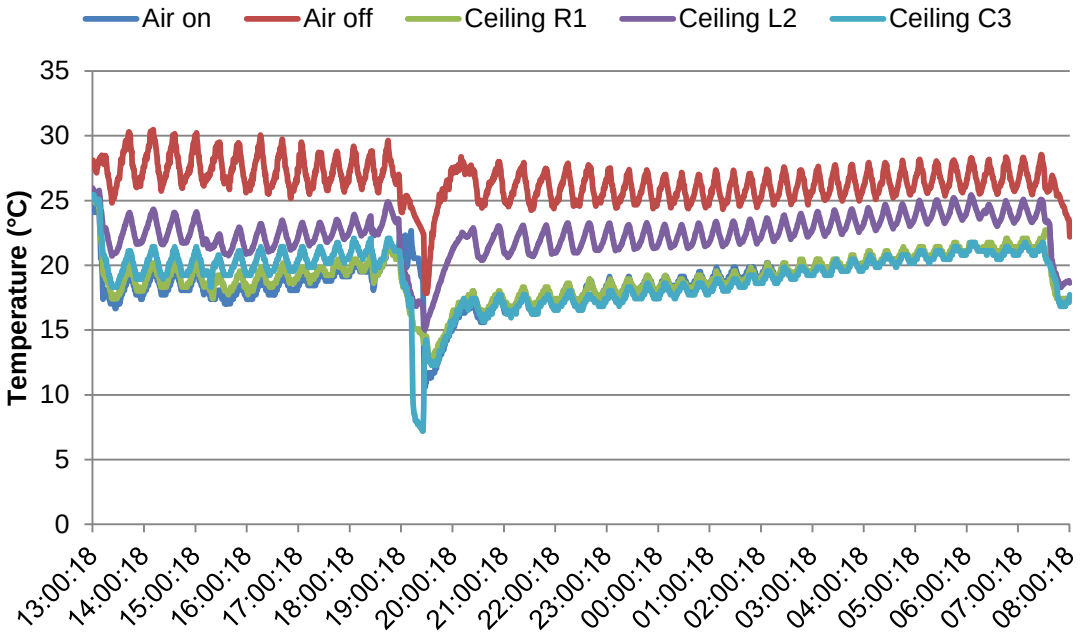

Figure 34. Graph showing temperature profile of air temperatures in the defrost room during a thawing cycle

Air temperatures immediately above the blocks of thawing fish dropped to approximately 5°C within a few hours of the fish being placed in the thawing room and then started to rise. At the end of the thawing cycle the air temperature immediately above a top fish block approached 20°C, and 15°C immediately above a fish block in the middle of a rack.

17.1.3.2 Fish temperatures

The surface temperature of the fish approached 0°C after 6 hours in the top block and after 9 hours in the middle block. In the middle shelf the surface temperature of the fish was above 4°C for the last 6 hours of the thawing process. Data from the surface of fish at the top position was lost after the packs were disturbed. At the end of the 18 hour thawing cycle centre/core temperatures within the fish blocks in both positions were still below 0°C (Figure 35 and Figure 36). However, the one recorded surface temperatures reached c.10°C by the end of thawing.

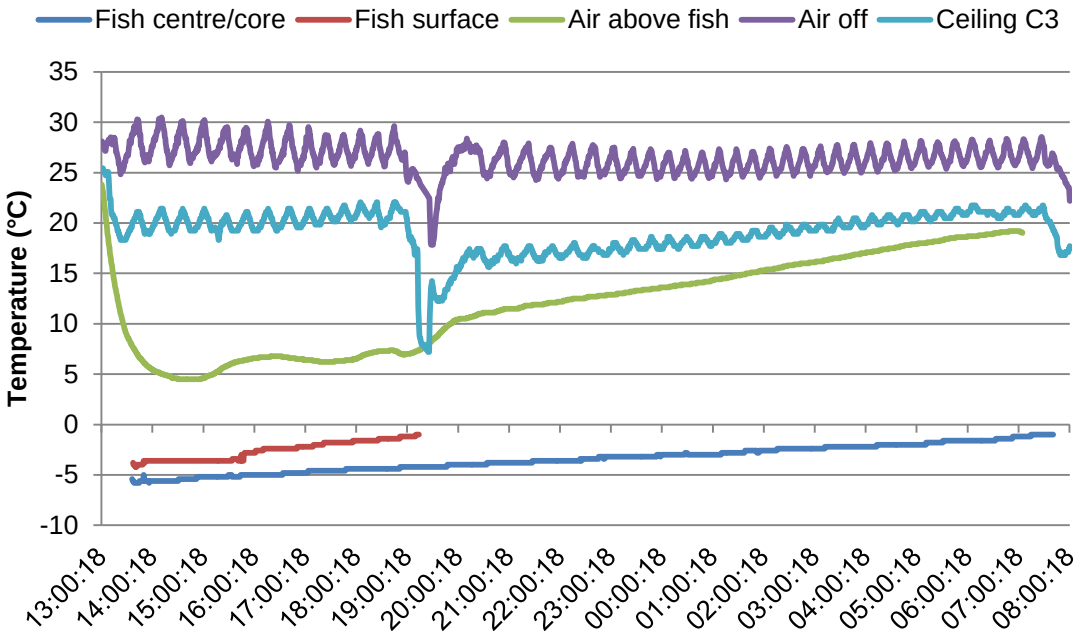

Figure 35. Temperatures during thawing of a frozen block of whole mackerel, located on the top shelf of the rack

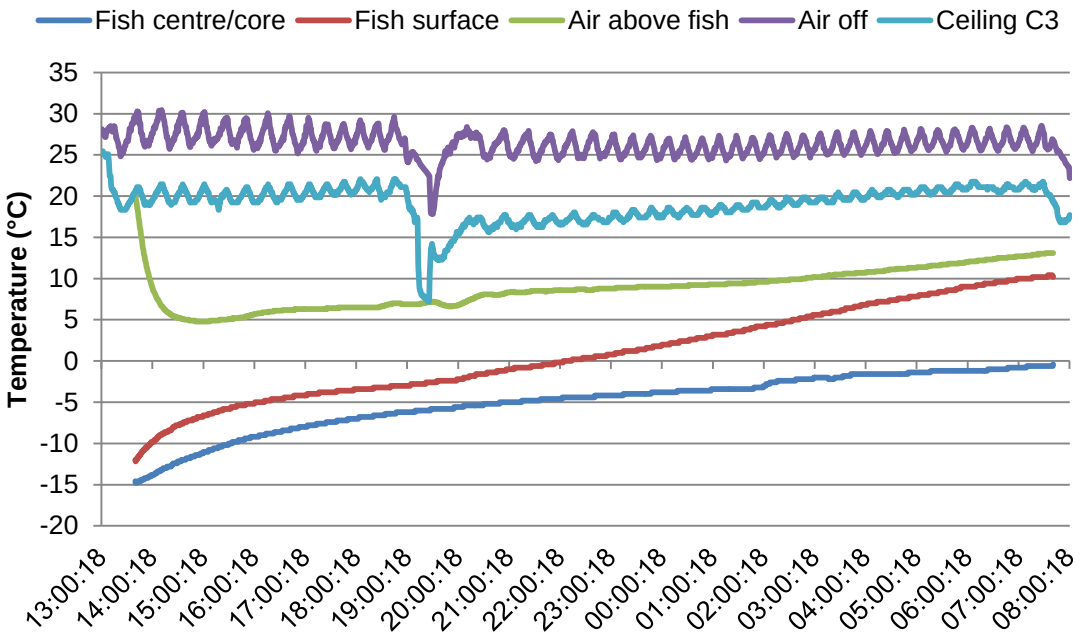

Figure 36. Temperatures during thawing of a frozen block of whole mackerel, located on the middle shelf of the rack

17.1.3.3 Airflow in the thawing room

The heater/humidifier unit used by the company directed the out-flowing air (air off) equally through two vents (left and right) in order to establish circulation within the room. However, the presence of racks and pallets within the room altered the circulation to the point at which air movement around some products was reduced to

zero, this would reduce the surface heat transfer and extend thawing times in these areas (Table 29).

Table 29. Air speed measurement within the defrost room *

Location	Air speed (m/s)		
	10 cm from ceiling	Mid height	10 cm from floor
1	1.02	0	0
2	0.5	0	0
3	0.5	0	0

*Air speed on to heater/humidifier: 5.15 m/s; air speed off left duct: 2.3 m/s; air speed off right duct: 2.5 m/s

17.1.3.4 Product surface temperatures during subsequent (wet-side) processing

The data (Table 30) indicated that whilst the product leaving the defrost room was below 2.2°C, all fish temperatures were above 6°C and some temperatures approached 11°C during subsequent processing.

Table 30. Product surface temperatures during subsequent processing

Process step		Product	Surface temperatures (°C)	Mean surface temperature (°C)
9	Post defrost	Whole mackerel from defrost room	0.5 to 2.2	1.63
11	Post defrost	Whole mackerel from chill store	6.7 to 8.6	7.58
12	Post filleting	Mackerel fillets	10.4 to 10.8	10.48
14	Post Brining	Herring	8.5 to 9.8	9.37

17.1.4 Conclusions

This limited trial looked at one commercial thawing method used at a SP producing smoked fish products. Since thawing is a very slow process at low ambient temperatures, high ambient temperatures of up to 25°C were used by the company to enable more rapid thawing during an overnight process. The thawing room was filled with racked fish blocks during the day (mainly the morning) and thawed overnight, the room being emptied in the morning. The temperatures measured show that this length of time was required, since deep centre temperatures of the fish at the centre of the blocks were still just below 0°C at the end of the process. Whilst centre/core temperatures of fish blocks generally remained low (<1°C) during the thawing process, surface temperatures were a concern and temperatures of up to 11°C were measured in whole fish after thawing. FDA guidance (FDA, 2011) is that previously frozen fish should not be exposed to temperatures above 4.4°C for “more than 12 hours, cumulatively, if any portion of that time is at temperatures above 21°C” or “more than 24 hours, cumulatively, as long as no portion of that time is at temperatures above 21°C”. The process used by this company used air temperatures in its thawing operation above 21°C and for more than 12 hours, however the air temperatures measured around the fish blocks were lower, <20°C (due to poor air circulation within the room). Time constraints unfortunately prevented further study.

17.2 Experimental investigation of chilling and warming rates of Atlantic mackerel and herring under simulated industrial conditions

17.2.1 Introduction

As already mentioned, the industrial survey and literature review revealed a lack of published experimental data on cooling and warming rates for herring and Atlantic mackerel under industrial processing conditions. The rates of temperature change are important to determine the periods of time that products remain at the histamine-forming elevated temperatures. A short series of practical trials were conducted to determine example cooling and warming rates for herring and Atlantic mackerel under a range of conditions.

17.2.2 Materials and methods

Whole Atlantic mackerel and herring were sourced from a local fish merchant. All fish were stored in ice in a chill room prior to use. The dimensions (length, width and depth at maximum cross-section, distance of maximum cross-section from nose) and weight of each fish was recorded. A summary of the dimensions and weighs of the experimental samples is given in Table 31.

Table 31. Summary of weights and dimensions of herring and Atlantic mackerel used in experimental trials

		Weight (g)	Length (mm)	Max width (mm)	Max thickness (mm)	Volume (mm^3) $x10^3$
Herring	Max	326	293	70	41	193
	Min	133	228	48	21	76
	Mean	258.5	256.6	62.3	32.2	137
	SD	53.5	18.6	5.5	4.5	34.5
Atlantic mackerel	Max	408	355	66	47	249
	Min	222	269	48	32	120
	Mean	318.9	310.2	55.4	40.1	183
	SD	48.1	23.4	4.1	3.6	32.6

Single point metal shrouded T-type (copper-constantan) probes (1.2 mm diameter, 100 mm length; RS Components Ltd, UK) were placed into each fish, one at approximate geometric centre of the fish at the centre of the maximum cross-section to measure the centre/core (slowest responding) temperature and in some cases a second just under the skin of the fish. A third T-type (copper-constantan) wire thermocouple was attached to the outside of the fish to measure the ambient conditions. Temperatures were recorded using a Comark Diligence EV2014 data logger (Comark Instruments, Norwich, UK) programmed to record the temperature every minute.

17.2.2.1 Cooling conditions used for Atlantic mackerel and herring

Replicate trials using whole fish were performed under conditions representing the main industrial cooling methods (air, ice, and immersion in ice and water) to provide indicative cooling time-temperature histories for the histamine formation modelling.

The instrumented fish were first placed into a water-bath running at 16±1°C to equalise the entire fish to the desired experimental starting temperature before cooling. Once equalised the fish were cooled using one of the following cooling methods:

1. Cooling fish in air (2°C). Instrumented fish were placed on a plastic tray inside an industrial chill room operating at 2°C±1˚C.
2. Cooling fish with ice. A thin layer of pebble ice was placed in a standard fish box with drainage holes and instrumented fish laid on top. Further layers of pebble ice were added until fish were completely covered. The boxes were monitored throughout the experimental period and more ice added to prevent fish becoming exposed.
3. Cooling fish with ice and water. A non-draining fish box was filled with pebble ice, and water added to completely fill the box. Instrumented fish were placed below the surface of the water/ice mix. Both non-agitated and agitated trials were carried out. Agitation was provided using compressed air.

Temperatures of all fish were monitored until centre/core temperatures were within 1°C of the cooling media temperature.

17.2.2.2 Warming conditions used for Atlantic mackerel and herring

A short series of trials were carried out replicate poor practice and/or warming behaviour to provide indicative data for histamine formation modelling.

The trials were carried out to replicate the warming up of previously chilled fish in either a processing environment (8±1°C under static or high air movement conditions), or an unrefrigerated processing hall (17±1°C).

The instrumented fish were first placed in fish boxes under ice in a chill room (2±1°C) to equalise the entire fish to the desired experimental starting temperature before warming. Once equalised the fish were warmed using one of the following cooling methods:

1. Warming fish in forced or static ambient air at 8±1°C. Instrumented fish were placed on a wire rack in a temperature controlled processing room. The static air samples were shielded to reduce airflow, whilst a fan was used to blow the room ambient air over the forced air samples at a mean velocity of 2.4 m/s.
2. Warming fish in static ambient air at 17±1°C. Instrumented fish were placed on a wire rack in a temperature controlled processing room.

Temperatures of all the fish were monitored until centre/core temperatures were within 1°C of the warming media temperature.

17.2.3 Results and discussion

17.2.3.1 Cooling of Atlantic mackerel and herring

Example cooling curves obtained from herring and mackerel under a range of conditions are shown in Figures 37 to 43**Error! Reference source not found.**. ean experimental cooling times for Atlantic mackerel and herring are given in Table 32. A range of times were seen for each method due to variation in fish sizes. As would be expected, Atlantic mackerel being larger and heavier than herring took longer to cool for all methods. Immersion cooling in an agitated mixture of ice and water was the most rapid method followed by cooling with ice and was relatively unaffected by ambient temperature of the environment in which the cooling takes place. Cooling in an unagitated mixture of ice and water however was slower and more variable (as shown in Figure 41), due to temperature stratification that occurs in the mixture due to the difference in water density at 4°C. Water is denser at 4°C

thus sinks to the bottom of the container which results in colder temperatures (around 0°C) at the surface of the mixture and a temperature of 4°C in the water below.

Table 32. Mean experimentally measured cooling times (centre temperature of individual whole fish from 16°C to <4°C) for Atlantic mackerel and herring cooled under different cooling regimes

Cooling Method	N =	Mean cooling time of core (centre) to <4°C, in minutes (SD)	
		Atlantic mackerel	Herring
Air at 2°C	4	61.6 (1.7)	48 (3.4)
Ice	4	43 (4.1)	21 (6.3)
Ice and water (non-agitated)	4	63 (19.5)	34 (13.5)
Ice and water (agitated)	4	30 (3.5)	-

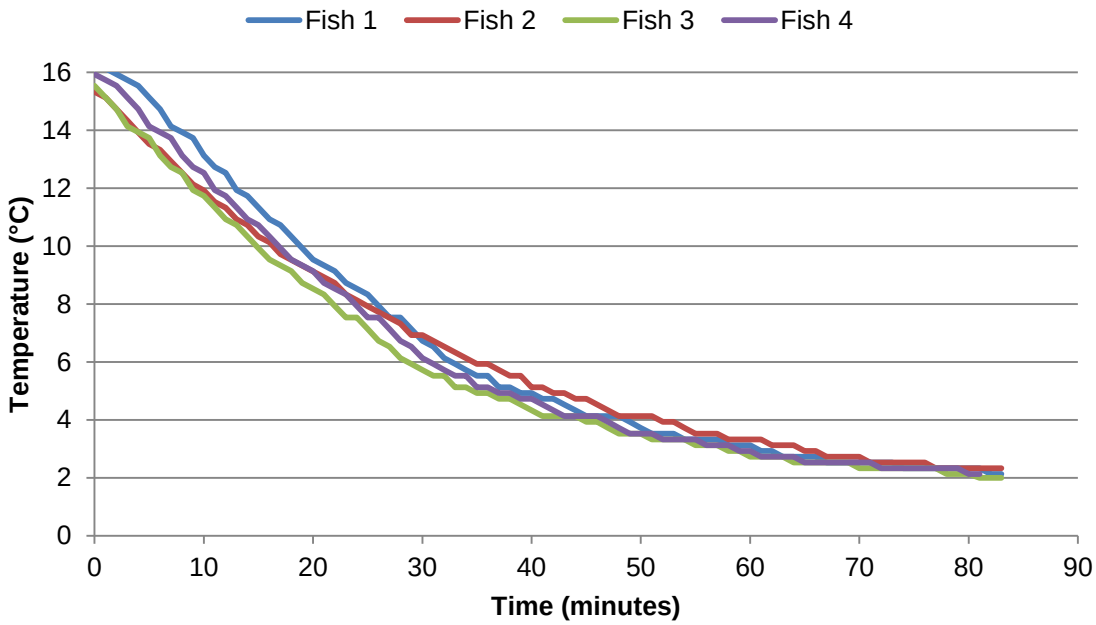

Figure 37. Experimental time-temperature histories measured at the thermal centre of 4 herrings cooled in air cooling (2°C ±1˚C)

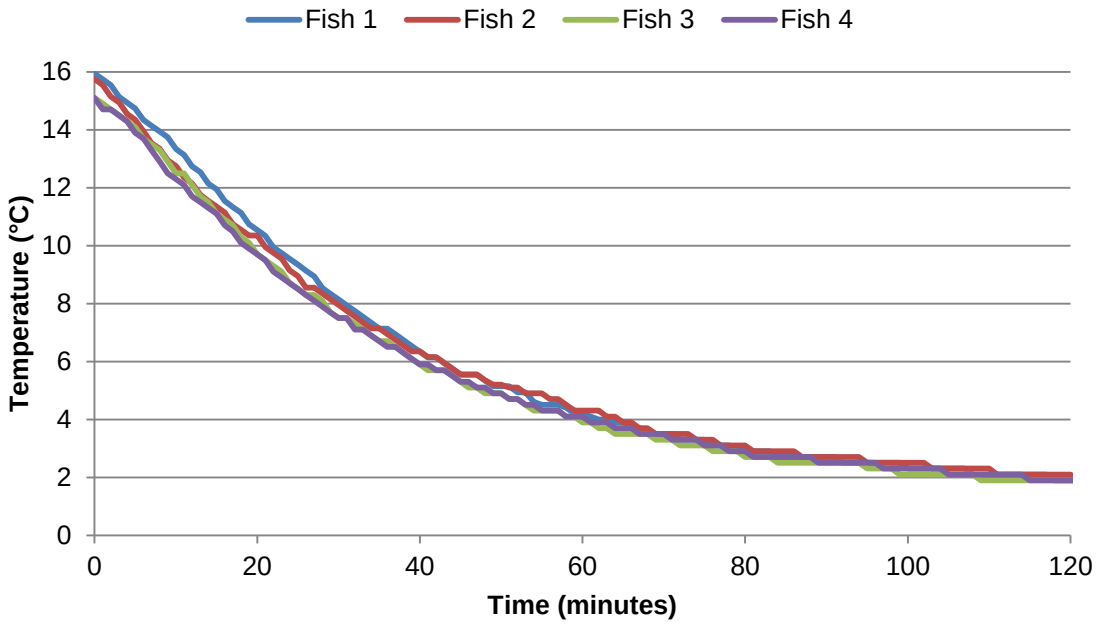

Figure 38. Experimental time-temperature histories measured at the thermal centre of 4 Atlantic mackerel cooled in air cooling (2°C ±1˚C)

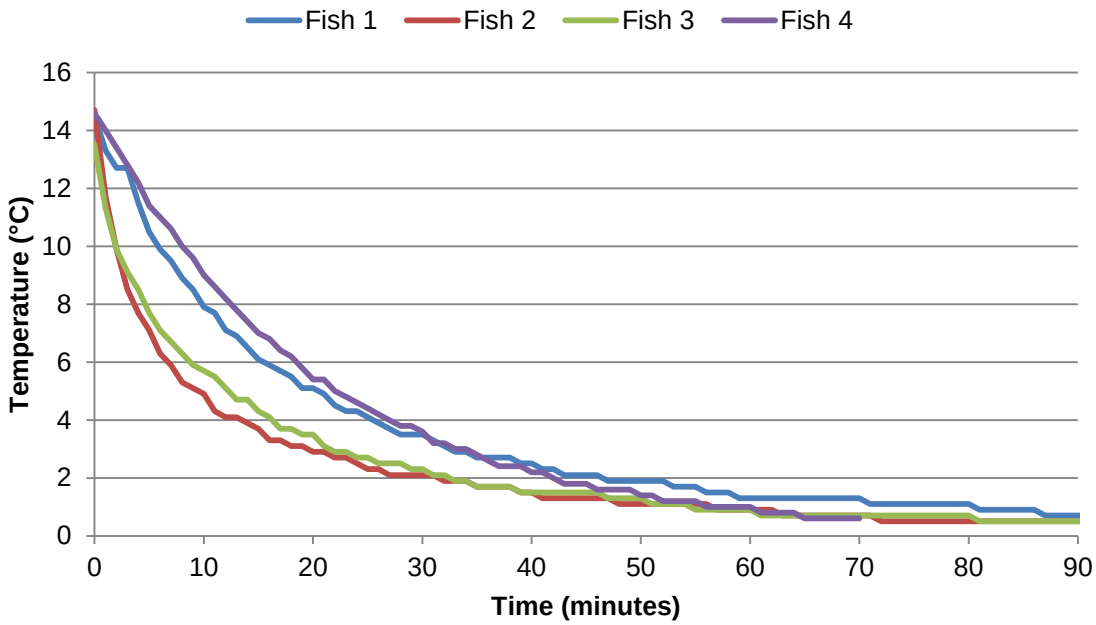

Figure 39. Experimental time-temperature histories measured at the thermal centre of 4 herrings cooled in ice (≈0°C)

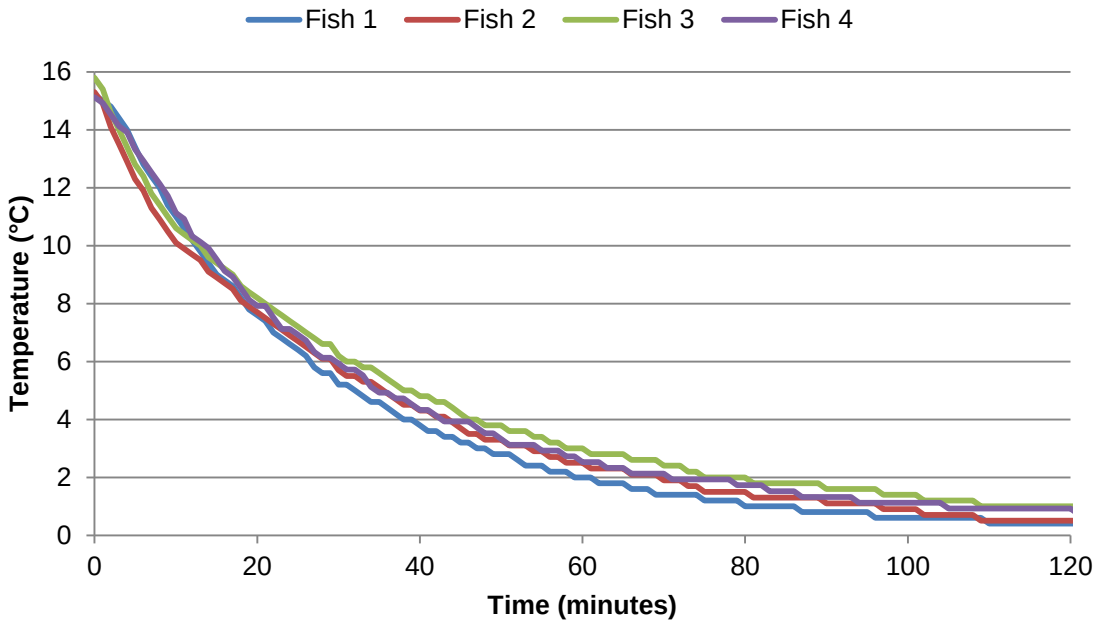

Figure 40. Experimental time-temperature histories measured at the thermal centre of 4 Atlantic mackerel cooled in ice (≈0°C)

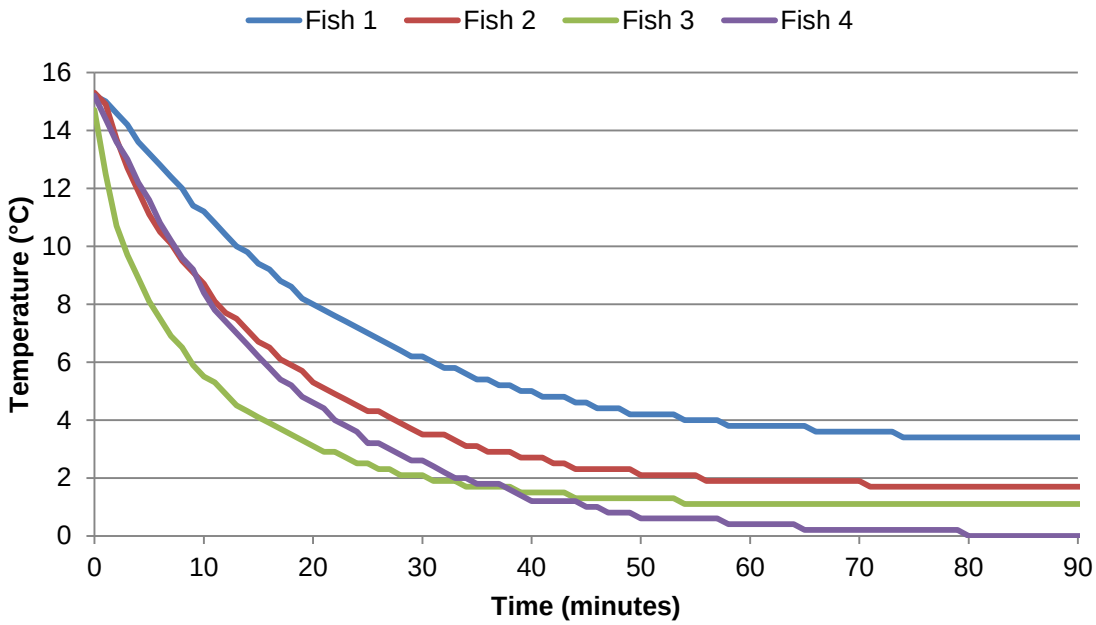

Figure 41. Experimental time-temperature histories measured at the thermal centre of 4 herrings cooled using non-agitated ice and water (≈0 to 4°C)

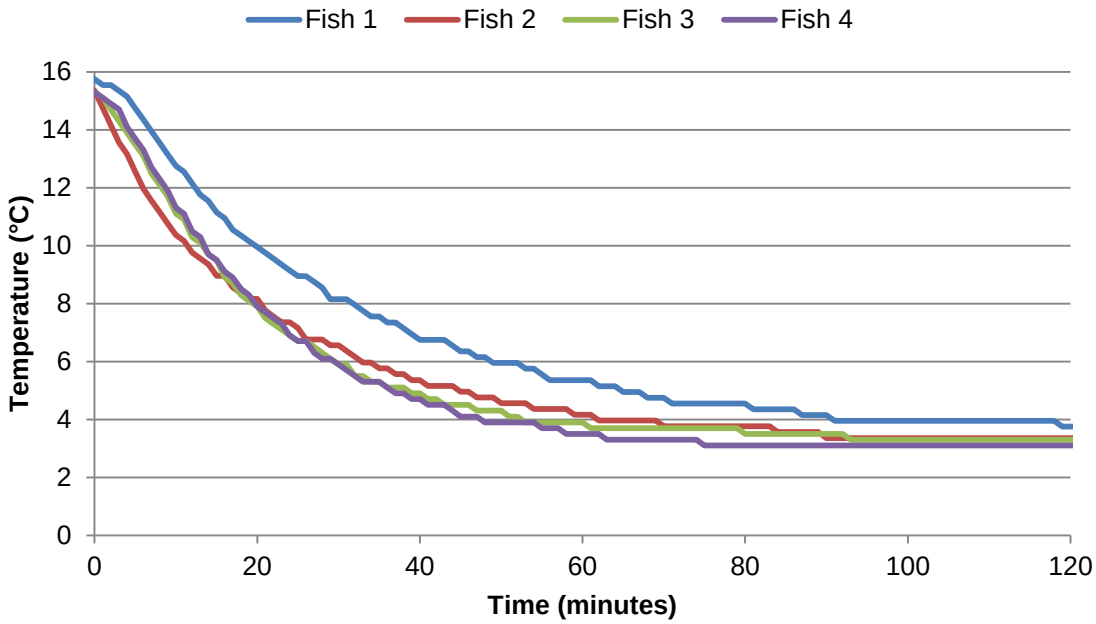

Figure 42. Experimental time-temperature histories measured at the thermal centre of 4 Atlantic mackerel cooled using non-agitated ice and water (≈0 to 4°C)

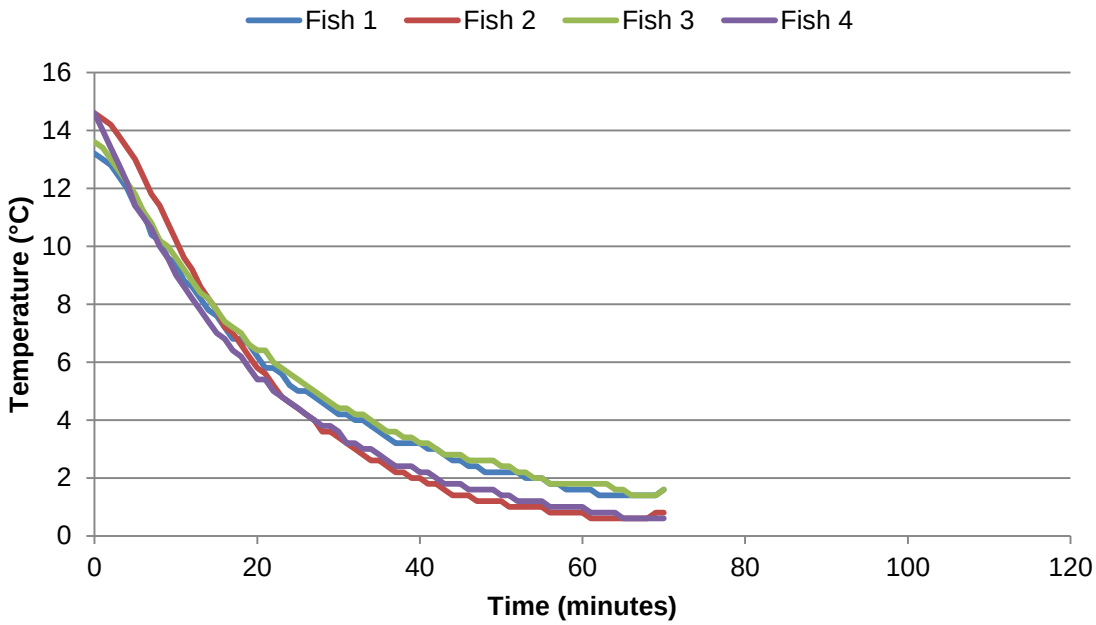

Figure 43. Experimental time-temperature histories measured at the thermal centre of 4 Atlantic mackerel cooled using agitated ice and water (≈0°C)

17.2.3.2 Warming of Atlantic mackerel and herring

Experimental warming temperature histories obtained from Atlantic mackerel and herring under a range of conditions are shown in Figures 44 to 47. Mean experimental warming times to >4°C for Atlantic mackerel and herring are given in Table 33. FDA guidance (FDA, 2011) is that once chilled fish should not be above 4.4°C for "more than 8 hours, cumulatively, as long as no portion of that time is at

temperatures above 21°C". These results show that within a processing environment, where the ambient air should be expected to be refrigerated, any exposed uniced fish can warm above 4°C in less than 11 minutes if exposed to high air velocities. If the fish is not exposed to high air velocities then warming will take on average 22 to 24 minutes in herrings and approximately 30 minutes in mackerel. In an unrefrigerated environment (17°C) fish will warm much faster (even under static air conditions) and centre/core temperatures exceed 4°C in approximately 15 minutes.

Table 33. Mean experimentally measured warming times (to >4°C) for Atlantic mackerel and herring warmed under different warming regimes

Cooling Method	N =	Mean warming time from 0°C to >4°C in minutes (SD)			
		Atlantic mackerel		Herring	
		Surface	Centre	Surface	Centre
Static air at 8±°C	2	29.5 (2.1)	31.1 (3.0)	21.5 (2.1)	24.0 (5.7)
Forced air at 8±1°C	2	2.3 (1.8)	15.1 (0.1)	11.4 (1.9)	11.9 (1.2)
Static air at 17±1°C	8	-	14.8 (2.0)	-	15.3 (2.7)

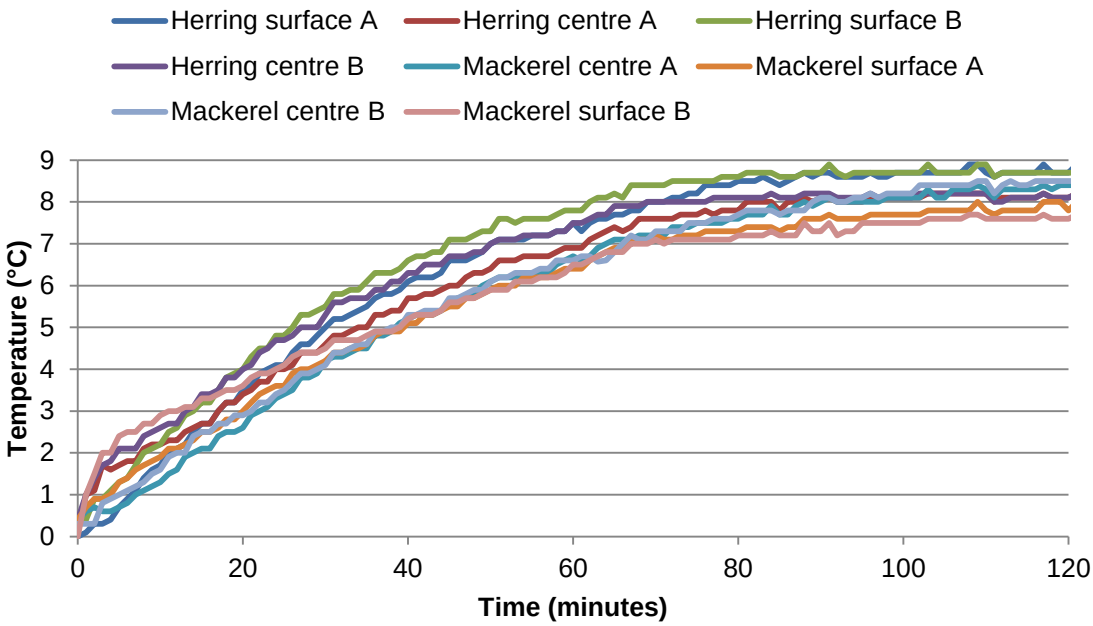

Figure 44. Example experimental time-temperature curves (n=2) for warming of chilled herring and Atlantic mackerel (fish thermal centre and surface temperatures) in static air at 8±1°C

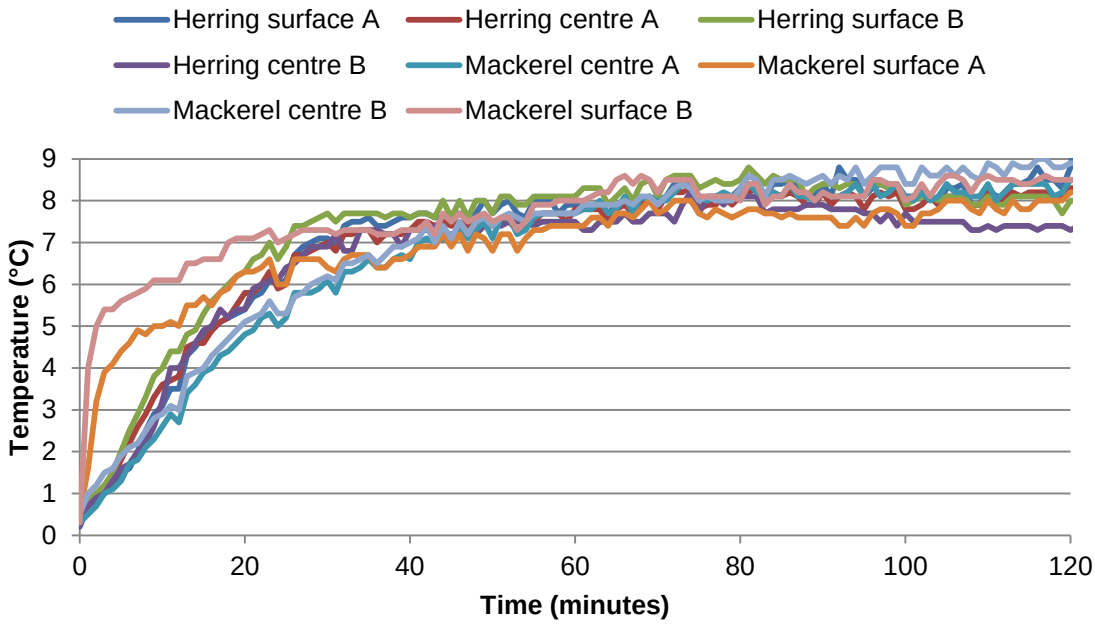

Figure 45. Example experimental time-temperature curves (n=2) for warming of chilled herring and Atlantic mackerel (fish thermal centre and surface temperatures) in forced air at 8±1°C

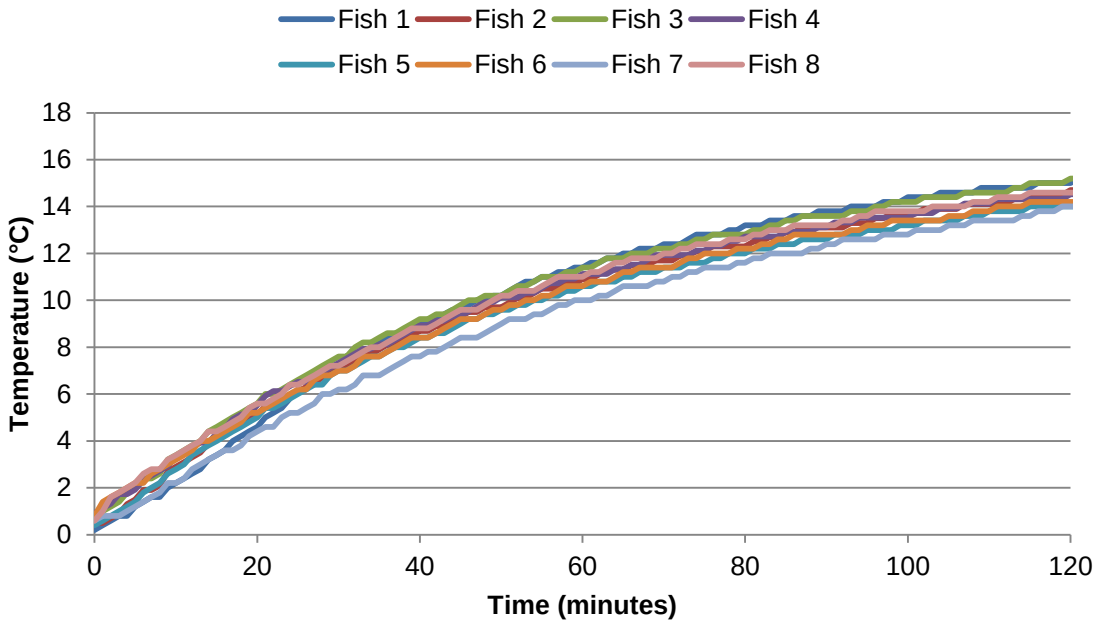

Figure 46. Example experimental time-temperature curves (n=8) for warming of chilled Atlantic mackerel (fish thermal centre temperatures) in static air at 17±1°C

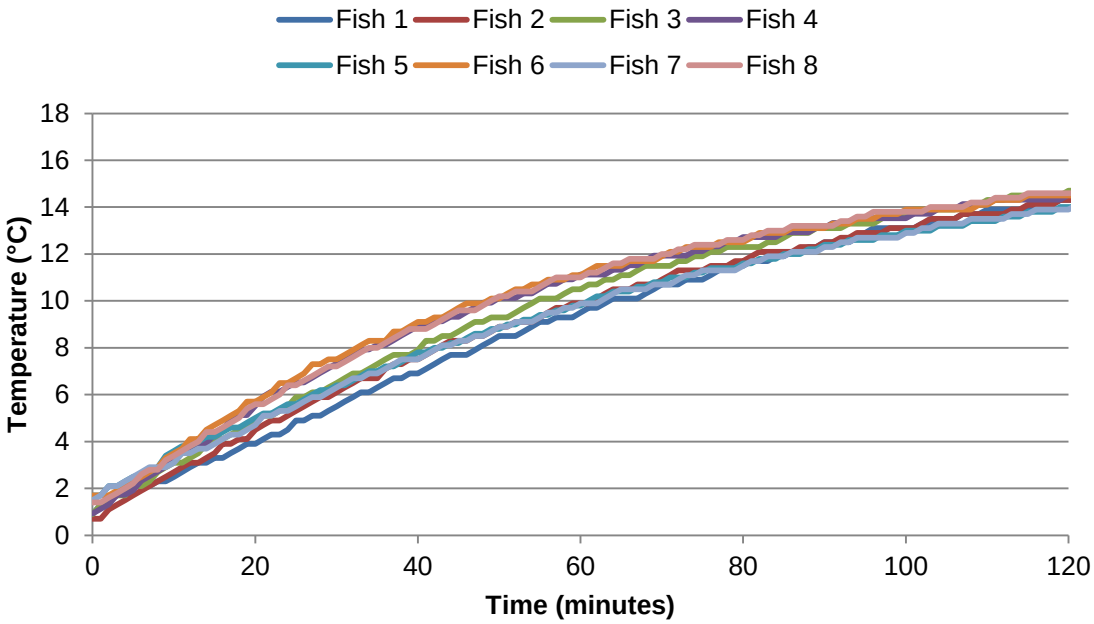

Figure 47. Example experimental time-temperature curves (n=8) for warming of chilled herring (fish thermal centre temperatures) in static air at 17±1°C

17.2.4 Conclusions

As already mentioned, FDA guidance (FDA, 2011) is that fish should be chilled "as soon as possible after harvest, but not more than 9 hours from the time of death", once chilled fish should not be above 4.4°C for "more than 8 hours, cumulatively, as long as no portion of that time is at temperatures above 21°C".

There are a number of processing steps in the cold chain from harvest to fork where fish temperatures will or can exceed 4.4°C.

At the point of harvest the fish will be at the temperature of the sea it was caught in. This can be as high as 17°C plus in in-shore fisheries in mid summer. The studies carried out have shown that if the initial primary cooling stage is carried out in air or unagitated ice/water then cooling times to 4°C will be approximately 60 minutes in mackerel and less with herrings.

Much of the fish of interest is frozen whole for distribution and it is very unlikely that fish temperatures will rise above 4°C during freezing and frozen distribution. After distribution the fish are thawed before further processing. During the thawing operation the surface of fish at exposed positions can spend the last 6 hours of the thawing process at temperatures above 4°C. However, most of the fish will still be well below 4°C at the end of the thawing process.

After thawing of frozen raw material and during processing of chilled raw material temperature rises are likely during operations such as filleting and brining and during any period spent in an environment above 4°C. At the commercial operation fish temperatures ranging from 7.5°C to 10.5°C were measured in these stages of processing. The experimental warming studies showed that in unrefrigerated air the core of fish would rise to aver 4°C in approximately 15 minutes. In a refrigerated forced air situation a similar rate of warming was measured. In a well controlled

operation processing times should be measured in minutes rather than hours so the time spent above 4°C is unlikely to exceed, or even approach, the recommended limit.

Retail display and domestic handling are two areas of potential concern where there is a lack of clear data. Fish displayed on ice is unlikely to rise above 4°C unless the top surface of the fish is fully exposed and subjected to high levels of radiant heat gain from lighting. From surveys of domestic handling and storage we know that the average domestic refrigerator temperature in the UK is approximately 6.2°C (James *et al*., 2008). This is a cause of concern if fish is held for many days at this temperature.

18. Appendix 10: Histamine modelling

18.1 Introduction

After capture, Atlantic mackerel and herring enter the supply chain where they undergo a series of time-temperature conditions before consumption. These time-temperature histories can vary widely dependent of the type of fishery, handling practices, seasonal temperatures, cooling methods used, any temperature abuse conditions, etc. As time-temperature is a key parameter in histamine formation, the impacts on ultimate histamine levels at consumption of a range of possible supply chain time-temperature histories was assessed through modelling.

18.2 Materials and methods

18.2.1 The SSSP model

The Seafood Spoilage and Safety Predictor (SSSP) v.3.1 model was used to predict the expected increase in histamine during a range of hypothetical time-temperature scenarios representing different fishery and supply chain practices.

The SSSP modelling software was developed by the National Institute of Aquatic Resources (DTU Aqua) at the Technical University of Denmark (DTU) and is available as freeware at http://sssp.dtuaqua.dk/. The model is primarily designed to predict histamine formation during storage and makes an assumption that product temperatures can change instantaneously. This is not a true reflection of the physics of heat transfer as heat needs to be conducted throughout a body for all of that body to attain the outside temperature. To modify the model for temperature transients, temperature change curves were incorporated into the model by using small time steps of gradually changing temperatures to attempt to simulate the true temperature experience of mackerel and herring throughout the catch to consumption chain. Whilst more accurately reflecting the true temperatures experienced by the histamine triggering organisms, the method is cumbersome and uses the model in a way that may give unexpected results. An update to the SSSP model that accommodates transient temperatures would aid further and fuller investigation of controls for histamine formation.

The SSSP program considers *M. morganii* and *M. psychrotolerans* growth and the subsequent catalysation of histamine formation. At higher temperatures mesophilic *M. morganii* are responsible for the majority of histamine formation whereas at lower temperatures growth and histamine formation by the psychrotolerant bacterium *M. psychrotolerans* is more important. The SSSP model embodies the studies of Emborg & Dalgaard (2008) and defines the growth rate models for *M. morganii* and *M. psychrotolerans* as:

$$\sqrt{\mu_{max}} = 0.039 \times (T - 2.8) \times (1 - e^{(0.301 \times (T - 44.7))})$$

and

$$\sqrt{\mu_{max}} = 0.023 \times (T - (-5.9)) \times (1 - e^{(0.450 \times (T - 33.1))})$$

respectively where

μ_{max} = maximum growth rate

T = temperature (°C)

Inspection of the model formulae show that zero growth rates will be achieved below T = 2.8°C and above T= 44.7°C for *M. morganii*, and below T = -5.9°C and above T= 33.1°C for *M. psychrotolerans*. Thus, histamine formation triggered by *M. morganii* could be negated if the fish could be maintained at the temperature of melting ice (0°C), however *M. psychrotolerans* could contribute to histamine formation at these temperatures.

18.2.2 Derivation of baseline time-temperature scenarios

A number of temperature scenarios, reflecting typical fisheries and supply chains were postulated as a basis for comparison modelling. These were based on the findings of the literature review (Section 9, Appendix 1), surveys (Sections 14 and 15, Appendices 6 and 7) and experimental work (Section 17, Appendix 9) carried out as part of this project. These are given in Table 34 for catch to processing and Table 35 for processing to consumption. The Type 1 scenario represents large boats with RSW tanks and integrated landings, and Type 2 represents small boats using iced fish in boxes and incorporating a market stage at landing. Type 3 is a nominal refrigerated (packaged) product distribution chain and Type 4 represents a fish counter sales with distribution at the temperature of melting ice. Scenarios were compiled for herring and Atlantic mackerel for summer and winter fishing using nominal seasonal mean temperatures (Table 36).

Table 34. Basic capture to processing temperature scenarios used for histamine modelling

	Catch to end processing Type 1 (major operators)	Catch to end processing Type 2 (small boat fisheries)
In net	4 h @ seawater temp	2 h @ seawater temp
Aboard boat	24 h in RSW @ -1°C	6 h under ice @ 0°C
Wholesale/market	none	12 h under ice @ 0°C
Reception store	12 h under ice @ 0°C	
Processing & packing	2 h air @ 12°C	

Table 35. Basic processing to domestic storage temperature scenarios used for histamine modelling

	Chilled distribution Type 3 (retail display cabinet)	Under ice distribution Type 4 (counter sales)
Transport to retailer	12 h air @ 2°C	12 h under ice @ 0°C
Retailer display	5 days air @ 4°C	5 days under ice @ 0°C
Transport to household	1 h air @ air temp	
Domestic storage	2 days air @ 6°C	

Table 36. Seasonal temperatures used in temperature scenarios (Sources: Met Office, 2012; Baxter *et al*., 2011)

	Temperature (°C)	
	Air	Seawater
Summer	20	18
Winter	6	4

The entire mass of a fish cannot change temperature instantaneously when moved into a different ambient temperature. The surface will be affected first and

depending on whether the fish is being heated or cooled the heat will flow into or out from the deeper regions. Thus, during cooling processes the surface will cool quickly, but the centre of the fish will take longer to attain the cooling medium temperature. Similarly, when warming, the surface will heat quicker than the centre/core. Thus, even when iced the centre of the fish will start at its live temperature (i.e. that of the surrounding seawater) and be reduced towards 0°C. During this time, the histamine-forming processes could be taking place. As already described (Section 17, Appendix 9) a series of practical experimental trials were carried out to determine these cooling and warming time-temperature histories in order to predict the effects of these changes on histamine formation using the model. The experimental time-temperature histories were used to calculate expected temperature change curves under different ambient conditions and/or starting temperatures using a Newtonian cooling/warming model.

These product time-temperature histories were used to enter a 'worst part' of the fish (i.e. the part that stays warmest longest and thus more likely to trigger histamine formation) into the temperature scenarios. The 'worst part' of a fish during a cooling process is the centre/core as it is slowest to cool, and the 'worst part' for a warming process is the fish surface as it is faster to warm. Histamine formation is a function of time and temperature; a maximum base line scenario (Summer Type 1 + Type 3) and minimum base line scenario (Winter Type 2 + Type 4) from capture to consumption were compiled. These baseline scenarios incorporated the 'worst case' true temperature curves from experimental results.

18.2.3 Model conditions

The temperature scenarios were entered into the SSSP model assuming initial concentrations of histidine of 2200 mg/kg for Atlantic mackerel and 220 mg/kg for herring (Klausen & Lund, 1986), initial concentrations of histamine 0 mg/kg (Mackie *et al*., 1997), and initial concentrations of *M. morganii* and *M. psychrotolerans* of 10 CFU/g (SSSP default).

18.3 Results

18.3.1 Baseline modelling for correctly operated supply chains

The final histamine levels as predicted by the SSSP model are given in Table 37, and graphical results for the maximum and minimum baselines are presented in Figure 48 to Figure 51.

Table 37. Final total histamine levels (mg/kg) predicted by the SSSP model for baseline temperature scenarios

	Final total histamine levels (mg/kg) predicted by the SSP model	
	Herring	Atlantic mackerel
Maximum Baseline	7.09	6.77
Minimum Baseline	0.03	0.03

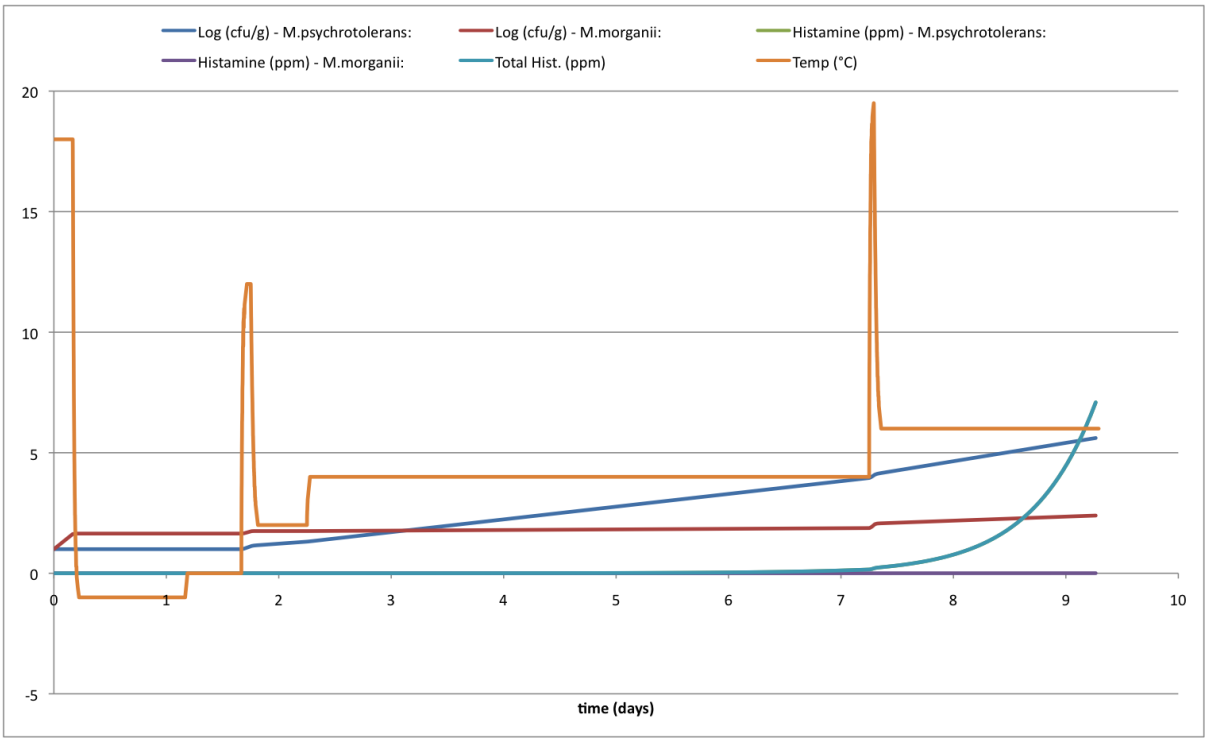

Figure 48. SSSP model of histamine formation for maximum (summer catch with chilled distribution) herring baseline temperature scenario

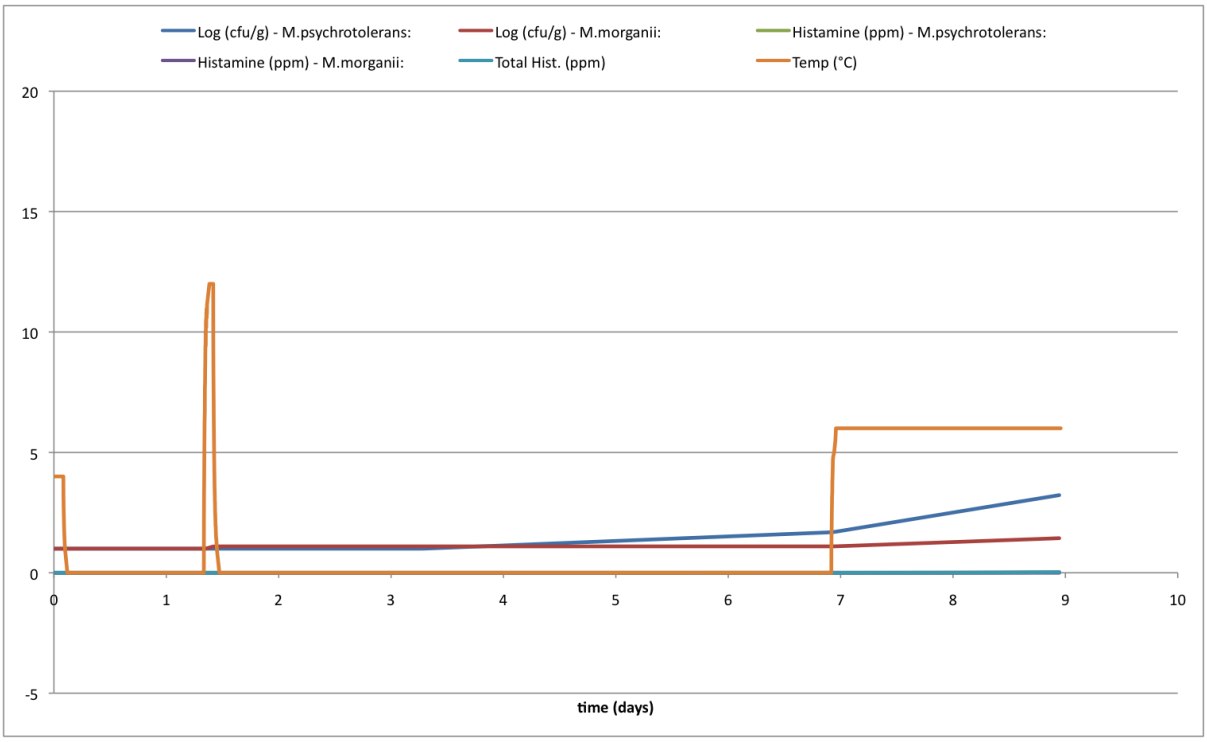

Figure 49. SSSP model of histamine formation for minimum (winter catch with under-ice distribution) herring baseline temperature scenario

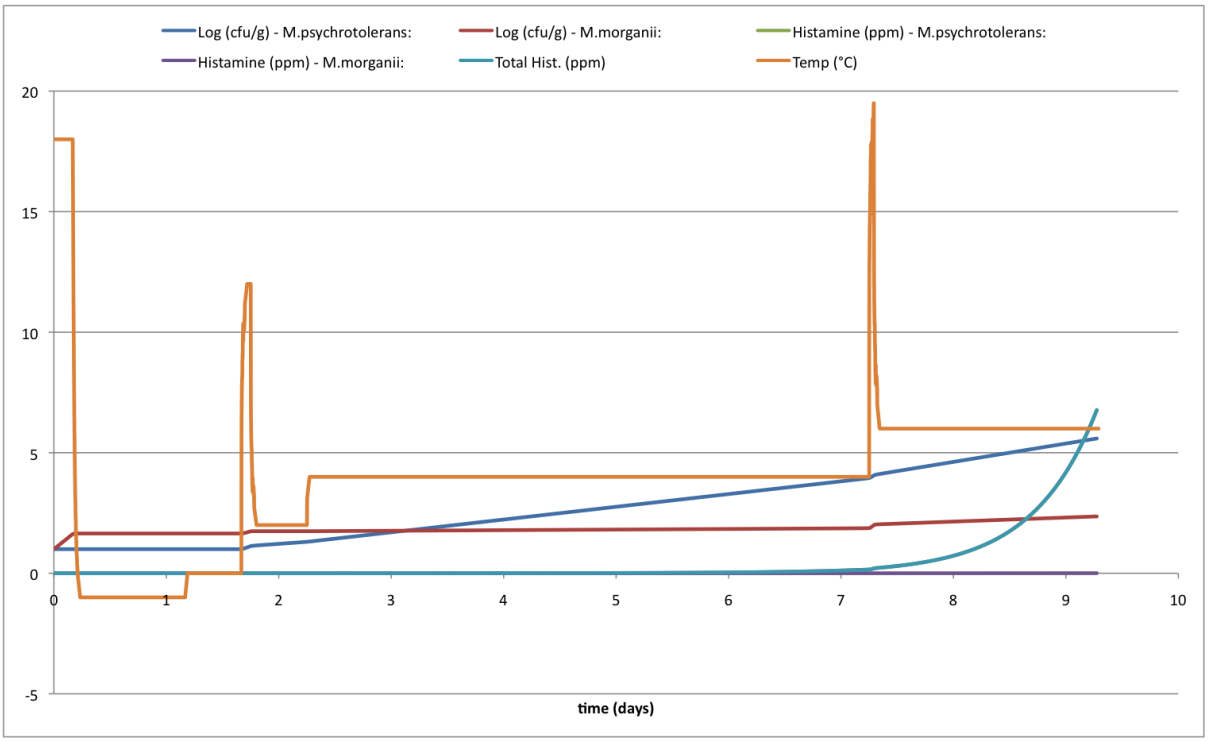

Figure 50. SSSP model of histamine formation for maximum (summer catch with chilled distribution) Atlantic mackerel baseline temperature scenario

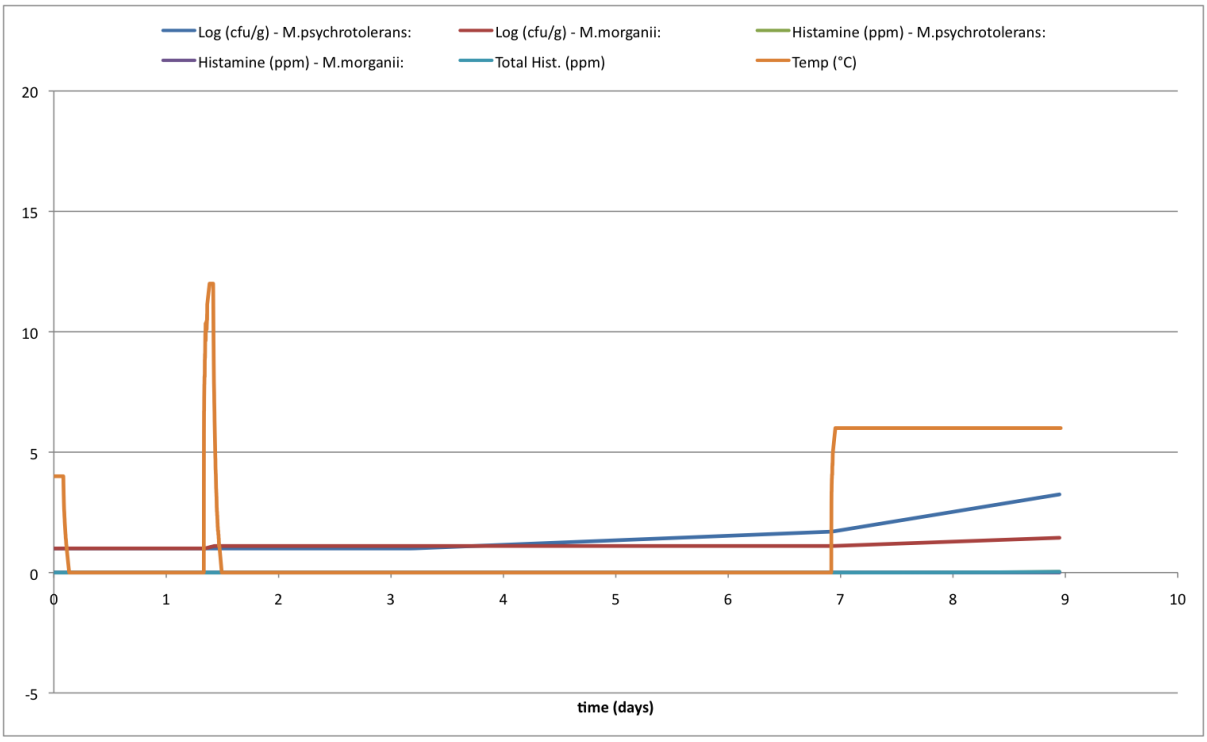

Figure 51. SSSP model of histamine formation for minimum (winter catch with under-ice distribution) Atlantic mackerel baseline temperature scenario

The model output for maximum time temperature scenario (summer catch with chilled distribution) as shown in Figure 48 and Figure 50 indicates histamine begins to become apparent after 7 days and final levels reach approximately 7 mg/kg.

These levels are well below the 50 mg/kg threshold. The model results using the minimum baseline scenario (winter catch with under-ice distribution) as shown in Figure 49 and Figure 51 indicate negligible (0.03 mg/kg) histamine formation.

Additional scenarios were modelled for the higher risk summer months representing the combinations of basic capture (large or small boat), and distribution (chilled or under-ice). The final histamine levels as predicted by the SSSP model are given in Table 38.

Table 38. Final total histamine levels (mg/kg) predicted by the SSSP model for summer temperature scenarios

Capture	Distribution	Final total histamine levels (mg/kg) predicted by the SSSP model	
		Herring	Atlantic mackerel
Large Boat (Type 1)	Chilled (Type 3)	6.9	6.9
Small Boat (Type 2)	Chilled (Type 3)	3.9	3.9
Large Boat (Type 1)	Under-ice (Type 4)	0.12	0.12
Small Boat (Type 2)	Under-ice (Type 4)	0.07	0.07

The results of this initial modelling suggested that there is no substantial risk of histamine formation in most normal capture to consumption supply chains provided that the temperature control measures operate correctly.

18.3.2 Anomaly modelling for poorly operated supply chains or accidental temperature abuse conditions

The model outputs from the previous section indicate that provided the temperature control chain performs correctly, histamine formation is limited. However, many deviations from the ideal are possible, and this section assesses the impact of some of the more likely perturbations.

18.3.2.1 Effects of period in sea post mortem and RSW tank duration for large boat (Type 1) fisheries

Fish that have been captured but not yet brought on board to be chilled will remain at seawater temperature for some time. This period of elevated temperature will have greatest effect in the summer months when mean seawater temperatures around Scotland can be up to c.18°C (Baxter *et al*., 2011). From the industrial survey it was found that large boats may hold fish in RSW tanks for up to 3 days. These effects were modelled for their effect on the final histamine levels based on the maximum baseline temperature scenario (Type 3 distribution).

Table 39. Final total histamine levels (mg/kg) predicted by the SSSP model for herring with Maximum Baseline Temperature Scenario

Period in sea at 18°C before chilling on board (h)	Duration in RSW tanks at -1°C (h)		
	24	48	72
0	2.1	2.9	3.8
4	6.9	9.3	12.4
8	22.2	29.8	39.8
12	70.2	93.4	123.8

Table 40. Final total histamine levels (mg/kg) predicted by the SSSP model for Atlantic mackerel with Maximum Baseline Temperature Scenario

Period in sea at 18°C before chilling on board (h)	Duration in RSW tanks at -1°C (h)		
	24	48	72
0	2.1	2.9	3.8
4	6.9	9.3	12.4
8	22.2	29.8	39.8
12	70.2	93.4	123.8

It can be seen that there is no substantial differences between herring (Table 39) and mackerel (Table 40) predictions despite a 10-fold difference in initial starting concentrations of histidine. Provided the RSW chilling starts within 8 hours of capture, and the remainder of the chill chain functions correctly, final levels even after a 3-day period in the RSW tanks are predicted to be less than the 50 mg/kg threshold. However, with longer in-sea periods, the histamine levels are higher and more likely to contribute to risk. This suggests that fish should be removed from the sea into on-board chilling as soon as possible to minimise histamine fish poisoning risk.

18.3.3 Effects of icing regime for small boat (Type 2) fisheries

Small boat fisheries chill fish after capture using ice in boxes. If not regularly re-iced, this ice will melt over time, exposing fish and allowing them to warm to the temperature of the ambient air. Taking a mean summer high air temperatures around Scotland of 20°C (Met Office, 2013), the effects of re-icing to various degrees was assessed in the SSSP model (Table 41).

Table 41. Final total histamine levels (mg/kg) predicted by the SSSP model with different icing regimes and under-ice (Type 4) distribution in summer

Time (h)		Final total histamine levels (mg/kg)	
Under Ice (0°C)	Exposed to ambient air (20°C)	Herring	Atlantic mackerel
6	0	0.07	0.06
3	3	0.18	0.18
0	6	0.5	0.5

Despite a 10-fold difference in starting concentrations of histidine used in the model there was a negligible difference between final levels of histamine predicted by the model in herring and Atlantic mackerel (more investigation into the mechanics of the SSSP model is required to identify the reason for this).

This modelling shows that the SSSP model does not predict that a short period of temperature abuse (6 hours at 20°C) at catching, as may occur with fish caught by in-shore boats during the summer months, has a appreciable effect on final histamine levels at the point of consumption provided that the fish is subsequently stored at a low temperatures throughout the rest of the supply chain. Provided this is the case and temperatures are kept low through the remainder of the supply chain then according to the model the small amount of histamine that does form does not reach a level that poses a risk. This finding is not inconsistent with regulatory and guidance advice that stresses the importance of rapid chilling after capture. Specific FDA guidance (FDA, 2011) is that at ambient temperatures of less than 28°C chilling

should take place “not more than 12 hours from the time of death”. The 6 hours used in this modelling is half of this maximum time. Nethertheless it is the authors opinions that boats should always be advised to chill directly after catch, and take sufficient ice, or provide sufficient refrigeration, in order to achieve this.

18.3.4 Effects of retail display on ice

In the retail store, some fish can be displayed on ice in serve-over iced counters. Whilst fish surfaces touching and under the ice will be close to 0°C, the exposed upper surfaces will be in contact with, and warmed by, the ambient air in the store. Further warming can be caused by radiant heating of the surfaces, especially from lighting sources in the cabinets and within the stores. Experimental measurements by the authors (unpublished commercial trials) show that these surfaces can reach c.9-10°C.

Although fish at the retailer will usually be stored fully under ice overnight, during the day they can spend up to 12 hours a day on a bed of ice on the retail display counter. These elevated temperatures (up to 10°C) whilst on display could contribute to histamine formation. This temperature scenario of 12 hours at 0°C then 12 hours at 10°C for each day of retail display was incorporated into a Type 4 distribution and modelled for Type 1 and Type 2 fisheries (Table 42).

Table 42. Final total histamine levels (mg/kg) predicted by the SSSP model incorporating effects of warming of exposed fish surface on iced counter display

	Final total histamine levels (mg/kg) predicted by the SSSP model	
Capture	Herring	Atlantic mackerel
Large Boat (Type 1)	94.4	94.4
Small Boat (Type 2)	54.9	54.9

The model results showed that in-store display on ice can potentially contribute to histamine formation, raising levels in both species to above the 50 mg/kg threshold by the end of the supply chain. Despite a 10-fold difference in starting concentrations of histidine used in the model there was no difference seen between the histamine levels predicted by the model for herring and Atlantic mackerel. These model results suggest that fish on display need to be regularly re-positioned and re-iced to reduce this risk caused by exposed surface warming in store.

18.3.5 Effects of domestic storage temperatures on histamine formation

The baseline temperature scenarios assume good domestic storage practices to maintain 6°C, however it has been shown that typical domestic storage temperatures can be up to 10°C (James *et al*., 2008). The effects of this on histamine formation were determined (Table 43).

Table 43. Final total histamine levels (mg/kg) predicted by the SSSP model for summer temperature scenarios with domestic storage at 10°C

Capture	Distribution	Final total histamine levels (mg/kg) predicted by the SSSP model for domestic storage at 10°C	
		Herring	Atlantic mackerel
Large boat (Type 1)	Chilled (Type 3)	102.6	102.6
Small boat (Type 2)	Chilled (Type 3)	59.6	59.6
Large boat (Type 1)	Under-ice (Type 4)	1.9	1.9
Small boat (Type 2)	Under-ice (Type 4)	1.1	1.1

As before, these results show no difference between herring and mackerel despite a 10-fold difference in starting concentrations of histidine. The modelling showed that provided the distribution chain runs at 0°C with fish being maintained at the temperature of melting ice (Type 4) there is little effect of domestic storage at 10°C. However, for a warmer distribution chain (Type 3), the SSSP model indicates the higher temperature domestic storage may raise histamine levels above the 50 mg/kg warning threshold. This modelling result suggests that consumers should be made aware of the potential effect of poor domestic storage on histamine formation risk.

18.4 Freezing and thawing effects on histamine formation

The industrial survey showed that much of the herring and Atlantic mackerel catch is frozen and processed at a later time. This in itself is not a control measure as once formed histamine cannot be destroyed. Additionally the fish is typically subjected to elevated temperatures during thawing. Experimental measurements made at processors recorded surface temperatures c.15°C for c.11 hours, followed by c.10°C for c.6 hours.

It was hoped to model the effects of the thawing process on histamine formation using the SSSP model but unknown anomalies in the model prevented useful outputs from being obtained. The underlying code of the SSSP model will need to be addressed before modelling of the freeze thaw process is possible.

It is conceivable that the elevated temperatures seen during thawing could potentially contribute to histamine formation risk, but modelling of this is not possible with the current SSSP model. Improvements to the current model were not within the scope of this risk management review study.

19. Appendix 11: Recommended controls

Most recommended controls for controlling histamine formation are based on those in Chapter 7 of the US FDA *Fish and Fishery Products Hazards and Control Guidance* (FDA, 2011). This recommends the following on-board controls:

"*Rapid chilling of scombrotoxin-forming fish immediately after death is the most important element in any strategy for preventing the formation of scombrotoxin (histamine), especially for fish that are exposed to warm waters or air, and for tunas which generate heat in their tissues. Some recommendations follow:*

- *Fish exposed to air or water temperatures above 83°F (28.3°C) should be placed in ice, or in refrigerated seawater, ice slurry, or brine of 40°F (4.4°C) or less, as soon as possible after harvest, but not more than 6 hours from the time of death; or*
- *Fish exposed to air and water temperatures of 83°F (28.3°C) or less should be placed in ice, or in refrigerated seawater, ice slurry, or brine of 40°F (4.4°C) or less, as soon as possible after harvest, but not more than 9 hours from the time of death; or*
- *Fish that are gilled and gutted before chilling should be placed in ice, or in refrigerated seawater, ice slurry, or brine of 40°F (4.4°C) or less, as soon as possible after harvest, but not more than 12 hours from the time of death; or*
- *Fish that are harvested under conditions that expose dead fish to harvest waters of 65°F (18.3°C) or less for 24 hours or less should be placed in ice, or in refrigerated seawater, ice slurry, or brine of 40°F (4.4°C) or less, as soon as possible after harvest, but not more than the time limits listed above, with the time period starting when the fish leave the 65°F (18.3°C) or less environment.*

Note: If the actual time of death is not known, an estimated time of the first fish death in the set may be used (e.g., the time the deployment of a longline begins)."

It recommends the following processing controls:

- "*Scombrotoxin-forming fish that have not been previously frozen or heat processed sufficiently to destroy scombrotoxin-forming bacteria should not be exposed to temperatures above 40°F (4.4°C) for:*
    - *More than 4 hours, cumulatively, if any portion of that time is at temperatures above 70°F (21.1°C); or*
    - *More than 8 hours, cumulatively, as long as no portion of that time is at temperatures above 70°F (21.1°C).*
- *Scombrotoxin-forming fish that have been previously frozen, or heat processed sufficiently to destroy scombrotoxin-forming bacteria and are subsequently handled in a manner in which there is an opportunity for recontamination with scombrotoxin- forming bacteria (e.g., contact with fresh fish, employees, or introduction of raw ingredients), should not be exposed to temperatures above 40°F (4.4°C) for:*
    - *More than 12 hours, cumulatively, if any portion of that time is at temperatures above 70°F (21.1°C); or*

    - *More than 24 hours, cumulatively, as long as no portion of that time is at temperatures above 70°F (21.1°C).*
- *Scombrotoxin-forming fish that have been heat processed sufficiently to destroy scombrotoxin-forming bacteria and enzymes and are not subsequently handled in a manner in which there is an opportunity for recontamination with scombrotoxin-forming bacteria (e.g., no contact with fresh fish, employees, or raw ingredients) are at low risk for further scombrotoxin (histamine) development.*"

20. References

Ababouch, L., Souibri, L., Rhaliby, K., Ouahdi, O., Battal, M. & Busta, F. F. (1996) Quality changes in sardines (*Sardina pilchardus*) stored in ice and at ambient temperature. *Food Microbiology*. Vol. 13, pp123-132

Abe, H., Dobson, G. P., Hoeger, U. & Parkhouse, W. S. (1985) Role of histidine-related compounds to intracellular buffering in fish skeletal muscle. *The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*. Vol. 249:4, ppR449-R454.

Adkin, A., Hartnett, E., Jordan, L., Newell, D. & Davison, H. (2006) Use of a systematic review to assist the development of campylobacter control strategies in broilers. *Journal of Applied Microbiology*. Vol. 100, pp306-315.

Al-Bulushi, I., Poole, S., Deeth, H. C. & Dykes, G. A. (2009) Biogenic amines in fish: roles in intoxication, spoilage, and nitrosamine formation - a review. *Critical Reviews in Food Science & Nutrition*. Vol. 49:4, pp369-377.

Alak, G., Hisar, S. A., Hisar, O. & Genccelep, H. (2011) Biogenic amines formation in Atlantic bonito (*Sarda sarda*) fillets packaged with modified atmosphere and vacuum, wrapped in chitosan and cling film at 4°C. *European Food Research and Technology*. Vol. 232:1, pp23-28.

Allen, D. G., Green, D. P., Bolton, G. E., Jaykus, L. A. & Cope, W. G. (2005) Detection and identification of histamine-producing bacteria associated with harvesting and processing mahimahi and yellowfin tuna. *Journal of Food Protection.* Vol. 68:8, pp1676-1682.

Anonymous (1997) Scombrotoxin food poisoning. *SCIEH Weekly Report*. Vol. 31, No. 97/16, pp77-79.

Antoine, F. R., Wei, C. I., Littell, R. C. & Marshall, M. R. (1999) HPLC method for analysis of free amino acids in fish using o-phthaldialdehyde precolumn derivatization. *Journal of Agricultural and Food Chemistry*. Vol. 47:12, pp5100-5107.

Antoine, F. R., Wei, C. I., Littell, R. C., Quinn, B. P., Hogle, A. D. & Marshall, M. R. (2001) Free amino acids in dark- and white-muscle fish as determined by o-phthaldialdehyde precolumn derivatization. *Journal of Food Science*. Vol. 66:1, pp72-77.

Antoine, F. R., Wei, C. I., Otwell, W. S., Sims, C. A., Littell, R. C., Hogle, A. D. & Marshall, M. R. (2004) Chemical analysis and sensory evaluation of mahi-mahi (*coryphaena hippurus*) during chilled storage. *Journal of Food Protection*. Vol. 67:10, pp2255-2262.

Archer, M., Edmonds, M. & George, M. (2008) *Seafood thawing*. Seafish report SR598, The Sea Fish Industry Authority.

Attaran, R. R. & Probst, F. (2002) Histamine fish poisoning: a common but frequently misdiagnosed condition. *Emergency Medicine Journal.* Vol. 19, pp474-475.

Aubourg, S. P., Quitral, V., Larraín, M. A., Rodríguez, A., Gómez, J., Maier, L. & Vinagre, J. (2007) Autolytic degradation and microbiological activity in farmed Coho salmon (*Oncorhynchus kisutch*) during chilled storage. *Food Chemistry*. Vol. 369-375.

Auerswald, L., Morren, C. & Lopata, A. L. (2006) Histamine levels in seventeen species of fresh and processed South African seafood. *Food Chemistry*. Vol. 98:2, pp231-239.

Aytac, S. A., Ozbas, Z. Y. & Vural, H. (2000) Effects of irradiation, antimicrobial agents and modified-atmosphere packaging on histamine production by *Morganella morganii* in mackerel fillets. *Archiv fuer Lebensmittelhygiene.* Vol. 51:1, pp28-30.

Bakar, J., Yassoralipour, A., Abu Bakar, F. & Rahman, R. A. (2010) Biogenic amine changes in barramundi (*lates calcarifer*) slices stored at 0°C and 4°C. *Food Chemistry*. Vol. 119:2, pp467-470.

Bartholomew, B. A., Berry, P. R., Rodhouse, J. C., Gilbert, R. J. & Murray, C. K. (1987) Scombrotoxic fish poisoning in Britain: features of over 250 suspected incidents from 1976 to 1986. *Epidemiology & Infection*. Vol. 99, pp775-782.

Bartholomew, B. A., Berry, P. R., Rodhouse, J. C., Gilbert, R. J. & Murray, C. K. (1987) Scombrotoxic fish poisoning in Britain - features of over 250 suspected incidents from 1976 to 1986. *Epidemiology and Infection*. Vol. 99:3, pp775-782.

Baxter, J. M., Boyd, I. L., Cox, M., Donald, A. E., Malcolm, S. J., Miles, H.,Miller, B., Moffat, C. F., (2011) *Scotland's Marine Atlas: Information for the national marine plan*. Marine Scotland, Edinburgh. pp. 191

Baygar, T. & Gokoglu, N. (2004) Determination of the changes in food composition and quality of tuna (*K. pelamis*) during canning process. *Gida*. Vol. 29:2, pp149-157.

Ben-Gigirey, B., Craven, C. & An, H. J. (1998) Histamine formation in albacore muscle analyzed by aoac and enzymatic methods. *Journal of Food Science*. Vol. 63:2, pp210-214.

Bermejo, A., Mondaca, M. A., Roeckel, M. & Marti, M. C. (2004) Bacterial formation of histamine in jack mackerel (*Trachurus symmetricus*). *Journal of Food Processing and Preservation*. Vol. 28:3, pp201–222.

Bermejo, A., Mondaca, M. A., Roeckel, M., Marti, M. C. (2003) Growth and characterization of the histamine-forming bacteria of jack mackerel (*Trachurus symmetricus*). *Journal of Food Processing and Preservation.* Vol. 26:6, pp401-414.

Beutling, D. M., Peconek, J. & Stan-Lotter, H. (2009) *Chromohalobacter beijerinckii*: A psychrophilic, extremely halotolerant and enzymatically active microbe from salted food with the capacity for biogenic amine production. *European Food Research and Technology*. Vol. 229:5, pp725-730.

Bolton, G. E., Bjornsdottir, K., Nielsen, D., Luna, P. F. & Green, D. P. (2009) Effect of high hydrostatic pressure on histamine forming bacteria in yellowfin tuna and mahi-mahi skinless portions. *Institute of Food Technologists (IFT) Conference 2009*. USA. Abstract no. 006-05. (Cited by Naila *et al*., 2010)

Bremer, P. J., Osborne, C. M., Kemp, R. A., Veghel, P. & Fletcher, G. C. (1998) Thermal death times of Hafnia alvei cells in a model suspension and in artificially contaminated hot-smoked kahawai (*Arripis trutta*). *Journal of Food Protection*. Vol. 61:8, pp1047-1051.

Brillantes, S. & Samosorn, W. (2001) Determination of histamine in fish sauce from Thailand using a solid phase extraction and high-performance liquid chromatography. *Fisheries Science*. Vol. 67:6, pp1163-1168.

Brillantes, S., Paknoi, S. & Totakien, A. (2002) Histamine formation in fish sauce production. *Journal of Food Science*. Vol. 67:6, pp2090-2094.

Cha, Y. J., Park, S. Y., Cho, W. J., Lee, Y. M., Kim, H. & Kim, J. S. (2001) Storage stability of seasoned-dried Pacific saury (imported product) treated with liquid smoke. *Journal of Food Science & Nutrition.* Vol. 6:4, pp235-239. (cited by Flectcher, 2010)

Chang, S.-C., Kung, H.-F., Chen, H.-C., Lin, C.-S. & Tsai, Y.-H. (2008) Determination of histamine and bacterial isolation in swordfish fillets (*Xiphias gladius*) implicated in a food borne poisoning. *Food Control*. Vol. 19, pp16–21.

Chang, S.-C., Lin, C.-S., Chen, Y.-L. Lee, Y.-H., & Tsai, Y.-H. (2010) Inhibition effect of chitosan on histamine-producing bacteria and its application in marlin flesh preservation. *Taiwanese Journal of Agricultural Chemistry and Food Science*. Vol. 48:1, pp46-54.

Chaouqy, N. E. & El Marrakchi, A. (2005) Chemical and bacteriological aspect of anchovy (Engraulis encrasicholus) stored in ice and at ambient temperature (20-25°C). *Revue de Médecine Vétérinaire*. Vol. 156:6, pp341-349.

Chen, H. C., Lee, Y. C., Hwang, D. F., Chiou, T. K. & Tsai, Y. H. (2011) Determination of histamine in mahi-mahi fillets (*coryphaena hippurus*) implicated in a foodborne poisoning. *Journal of Food Safety*. Vol. 31:3, pp320-325.

Chen, H. C., Lee, Y. C., Lin, C. M., Hwang, D. F. & Tsai, Y. H. (2010) Determination of histamine and bacterial isolation in marlin fillets (*Makaira nigricans*) implicated in a foodborne poisoning. *Journal of Food Safety*. Vol. 30:3, pp699-710.

Chen, H.-C., Kung, H.-F., Chen, W.-C., Lin, W.-F., Hwang, D.-F., Lee, Y.-C. & Tsai, Y.-H. (2008) Determination of histamine and histamine-forming bacteria in tuna dumpling implicated in a food-borne poisoning. *Food Chemistry*. Vol. 106:2, pp612-618.

Chotimarkorn, C. (2011) Quality changes of anchovy (*Stolephorus heterolobus*) under refrigerated storage of different practical industrial methods in Thailand. *Journal of Food Science & Technology.* DOI 10.1007/s13197-011-0505-y. vol. pages.

Clark, R. F., Williams, S. R., Nordt, S. P. & Manoguerra, A. S. (1999) A review of selected seafood poisonings. *Undersea and Hyperbaric Medicine*. Vol. 26:3, pp175-184.

Dalgaard, P., Emborg, J., Kjølby, A., Sørensen, N. D. & Ballin, N. Z. (2008) Histamine and biogenic amines: formation and importance in seafood. In: Børresen, T. (Ed.), *Improving Seafood Products for the Consumer*. Woodhead Publishing Ltd, Cambridge.

Dalgaard, P., Madsen, H. L., Samieian, N. & Emborg, J. (2006) Biogenic amine formation and microbial spoilage in chilled garfish (*Belone belone belone*) - effect of modified atmosphere packaging and previous frozen storage. *Journal of Applied Microbiology*. Vol. 101:1, pp80-95.

de la Hoz, L., López-Gálvez, D. E., Fernández, M., Hierro, E. & Ordóñez, J. A. (2000) Use of carbon dioxide enriched atmospheres in the refrigerated storage (2°C) of salmon (*Salmo salar*) steaks. *European Food Research and Technology*. Vol. 210:3, pp179-188.

DEFRA (2010) Charting Progress 2 Report. Retrieved 2012 24-February from DEFRA: http://chartingprogress.defra.gov.uk/feeder/Section_3.2_Temperature_and_Salinity.pdf

DEFRA (2011) DEFRA-Overseas Trade. Retrieved 2012 10-May from DEFRA: http://www.defra.gov.uk/statistics/foodfarm/food/overseastrade/.

Du, W. X., Huang, T. S., Kim, J., Marshall, M. R. & Wei, C. I. (2001) Chemical, microbiological, and aromascan evaluation of mahi-mahi fillets under various storage conditions. *Journal of Agricultural and Food Chemistry*. Vol. 49:1, pp527-534.

Du, W. X., Lin, C. M., Phu, A. T., Cornell, J. A., Marshall, M. R. & Wei, C. I. (2002) Development of biogenic amines in yellowfin tuna (*Thunnus albacares*): effect of storage and correlation with decarboxylase-positive bacterial flora. *Journal of Food Science.* Vol. 67:1, pp292-301.

Economou, V., Brett, M. M., Papadopoulou, C., Frillingos, S. & Nichols, T. (2007) Changes in histamine and microbiological analyses in fresh and frozen tuna muscle during temperature abuse. *Food Additives & Contaminants*. Vol. 24:8, pp820-832.

Edmunds, W. J. & Eitenmiller, R. R. (1975) Effect of storage time and temperature on histamine content and histidine decarboxylase activity of aquatic species. *Journal of Food Science*. Vol. 40, pp516-519. (cited by Emborg, 2008)

EFSA (2010) Revision of the joint AFC/BIOHAZ guidance document on the submission of data for the evaluation of the safety and efficacy of substances for the removal of microbial surface contamination of foods of animal origin intended for human consumption. *EFSA Journal*. Vol. 8:4, 1544 [32pp.]

Emborg, J. (2008) *Gaps in available information on biogenic amines in seafood*. SEAFOODplus Publication Series, Report 3.4.1 – 2008, ISBN 978-87-7075-004-2.

Emborg, J. & Dalgaard, P. (2006) Formation of histamine and biogenic amines in cold-smoked tuna: an investigation of psychrotolerant bacteria from samples implicated in cases of histamine fish poisoning. *Journal of Food Protection*. Vol. 69:4, pp897-906.

Emborg, J. & Dalgaard, P. (2008a) Modelling the effect of temperature, carbon dioxide, water activity, and pH on growth and histamine formation by *Morganella psychrotolerans*. *International Journal of Food Microbiology*. Vol. 128:2, pp226-233.

Emborg, J. & Dalgaard, P. (2008b) Growth, inactivation and histamine formation of *Morganella psychrotolerans* and *Morganella morganii* – development and evaluation of predictive models. *International Journal of Food Microbiology*. Vol. 128:2, pp234-243.

Emborg, J., Dalgaard, P. & Ahrens, P. (2006) *Morganella psychrotolerans* sp. nov., a histamine-producing bacterium isolated from various seafoods. *International Journal of Systematic & Evolutionary Microbiology*. Vol. 56:10, pp2473-2479.

Emborg, J., Laursen, B. G. & Dalgaard, P. (2005) Significant histamine formation in tuna (*Thunnus albacares*) at 2°C - effect of vacuum- and modified atmosphere-packaging on psychrotolerant bacteria. *International Journal of Food Microbiology.* Vol. 101:3, pp263-279.

Emborg, J., Laursen, B. G., Rathjen, T. & Dalgaard, P. (2002) Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere-packed salmon (*Salmo salar*) at 2°C. *Journal of Applied Microbiology.* Vol. 92:4, pp790-799.

Enes Dapkevicius, M. L. N., Nout, M. J. R., Rombouts, F. M., Houben, J. H. & Wymenga, W. (2000) Biogenic amine formation and degradation by potential fish silage starter microorganisms. *International Journal of Food Microbiology.* Vol. 57:1,2, pp107-114.

Erkan, N. & Özden, O. (2008) Quality assessment of whole and gutted sardines (*Sardina pilchardus*) stored in ice. *International Journal of Food Science & Technology*. Vol. 43:9, pp1549-1559.

Etienne, M. (2006) *Tracability; Project 6.3 – Valid; Methodology for histamine and biogenic amines analysis.* (http://archimer.ifremer.fr/doc/2006/rapport-6484.pdf)

EU (2004) Regulation (EC) No 853/2004 of The European Parliament and of The Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. *Official Journal of the European Union*.

EU (2005) Regulation (EC) No 2073/2005 of The European Parliament and of The Council of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*.

Evans, J. A., Scarelli, S. & Swain, M. V. L. (2007) Temperature and energy performance of refrigerated retail display and commercial catering cabinets under test conditions. *International Journal of Refrigeration.* Vol. 30, pp398-408.

Fadhlaoui-Zid, K., Curiel, J. A., Landeta, G., Fattouch, S., Reverón, I., de las Rivas, B., Sadok, S. & Muñoz, R. (2012) Biogenic amine production by bacteria isolated from ice-preserved sardine and mackerel. *Food Control*. Vol. 25:1, pp89-95.

FAO-WHO (2012) *Joint FAO/WHO Expert Meeting on the Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products*. 23-27 July 2012, FAO Headquarters, Rome, Italy.

FDA Closure Letter (2011) Retrieved 2012 8-March from FDA: http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/ucm256560.htm

FDA Import Refusal Report (2004) Retrieved 2012 8-March from FDA: http://www.accessdata.fda.gov/scripts/importrefusals/ir_detail.cfm?EntryId=862-0178743-8&DocId=2&LineId=2&SfxId=

FDA Warning Letter (2011) Retrieved 2012 8-March from FDA: http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/2011/ucm253621.htm

Feldman, K. A., Werner, S. B., Cronan, S., Hernandez, M., Horvath, A. R., Lea, C. S., Au, A. M. & Vugia, D. J. (2005) A large outbreak of scombroid fish poisoning associated with eating Escolar fish (*Lepidocybium flavobrunneum*). *Epidemiology & Infection*. Vol. 133:1, pp29-33.

Fernandez-No, I. C., Bohme, K., Calo-Mata, P. & Barros-Velazquez, J. (2011) Characterisation of histamine-producing bacteria from farmed blackspot seabream (*Pagellus bogaraveo*) and turbot (*Psetta maxima*). *International Journal of Food Microbiology*. Vol. 151:2, pp182-9.

Fletcher, G. C. (2010) *Research of relevance to histamine poisoning in New Zealand*. MAF Technical Paper No: 2011/70, ISBN 978-0-478-38709-4 (online).

Fletcher, G. C., Bremer, P. J., Summers, G. & Osborne, C. M. (1998) *Guidelines for the safe preparation of hot-smoked seafood in New Zealand.* Christchurch, New Zealand Institute for Crop & Food Research Limited. (Cited by Fletcher, 2010)

Fonberg-Broczek, M., Sawilska-Rautenstrauch, D., Windyga, B., Sciezynska, H., Jedra, M., Badowski, P. & Urbanek-Karlowska, B. (2003) Histamine and tyramine content depending on microbiological quality of salted herrings stored at different temperatures. *Roczniki Panstwowego Zakladu Higieny*. Vol. 54:1, pp87-95.

Foo, L. Y. (1975) The content of histamine and fish food poisoning. *New Zealand Medical Journal*. Vol. 82, pp381-383. (cited by Fletcher, 2010)

Food and Drug Administration (FDA) (1995) *Decomposition and histamine - raw, frozen tuna and mahi-mahi; canned tuna; and related species*. Compliance Policy Guide 540.525. Washington, Food and Drug Administration.

Food and Drug Administration (FDA) (2011) *Fish and fishery products hazards and controls guidance*. Fourth Edition. Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Food Safety, Washington, DC, USA.

Food Standards Agency (UK) (2012) Approved Premises (Scotland). Retrieved 2012 5-06 from http://www.food.gov.uk/multimedia/pdfs/fishestsscot.pdf

Frank, H. A. & Yoshinaga, D. H. (1987) Table for estimating histamine formation in skipjack tuna, *Katsuwonus pelamis*, at low nonfreezing temperatures. *Marine Fisheries Review*. Vol. 49:4, pp67-74.

Frank, H. A., Yoshinaga, D. H. & Nip, W. K. (1981) Histamine formation and honeycombing during decomposition of skipjack tuna, *Katsuwonus pelamis*, at elevated temperatures. *Marine Fisheries Review.* Vol. 43:10, pp9-14.

Frank, H. A., Yoshinaga, D. H. & Wu, I.-P. (1983) Nomograph for estimating histamine formation in skipjack tuna at elevated temperatures. *Marine Fisheries Review.* Vol. 45, pp4-6.

Gessner, B., Hokama, Y. & Isto, S. (1996) Scombrotoxicosis-like illness following the ingestion of smoked salmon that demonstrated low histamine levels and high toxicity on mouse bioassay. *Clinical Infectious Diseases*. Vol. 23, pp1316-1318.

Gillespie, I., Adak, G. K., O'Brien, S. J., Brett, M. M. & Bolton, F. J. (2001) General outbreaks of infectious intestinal disease associated with fish and shellfish, England and Wales, 1992-1999. *Communicable Disease and Public Health*. Vol. 4:2, pp117-123.

Gingerich, T. M., Lorca, T., Flick, G. J. Jr., McNair, H. M. & Pierson, M. D. (2001) Isolation of histamine-producing bacteria from fish-processing facilities and fishing vessels. *Journal of Aquatic Food Product Technology*. Vol. 10:3, pp61-66.

Gökoğlu, N. (2003) Changes in biogenic amines during maturation of sardine *sardina pilchardus* marinade. *Fisheries Science*. Vol. 69:4, pp823-829.

Gökoğlu, N., Yerlikaya, P. & Cengiz, E. (2003) Biogenic amines formation in sardine marinade during refrigerated storage. *Journal of Food Biochemistry*. Vol. 27:5, pp435-447.

Gökoğlu, N., Yerlikaya, P. & Cengiz, E. (2004) Changes in biogenic amine contents and sensory quality of sardine (*Sardina pilchardus*) stored at 4°C and 20°C. *Journal of Food Quality*. Vol. 27:3, pp221-231.

Gonzaga, V. E., Lescano, A. G., Huaman, A. A., Salmon-Mulanovich, G. & Blazes, D. L. (2009) Histamine levels in fish from markets in Lima, Peru. *Journal of Food Protection*. Vol. 72:5, pp1112-1115.

Graham, J., Johnston, W. A. & Nicholson, F. J. (1992) *Ice in Fisheries*. FAO Fisheries Technical Paper. No. 331. Rome, FAO. 1992. 75p.

Gram, L. & Dalgaard, P. (2002) Fish spoilage bacteria – problems and solutions. *Current Opinion in Biotechnology*. Vol. 13:3, pp262–266.

Gram, L.& Huss, H. H. (1996) Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology*. Vol. 33(1), pp121-137.

Guillén-Velasco, S., Ponce-Alquicira, E., Farres-Gonzalez Saravia, A. & Guerrero-Legarreta, I. (2004) Histamine production by two Enterobacteriaceae strains isolated from tuna (*Thunnus thynnus*) and jack mackerel (*Trachurus murphyi*). *International Journal of Food Properties*. Vol. 7:1, pp91-103.

Guizani, N., Al-Busaidy, M. A., Al-Belushi, I. M., Mothershaw, A. & Rahman, M. S. (2005) The effect of storage temperature on histamine production and the freshness of yellowfin tuna (*Thunnus albacares*). *Food Research International*. Vol. 38:2, pp215-222.

Health Protection Agency (2010) *Health Protection Report*. Vol. 4:32. (http://www.hpa.org.uk/hpr/archives/2010/hpr3210.pdf).

Health Protection Scotland (2012) eWeekly Report. Retrieved 2012 26-February from Health Protection Scotland: http://www.hps.scot.nhs.uk/ewr/subjectsummary.aspx?subjectid=160.

Henderson, L., Gregory, J. & Swan, G. (2002) *The National Diet & nutrition Survey: adults aged 19 to 64 years. Types and quantities of food consumed*. Volume 1. ISBN 0 11 621566 6. (http://food.gov.uk/multimedia/pdfs/ndnsprintedreport.pdf).

Horner, W. F. A. (1997) Preservation of fish by curing (drying, salting and smoking). In: G.M. Hall (Ed.). *Fish Processing Technology*. Second Edition. Blackie Academic and Professional, London: pp32-73.

Hsu, H. H., Chuang, T. C., Lin, H. C., Huang, Y. R., Lin, C. M., Kung, H. F. & Tsai, Y. H. (2009) Histamine content and histamine-forming bacteria in dried Milkfish (*Chanos chanos*) products. *Food Chemistry*. Vol. 114:3, pp933-938.

Hungerford, J. M. (2010) Scombroid poisoning: A review. *Toxicon*. Vol. 56, pp231-243.

Hungerford, J. M. & Wu, W.-H. (2012) Comparison of three rapid test kits for histamine in fish: BiooScientific Max Signal enzymatic assay, Neogen Veratox ELISA, and the Neogen Reveal Histamine screening test. *Food Control*. Vol. 25:2, pp448-457.

James, S. J. & James, C. (2013) Chilling. *Encyclopedia of Food Safety* (FOSA), edited by Y. Motarjemi & L. Gorris. Elsevier. IN PRESS.

James, S. J., Evans, J. E. & James, C. (2008) A review of the performance of domestic refrigerators. *Journal of Food Engineering*. Vol. 87, pp2-10.

Jeya Shakila, R. & Vasundhara, T. S. (2002) Formation of histamine and other biogenic amines during storage of freshwater fish chunks. *Asian Fisheries Science*. Vol. 15:1, pp1-6.

Jeya Shakila, R., Jeyasekaran, G. & Vijayalakshmi, S. K. (2005b) Effect of vacuum packaging on the quality characteristics of seer fish (*Scomberomorus commersonii*) chunks during refrigerated storage. *Journal of Food Science & Technology*. Vol. 42:5, pp438-443. (Cited by Fletcher, 2010)

Jeya Shakila, R., Jeyasekaran, G., Vyla, S. A. P. & Kumar, R. S. (2005a) Effect of delayed processing on changes in histamine and other quality characteristics of 3 commercially canned fishes. *Journal of Food Science*. Vol. 70:1, ppM24-M29.

Jeya Shakila, R., Vasundhara, T. S. & Vijaya Rao, D. (1996) Effect of spices on the biogenic amine formation and other quality characteristics of Indian mackerel during refrigerated storage. *Asian Fisheries Science*. Vol. 9, pp191-199.

Jeya Shakila, R., Vijayalakshmi, K. & Jeyasekaran, G. (2003a) Changes in histamine and volatile amines in six commercially important species of fish of the Thoothukkudi coast of Tamil Nadu, India stored at ambient temperature. *Food Chemistry*. Vol. 82:3, pp347-352.

Jeya Shakila, R., Vijayalakshmi, K. & Jeyasekaran, G. (2003b) Changes in amine forming bacteria and histamine in yellowfin tuna (*Thunnus albacares*) through the smoking process. *Journal of Aquatic Food Product Technology*. Vol. 12:3, pp43-56.

Jeyasekaran, G., Ganesan, P., Maheswari, K., Jeya Shakila, R. & Sukumar, D. (2004) Effect of delayed icing on the microbiological quality of tropical fish: barracudas (*Sphyraena barracuda*). *Journal of Food Science*. Vol. 69:7, ppM197-M200.

Jiang, J. J., Li, H. B., Zeng, Q. X., Sun, G. Y. & Zhu, Z. W. (2010) Effects of temperature and salinity on contents of the precursors of N-nitroso compounds in fish sauce. *Modern Food Science & Technology*. Vol. 26:3, pp234-237.

Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission (2010) *Proposed draft standard for smoked fish, smoke-flavoured fish and smoke-dried fish (at step 5 of the Procedure)*. Report of the thirtieth session of the Codex Committee on Fish and Fishery Products. Rome, Codex Alimentarius Commission. Pp. 81-89.

Jørgensen, L. V., Dalgaard, P. & Huss, H. H. (2000) Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. *Journal of Agricultural and Food Chemistry*. Vol. 48:6, pp2448-2453.

Joshi, P. A. & Bhoir, V. S. (2011) Study of histamine forming bacteria in commercial fish samples of Kalyan City. *International Journal of Current Scientific Research*. Vol. 1:2, pp39-42.

Julshamn, K. (2008) *Quality of cured and smoked salmon and trout generally good*. [Online] Available at: http://www.nifes.no/index.php?page_id=377&article_id=2635&lang_id=2 [Accessed 19 December 2012].

Kan, K., Ushiyama, H., Shindo, T., Uehara, S. & Yasuda, K. (2000) Outbreak of histamine poisoning due to ingestion of fish, 'abura-sokomutsu' (*Lepidocybium flavobrunneum*). *Journal of the Food Hygienic Society of Japan (Shokuhin Eiseigaku Zasshi).* Vol. 41:2, pp116-121.

Kanki, M., Yoda, T., Ishibashi, M. & Tsukamoto, T. (2004) *Photobacterium phosphoreum* caused a histamine fish poisoning incident. *International Journal of Food Microbiology*. Vol. 92:1, pp79-87.

Kanki, M., Yoda, T., Tsukamoto, T. & Baba, E. (2007) Histidine decarboxylases and their role in accumulation of histamine in tuna and dried saury. *Applied and Environmental Microbiology*. Vol. 73:5, pp1467-1473.

Kanki, M., Yoda, T., Tsukamoto, T. & Shibata, T. (2002) *Klebsiella pneumoniae* produces no histamine: *Raoultella planticola* and *Raoultella ornithinolytica* strains are histamine producers. *Applied and Environmental Microbiology*. Vol. 68:7, pp3462-3466.

Kilinc, B. & Cakli, S. (2005) Chemical, enzymatical and textural changes during marination and storage period of sardine (*Sardina pilchardus*) marinades. *European Food Research and Technology*. Vol. 221:6, pp821-827.

Kim, M.-K., Mah, J.-H. & Hwang, H.-J. (2009) Biogenic amine formation and bacterial contribution in fish, squid and shellfish. *Food Chemistry*. Vol. 116:1, pp87-95.

Kim, S. H., An, H. & Price, R. J. (1999) Histamine formation and bacterial spoilage of albacore harvested off the US northwest coast. *Journal of Food Science*. Vol. 64:2, pp340-343.

Kim, S. H., An, H. J., Field, K. G., Wei, C. I., Velazquez, J. B., Ben-Gigirey, B., Morrissey, M. T., Price, R. J. & Pitta, T. P. (2003a) Molecular detection of a histamine former, *Morganella morganii*, in albacore, mackerel, sardine, and a processing plant. *Journal of Food Science*. Vol. 68:2, pp453-457.

Kim, S. H., Barros-Velazquez, J., Ben-Gigrey, B., Eun, J. B., Jun, S. H., Wie, C. I. & An, H. J. (2003b) Identification of the main bacteria contributing to histamine formation in seafood to ensure product safety. *Food Science & Biotechnology*. Vol. 12:4, pp451-460.

Kim, S. H., Ben-Gigirey, B., Barros-Velazquez, J., Price, R. J. & An, H. J. (2000) Histamine and biogenic amine production by *Morganella morganii* isolated from temperature-abused albacore. *Journal of Food Protection*. Vol. 63:2, pp244-251.

Kim, S. H., Eun, J. B., Chen, T. Y., Wei, C. I., Clemens, R. A. & An, H. (2004b) Evaluation of histamine and other biogenic amines and bacterial isolation in canned anchovies recalled by the US FDA. *Journal of Food Science*. Vol. 69:6, ppM157-M162.

Kim, S. H., Field, K. G., Chang, D. S., Wei, C. I. & An, H. J. (2001a) Identification of bacteria crucial to histamine accumulation in Pacific mackerel during storage. *Journal of Food Protection*. Vol. 64:10, pp1556-1564.

Kim, S. H., Field, K. G., Morrissey, M. T., Price, R. J., Wei, C. I. & An, H. J. (2001b) Source and identification of histamaine-producing bacteria from fresh and temperature-abused albacore. *Journal of Food Protection*. Vol. 64:7, pp1035-1044.

Kim, S. H., Price, R. J., Morrissey, M. T., Field, K. G., Wei, C. I. & An, H. (2002b) Occurrence of histamine-forming bacteria in albacore and histamine accumulation in muscle at ambient temperature. *Journal of Food Science*. Vol. 67:4, pp1515-1521.

Kim, S. H., Price, R. J., Morrissey, M. T., Field, K. G., Wei, C. I. & An, H. (2002a) Histamine production by *Morganella morganii* in mackerel, albacore, mahi-mahi, and salmon at various storage temperatures. *Journal of Food Science*. Vol. 67, pp1522-1528.

Kim, S.-H., Wei, C.-I., Clemens, R. A. & Haejung, A. (2004a) Review: Histamine accumulation in seafoods and its control to prevent outbreaks of scombroid poisoning. *Journal of Aquatic Food Product Technology*. Vol. 13:4, pp81-100.

Kimura, B., Konagaya, Y. & Fujii, T. (2001) Histamine formation by *Tetragenococcus muriaticus*, a halophilic lactic acid bacterium isolated from fish sauce. *International Journal of Food Microbiology*. Vol. 70:1, 2, pp71-77.

Klausen, N. K. & Lund, E. (1986) Formation of biogenic-amines in herring and mackerel. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung.* Vol. 182:6, pp459-463.

Kongpun, O. & Suwansakornkul, P. (2000) Histamine formation during salting of Spanish mackerel (*Scomberomorus commersoni*). *Journal of Aquatic Food Product Technology*. Vol. 9:1, pp21- 30.

Koral, S. & Köse, S. (2012) The effect of filleting and ice application on the quality and safety of atlantic bonito (*sarda sarda*) at refrigerated storage. *International Journal of Food Science & Technology*. Vol. 47:1, pp210-220.

Korashy, N. T. & Farag, H. El-S. (2005) Histamine and histamine producing bacteria in some local and imported fish and their public health significance. *Research Journal of Agriculture and Biological Sciences*. Vol. 1:4, pp329-336.

Köse, S. (2010) Evaluation of seafood safety health hazards for traditional fish products: preventive measures and monitoring issues. *Turkish Journal of Fisheries and Aquatic Sciences*. Vol. 10, pp139-160.

Kuda, T. & Miyawaki, M. (2010) Reduction of histamine in fish sauces by rice bran nuka. *Food Control.* Vol. 21:10, pp1322-1326.

Kung, H. F., Chen, L. T., Liao, H. J., Lin, C. S., Liaw, E. T., Chen, W. C. & Tsai, Y. H. (2008) Chemical characterisation and histamine-forming bacteria in salted mullet roe products. *Food Chemistry*. Vol. 110:2, pp480-485.

Kung, H. F., Wang, T. Y., Huang, Y. R., Lin, C. S., Wu, W. S., Lin, C. M. & Tsai, Y. H. (2009) Isolation and identification of histamine-forming bacteria in tuna sandwiches. *Food Control*. Vol. 20:11, pp1013-1017.

Lakshmisha, I. P., Ravishankar, C. N., Ninan, G., Mohan, C. O. & Gopal, T. K. S. (2008) Effect of freezing time on the quality of Indian mackerel (*Rastrelliger kanagurta*) during frozen storage. *Journal of Food Science*. Vol. 73:7, ppS345-S353.

Lampila, L. E. & Tom, P. D. (2009) Scombrotoxin (Histamine) Formation. Chapt 27. *Compendium of fish and fishery product processes, hazards, and controls*. Davis, Sea Grant Extension Program, Food Science & Technology Department, University of California. (http://seafood.ucdavis.edu/haccp/compendium/chapt27.htm)

Lee, H., Kim, S. H., Wei, C. I., Jun, S. H., Eun, J. B. & An, H. (2005) Histamine and other biogenic amines and bacterial isolation in retail canned anchovies. *Journal of Food Science.* Vol. 70:2, ppC145-C150.

Lee, Y.-C., Kung, H.-F., Lin, C.-S., Hwang, C.-C., Lin, C.-M. & Tsai, Y.-H. (2012) Histamine production by *Enterobacter aerogenes* in tuna dumpling stuffing at various storage temperatures. *Food Chemistry*. Vol. 131:2, pp405-412.

Lehane, L. & Olley, J. (1999) *Histamine (Scombroid) Fish Poisoning: a review in a risk-assessment framework*. National Office of Animal and Plant Health, Canberra.

Lehane, L. & Olley, J. (2000) Histamine fish poisoning revisited. *International Journal of Food Microbiology.* Vol. 58, pp1–37.

Lokuruka, M. N. I. & Regenstein, J. M. (2004) Biogenic amines in iced and temperature-abused tropical fish: a comparative study with temperate Atlantic mackerel. *Journal of Aquatic Food Product Technology*. Vol. 13:1, pp87-99.

Lokuruka, M. N. I. & Regenstein, J. M. (2005) Icing practices' impact on scombrotoxin formation in Atlantic mackerel. *Journal of Aquatic Food Product Technology*. Vol. 14:4, pp23-36.

Lokuruka, M. N. I. & Regenstein, J. M. (2007) Handling and storage of Atlantic mackerel (*Scomber scombrus*) on biogenic amine production. *Journal of Aquatic Food Product Technology*. Vol. 15:4, pp17-33.

Lopez-Caballero, M. E., Alvarez-Torres, M. D., Sanchez-Fernandez, J. A. & Moral, A. (2002) Photobacterium phosphoreum isolated as a luminescent colony from spoiled fish, cultured in model system under controlled atmospheres. *European Food Research & Technology*. Vol. 215:5, pp390-395.

López-Sabater, E. I., Rodríguez-Jerez, J. J., Hernández-Herrero, M. & Mora-Ventura, M. T. (1996) Incidence of histamine-forming bacteria and histamine content in scombroid fish species from retail markets in the Barcelona area. *International Journal of Food Microbiology*. Vol. 28:3, pp411-418.

Lorca, T. A., Gingerich, T. M., Pierson, M. D., Flick, G. J., Hackney, C. R. & Sumner, S. S. (2001) Growth and histamine formation of *Morganella morganii* in determining the safety and quality of inoculated and uninoculated bluefish (*Pomatomus salatrix*). *Journal of Food Protection*. Vol. 64:12, pp2015-2019.

Mackie, I. M., Pirie, L., Ritchie, A. H. & Yamanaka, H. (1997) The formation of non-volatile amines in relation to concentrations of free basic amino acids during postmortem storage of the muscle of scallop (*Pecten maximus*), herring (*Clupea harengus*) and mackerel (*Scomber scombrus*). *Food Chemistry*. Vol. 60:3, pp291-295.

Mah, J. H. & Hwang, H. J. (2009a) Effects of food additives on biogenic amine formation in myeolchi-jeot, a salted and fermented anchovy (*Engraulis japonicus*). *Food Chemistry*. Vol. 114:1, pp168-173.

Mah, J. H. & Hwang, H. J. (2009b) Inhibition of biogenic amine formation in a salted and fermented anchovy by *Staphylococcus xylosus* as a protective culture. *Food Control*. Vol. 20:9, pp796-801.

Mah, J. H., Han, H. K., Oh, Y. J., Kim, M. G. & Hwang, H. J. (2002) Biogenic amines in jeotkals, Korean salted and fermented fish products. *Food Chemistry*. Vol. 79:2, pp239-243.

Mah, J. H., Kim, Y. J. & Hwang, H. J. (2009) Inhibitory effects of garlic and other spices on biogenic amine production in Myeolchi-jeot, Korean salted and fermented anchovy product. *Food Control*. Vol. 20:5, pp449-454.

Mahendradatta, M. (2003) The change of histamine content in some fish-based foods during storage. *Indonesian Food & Nutrition Progress*. Vol. 10:1, pp54-61.

Marine Management Organisation (MMO) (2011) UK Sea Fisheries Statistics 2010 - Tables. Retrieved 2012 February from http://www.marinemanagement.org.uk/fisheries/statistics/annual2010.htm

Marine Management Organisation (MMO) (2012a) Catch Limits 10m and under. Retrieved 2012 25-June from Marine Management Organisation: http://marinemanagement.org.uk/fisheries/management/quotas_catch_under10.htm

Marine Management Organisation (MMO) (2012b) UK Vessel lists. Retrieved 2011 7-Nov from Marine management Organisation: http://marinemanagement.org.uk/fisheries/statistics/vessel.htm

Marine Stewardship Council (2012) *Scottish Pelagic Sustainability Group Ltd western component of north east Atlantic mackerel*. Retrieved 2012 23-May from Marine Stewardship Council: http://www.msc.org/track-a-fishery/certified/north-east-atlantic/scottish-pelagic-sustainability-group-ltd-spsg

Mavromatis, P. & Quantick, P. (2002) Histamine toxicity and scombroid fish poisoning: A review. In: Alasalvar C., Taylor T. ed. *Seafoods - Technology, Quality and nutraceutical applications*. New York, Springer, pp89-103. (Cited by Fletcher, 2010)

Mbarki, R., Miloud, N. B., Selmi, S., Dhib, S. & Sadok, S. (2009) Effect of vacuum packaging and low-dose irradiation on the microbial, chemical and sensory characteristics of chub mackerel (*Scomber japonicus*). *Food Microbiology*. Vol. 26:8, pp821-826.

Mbarki, R., Sadok, S. & Barkallah, I. (2008) Influence of gamma irradiation on microbiological, biochemical, and textural properties of bonito (*Sarda sarda*) during chilled storage. *Food Science & Technology International*. Vol. 14:4, pp367-373.

McCarthy, S. & Burkhardt III, W. (2012) Efficacy of electrolyzed oxidizing water against listeria monocytogenes and *morganella morganii* on conveyor belt and raw fish surfaces. *Food Control*. Vol. 24:1-2, pp214-219.

McLauchlin, J., Little, C. L., Grant, K. A. & Mithani, V. (2006) Scombrotoxic fish poisoning. *Journal of Public Health*. Vol. 28:1, pp61-62.

McMeekin, T. A., Olley, J., Ross, T. & Ratskowsky, D. A. (1993) A thermodynamic approach to bacterial growth (Chapter 10). *Perspective microbiology: Theory and application,* Research Studies Press, Wiley, Taunton, New York. (Cited by Fletcher, 2010).

Mendes, R., Silva, H. A., Nunes, M. L. & Empis, J. M. A. (2000) Deteriorative changes during ice storage of irradiated blue jack mackerel (*Trachurus picturatus*). *Journal of Food Biochemistry*. Vol. 24:2, pp89-105.

Mendes, R., Silva, H. A., Nunes, M. L. & Empis, J. M. A. (2005) Effect of low-dose irradiation and refrigeration on the microflora, sensory characteristics and biogenic amines of Atlantic horse mackerel (*Trachurus trachurus*). *European Food Research & Technology*. Vol. 221, pp329-335.

Mermelstein, N. H. (2010) Analyzing for histamine in seafood. *Food Technology*. Vol. 64:2, pp65-70.

Met Office (2013) *Northern Scotland regional mapped climate averages, August 1971-2000*. http://www.metoffice.gov.uk/climate/uk/averages/regmapavge.html#nscotland.

Middlebrooks, B. L., Toom, P. M., Douglas, W. L., Harrison, R. E. & McDowell, S. (1988) Effects of storage time and temperature on the microflora and amine development in Spanish mackerel (*Scomberomorus- maculatus*). *Journal of Food Science*. Vol. 53:4, pp1024-1029. (Cited by Emborg, 2008)

Miki, M., Ishikawa, T. & Okayama, H. (2005) An outbreak of histamine poisoning after ingestion of the ground saury paste in eight patients taking isoniazid in tuberculosis ward. *Internal Medicine*. Vol. 44:11, pp1133-1136.

Millán, R., Izquierdo, P., Allara, M., Torres, G., García, A. & Barboza, Y. (2003) Effect of temperature and storage time on microbial quality and histamine production in Lisa (*Mugil curema*). *Revista Científica*. FCV-LUZ / Vol. XIII, Nº 5, pp339-346.

Mitchell, J. W. (1993) *Scombrotoxic fish poisoning*. A report prepared for the Ministry of Health. Institute of Environmental Health and Forensic Sciences Limited, Mt Albert Science Centre, NZ, 18 pp. (Cited by Fletcher, 2010)

Mohamed, R., Livia, S. S., Hassan, S., Soher, E. S. & Ahmed-Adel, E. B. (2009) Changes in free amino acids and biogenic amines of Egyptian salted-fermented fish (feseekh) during ripening and storage. *Food Chemistry*. Vol. 115:2, pp635-638.

Mohan, C. O., Ravishankar, C. N., Srinivasa Gopal, T. K., Ashok Kumar, K. & Lalitha, K. V. (2009) Biogenic amines formation in seer fish (*Scomberomorus commerson*) steaks packed with O2 scavenger during chilled storage. *Food Research International*. Vol. 42:3, pp411-416.

Naila, A., Flint, S., Fletcher, G. C., Bremer, P. J. & Meerdink, G. (2011) Biogenic amines and potential histamine - forming bacteria in rihaakuru (a cooked fish paste). *Food Chemistry*. Vol. 128:2, pp479-484.

Naila, A., Flint, S., Fletcher, G., Bremer, P. & Meerdink, G. (2010) Control of biogenic amines in food-existing and emerging approaches. *Journal of Food Science*. Vol. 75:7, ppR139-R150.

Napier, I. R. (2011) Fish Landings in Shetland and by Shetland Fleet 2010. Retrieved 2012 3-March from http://www.nafc.ac.uk/WebData/Files/Report%20-%20Landings%20-%202010.pdf

Niven, C. F., Jeffrey, M. B. & Corlett, D. A. (1981) Differential plating medium for quantitative detection of histamine-producing bacteria. *Applied and Environmental Microbiology*. Vol. 41, pp321-322.

Ohashi, M. (2002) Determination of K-value and histamine of mackerel and tuna, by using the oxygen-sensor method. *Journal of the Food Hygienic Society of Japan (Shokuhin Eiseigaku Zasshi)*. Vol. 43:1, pp39-43.

Ohnuma, S., Higa, M., Hamanaka, S., Matsushima, K. & Yamamuro, W. (2001) An outbreak of allergy-like food poisoning. *Internal Medicine*. Vol. 40:8, pp833-835.

Okuzumi, M., Fukumoto, I., & Fujii, T. (1990) Changes in bacterial flora and polyamines contents during storage of horse mackerel meat. *Bulletin of the Japanese Society of Scientific Fisheries*. Vol. 56, pp1307-1312. (cited by Kim *et al*., 2009)

Olgunoglu, I. A., Özogul, F., Özogul, Y. & Kuley, E. (2009) Chemical, sensory and microbiological assessment of marinated anchovy (*Engraulis engrasicholus* L., 1758) fillets stored at 1±1°C. *Advances in Food Sciences*. Vol. 31:2, pp102-108.

Osako, K., Kurokawa, T., Kuwahara, K. & Nozaki, Y. (2004) Seasonal variations in taurine and histidine levels of horse mackerel caught in the East China Sea. *Fisheries Science*. Vol. 70, pp1180-1182.

Osborne, C. M. & Bremer, P. J. (2000) Application of the bigelow (z-value) model and histamine detection to determine the time and temperature required to eliminate *Morganella morganii* from seafood. *Journal of Food Protection*. Vol. 63:2, pp277-280.

Özogul, F. & Özogul, Y. (2005) Formation of biogenic amines by Gram-negative rods isolated from fresh, spoiled, VP-packed and MAP-packed herring (*Clupea harengus*). *European Food Research & Technology*. Vol. 221:5, pp575-581.

Özogul, F. & Özogul, Y. (2006) Biogenic amine content and biogenic amine quality indices of sardines (*Sardina pilchardus*) stored in modified atmosphere packaging and vacuum packaging. *Food Chemistry*. Vol. 99:3, pp574-578.

Özogul, F., Kuley, E. & Kenar, M. (2011) Effects of rosemary and sage tea extract on biogenic amines formation of sardine (*Sardina pilchardus*) fillets. *International Journal of Food Science and Technology*. Vol. 46:4, pp761-766.

Özogul, F., Polat, A. & Özugul, Y. (2004) The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*). *Food Chemistry*. Vol. 85:1, pp49-57.

Özogul, F., Taylor, K. D. A., Quantick, P. & Özogul, Y. (2002a) Biogenic amines formation in Atlantic herring (*Clupea harengus*) stored under modified atmosphere packaging using a rapid HPLC method. *International Journal of Food Science & Technology*. Vol. 37:5, pp515-522.

Özogul, F., Taylor, K. D. A., Quantick, P. & Özogul, Y. (2002b) Changes in biogenic amines in herring stored under modified atmosphere and vacuum pack. *Journal of Food Science*. Vol. 67:7, pp2497-2501.

Özogul, Y., Özogul, F. & Gökbulut, C. (2006) Quality assessment of wild European eel (*Anguilla anguilla*) stored in ice. *Food Chemistry.* Vol. 95, pp458-465.

Paleologus, E. K. Savvaidis, I. N. & Kontominas, M. G. (2004) Biogenic amines formation and its relation to microbiological and sensory attributes in ice-stored whole, gutted and filleted Mediterranean Sea bass (*Dicentrarchus labrax*). *Food Microbiology*. Vol. 21, pp549–557.

Patterson, M. F. (2005) A review: microbiology of pressure-treated foods. *Journal of Applied Microbiology*. Vol. 98, pp400–9.

Periago, M. J., Rodrigo, J., Ros, G., Rodriguez-Jerez, J. J. & Hernandez-Herrero, M. (2003) Monitoring volatile and nonvolatile amines in dried and salted roes of tuna (*Thunnus thynnus* L.) during manufacture and storage. *Journal of Food Protection*. Vol. 66:2, pp335-340.

Petaja, E., Eerola, S. & Petäjä, P. (2000) Biogenic amines in cold-smoked fish fermented with lactic acid bacteria. *European Food Research & Technology*. Vol. 210:4, pp280-285.

Phuvasate, S. & Su, Y. C. (2010) Effects of electrolyzed oxidizing water and ice treatments on reducing histamine-producing bacteria on fish skin and food contact surface. *Food Control*. Vol. 21:3, pp286-291.

Pirani, S., Bernardi, C., Cantoni, C. & Longhi, D. (2010) First signalling of high content of histamine in missoltini (Alosa agone). *Industrie Alimentari*. Vol. 49:500, pp9-12.

Pirazzoli, P., Incerti, I., Gola, S. & Scaramuzza, N. (2006) Effect of salt concentration and storage temperature on shelf life and histamine formation in anchovy paste. *Industria Conserve*. Vol. 81:3, pp281-296.

Pons-Sanchez-Cascado, S., Bover-Cid, S., Veciana-Nogués, M. T. & Vidal-Carou, M. C. (2005a) Amino acid-decarboxylase activity of bacteria isolated from ice-preserved anchovies. *European Food Research & Technology*. Vol. 220:3,4, pp312-315.

Pons-Sanchez-Cascado, S., Veciana-Nogués, M. T. & Vidal-Carou, M. C. (2003) Effect of delayed gutting on biogenic amine contents during ripening of European anchovies. *European Food Research & Technology*. Vol. 216:6, pp489-493.

Pons-Sanchez-Cascado, S., Veciana-Nogués, M. T., Bover-Cid, S., Marine-Font, A. & Vidal-Carou, M. C. (2005b) Volatile and biogenic amines, microbiological counts, and bacterial amino acid decarboxylase activity throughout the salt-ripening process of anchovies (*Engraulis encrasicholus*). *Journal of Food Protection*. Vol. 68:8, pp1683-1689.

Poonsap, V. (2000) The processing of Thai fish sauce. *INFOFISH International*. Vol. 5, pp49-53.

Prester, L., Macan, J., Varnai, V. M., Orct, T., Vukusic, J. & Kipcic, D. (2009) Endotoxin and biogenic amine levels in Atlantic mackerel (*Scomber scombrus*), sardine (*Sardina pilchardus*) and mediterranean hake (*Merluccius merluccius*) stored at 22°C. *Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment*. Vol. 26:3, pp355-362.

Purnell, G. & James, C. (2012) Advances in food surface pasteurization by thermal methods. Chpt 8, pp241-273. *Microbial Decontamination in the Food Industry: Novel Methods and Applications*, edited by Demirci, A. & Ngadi, M. O. Woodhead Publishing Ltd.

RASFF Database Portal (2012) Retrieved 2012 8-March from RASFF: https://webgate.ec.europa.eu/rasff-window/portal/index.cfm?event=searchForm#

Rezaei, M., Montazeri, N., Langrudi, H. E., Mokhayer, B., Parviz, M. & Nazarinia, A. (2007) The biogenic amines and bacterial changes of farmed rainbow trout (*Oncorhynchus mykiss*) stored in ice. *Food Chemistry*. Vol. 103, pp150-154.

Rodtong, S., Nawong, S. & Yongsawatdigul, J. (2005) Histamine accumulation and histamine-forming bacteria in Indian anchovy (*Stolephorus indicus*). *Food Microbiology*. Vol. 22:5, pp475-482.

Rogers, P. L. & Staruszkiewicz, W. F. (2000) Histamine test kit comparison. *Journal of Aquatic Food Product Technology.* Vol 9:2, pp5-17.

Rosma, A., Afiza, T. S., Wan Nadiah, W. A., Liong, M. T. & Gulam, R. R. A. (2009) Microbiological, histamine and 3-MCPD contents of Malaysian unprocessed 'budu'. *International Food Research Journal*. Vol. 16:4, pp589-594.

Rossano, R., Mastrangelo, L., Ungaro, N. & Riccio, P. (2006) Influence of storage temperature and freezing time on histamine level in the European anchovy *Engraulis encrasicholus* (L., 1758): a study by capillary electrophoresis. *Journal of Chromatography B*. Vol. 830:1, pp161-164.

Rossi, S., Lee, C., Ellis, P. C. & Pivarnik, L. F. (2002) Biogenic amines formation in Bigeye tuna steaks and whole Skipjack tuna. *Journal of Food Science*. Vol. 67:6, pp2056-2060.

Ruiz-Capillas, C. & Moral, A. (2004) Free amino acids and biogenic amines in red and white muscle of tuna stored in controlled atmospheres. *Amino Acids*. Vol. 26:2, pp125-132.

Ruiz-Capillas, C. & Moral, A. (2005) Sensory and biochemical aspects of quality of whole bigeye tuna (*Thunnus obesus*) during bulk storage in controlled atmospheres. *Food Chemistry*. Vol. 89:3, pp347-354.

Sato, T., Fujii, T., Masuda, T. & Okuzumi, M. (1994) Changes in numbers of histamine – metabolic bacteria and histamine content during storage of common mackerel. *Fisheries Science*. Vol. 60:3, pp299–302.

Scottish Government (2011) Pelagic. Web: http://www.scotland.gov.uk/Topics/marine/marine-environment/species/fish/pelagic Accessed May 2011

Scottish Government (2011) Scottish Sea Fisheries Statistics 2010. Retrieved 2012 12-Jan from Scottish Government: http://www.scotland.gov.uk/Publications/2011/09/09155012/0

Seafood Scotland (2011) http://www.seafoodscotland.org. Accessed May 2011

Selmi, S., Monser, L. & Sadok, S. (2008) The influence of local canning process and storage on pelagic fish from tunisia: Fatty acid profiles and quality indicators. *Journal of Food Processing and Preservation*. Vol. 32:3, pp443-457.

SFPA (2012) SFPA Homepage. Retrieved 2012 10-May from SFPA: http://www.scottishpelagic.co.uk/

Shirai, N., Terayama, M. & Takeda, H. (2002) Effect of season on the fatty acid composition and free amino acid content of the sardine *Sardinops melanostictus*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*. Vol. 131:3, pp387-393.

Silva, C. C. G., Da Ponte, D. J. B. & Dapkevicius, M. (1998) Storage temperature effect on histamine formation in big eye tuna and skipjack. *Journal of Food Science*. Vol. 63:4, pp644-647.

Silva, T. M., Sabaini, P. S., Evangelista, W. P. & Gloria, M. B. A. (2011) Occurrence of histamine in brazilian fresh and canned tuna. *Food Control*. Vol. 22:2, pp323-327.

Silveira, N. F. A., Leitao, M. F. F., Baldini, V. L. S. & Teixeira Filho, A. R. (2001) Histamine producing bacteria and potential for its formation in fresh river fishes. *Brazilian Journal of Food Technology.* Vol. 4, pp19-25.

Smith-Palmer, A. (2012) Gastro-intestinal and foodborne infections. *HPS Weekly Report*. Vol. 46, No. 2012/13, pp100-101.

Smith-Palmer, A. & Cowden, J. (2002) Gastro-intestinal infections. *SCIEH Weekly Report*. Vol. 36, No. 2002/13, pp90-91.

Smith-Palmer, A. & Cowden, J. (2003) Gastro-intestinal infections. *SCIEH Weekly Report*. Vol. 37, No. 2003/13, pp76-78.

Smith-Palmer, A. & Cowden, J. (2009) Gastro-intestinal and foodborne infections: General outbreaks of infectious intestinal disease reported to HPS during 2008. *HPS Weekly Report*. Vol. 43, No. 2009/48, pp448-455.

Smith-Palmer, A. & Cowden, J. (2010) Gastro-intestinal and foodborne infections: General outbreaks of infectious intestinal disease reported to HPS during 2009. *HPS Weekly Report*. Vol. 44, No. 2010/49, pp485-491.

Starkusziewicz, W. F., Barnett, J. D., Rogers, P. L., Benner, R. A., Wong, L. L. & Cook, J. (2004) Effects of on-board and dockside handling on the formation of biogenic amines in mahi mahi (*Coryphaena hippurus*), skipjack tuna (*Katsuwonus pelamis*), and yellowfin tuna (*Thunnus albacares*). *Journal of Food Protection*. Vol. 67, pp134–141.

Takahashi, H., Kimura, B., Yoshikawa, M. & Fujii, T. (2003) Cloning and sequencing of the histidine decarboxylase genes of Gram-negative, histamine-producing bacteria and their application in detection and identification of these organisms in fish. *Applied & Environmental Microbiology*. Vol. 69:5, pp2568-2579.

Tao, Z. H., Sato, M., Yamaguchi, T. & Nakano, T. (2009) Formation and diffusion mechanism of histamine in the muscle of tuna fish. *Food Control*. Vol. 20:10, pp923-926.

Tao, Z. H., Sato, M., Zhang, H. M., Yamaguchi, T. & Nakano, T. (2011) A survey of histamine content in seafood sold in markets of nine countries. *Food Control*. Vol. 22:3-4, pp430-432.

Taylor, S. L. (1986) Histamine food poisoning: Toxicology and clinical aspects. *CRC Critical Reviews in Toxicology*. Vol. 17:2, pp91-128.

Taylor, S. L. & Speckhard, M. W. (1983) Isolation of histamine-producing bacteria from frozen tuna. *Marine Fisheries Review*. Vol. 45:4-6, pp35-39.

Thadhani, V. M., Jansz, E. R. & Peiris, H. (2002) Effect of exogenous histidine and Garcinia cambogia on histamine formation in skipjack (*Katsuwonus pelamis*) homogenates. *International Journal of Food Sciences & Nutrition*. Vol. 53:1, pp29-34.

Torres, G., Izquierdo, P., Allara, M. & García, A. (2003) The effect of temperature and storage time on the growth of histamine producing bacteria in two species of fish: lisa *(Mugil curema)* and robalo *(Centropomus undecimalis). Revista Científica*. FCV-LUZ / Vol. XIII, Nº 4, pp263-268.

Tsai, Y. H., Chang, S. C., Kung, H. F., Wei, C-I. & Hwang, D. F. (2005c) Histamine production by Enterobacter aerogenes in sailfish and milkfish at various storage temperatures. *Journal of Food Protection*. Vol. 68:8, pp1690-1695.

Tsai, Y. H., Hsieh, H. S., Chen, H. C., Cheng, S. H., Chai, T. & Hwang, D. F. (2007b) Histamine level and species identification of billfish meats implicated in two food-borne poisonings. *Food Chemistry*. Vol. 104:4, pp1366-1371.

Tsai, Y. H., Kung, H. F., Chen, H. C., Chang, S. C., Hsu, H. H. & Wei, C-I. (2007a) Determination of histamine and histamine-forming bacteria in dried milkfish (*Chanos chanos*) implicated in a food-borne poisoning. *Food Chemistry*. Vol. 105:3, pp1289-1296.

Tsai, Y. H., Kung, H. F., Lee, T. M., Chen, H. C., Chou, S. S., Wei, C.-I. & Hwang, D. F. (2005b) Determination of histamine in canned mackerel implicated in a foodborne poisoning. *Food Control*. Vol. 16:7, pp579-585.

Tsai, Y. H., Kung, H. F., Lee, T. M., Lin, G. T., Hwang, D. F. (2004) Histamine-related hygienic qualities and bacteria found in popular commercial scombroid fish fillets in Taiwan. *Journal of Food Protection*. Vol. 67:2, pp407-412.

Tsai, Y. H., Lin, C. Y., Chang, S. C., Chen, H. C., Kung, H. F., Wei, C-I. & Hwang, D. F. (2005a) Occurrence of histamine and histamine-forming bacteria in salted mackerel in Taiwan. *Food Microbiology*. Vol. 22:5, pp461-467.

Tsai, Y. H., Lin, C. Y., Chien, L. T., Lee, T. M., Wei, C. I. & Hwang, D. F. (2006) Histamine contents of fermented fish products in Taiwan and isolation of histamine-forming bacteria. *Food Chemistry*. Vol. 98:1, pp64-70.

Tsironi, T., Gogou, E., Velliou, E. & Taoukis, P. S. (2008) Application and validation of the tti based chill chain management system smas (safety monitoring and assurance system) on shelf life optimization of vacuum packed chilled tuna. *International Journal of Food Microbiology*. Vol. 128:1, pp108-115.

UK Food Surveillance System (2012) Retrieved 2012 8-March from FSA: http://food.gov.uk/enforcement/monitoring/fss/

Veciana-Nogués, M. T., Bover-Cid, S., Mariné-Font, A. & Vidal-Carou, M. C. (2004) Biogenic amine production by *Morganella morganii* and *Klebsiella oxytoca* in tuna. *European Food Research & Technology*. Vol. 218:3, pp284-288.

Veciana-Nogués, M. T., Mariné-Font, A. & VidalCarou, M. C. (1997) Biogenic amines in fresh and canned tuna. Effects of canning on biogenic amine contents. *Journal of Agricultural and Food Chemistry*. Vol. 45:11, pp4324-4328. (cited by Emborg, 2008)

Visciano, P., Campana, G., Annunziata, L., Vergara, A. & Ianieri, A. (2007) Effect of storage temperature on histamine formation in *Sardina pilchardus* and *Engraulis encrasicolus* after catch. *Journal of Food Biochemistry*. Vol. 31:5, pp577-588.

Visciano, P., Vergara, A., Ianieri, A., Campana, G. & Annunziata, L. (2004) Storage temperature effects on histamine production in fresh fish. *Industrie Alimentari.* Vol. 43:442, pp1275-1280. (Cited by Fletcher, 2010)

Vosikis, V., Papageorgopoulou, A., Economou, V., Frillingos, S. & Papadopoulou. C. (2008) Survey of the histamine content in fish samples randomly selected from the Greek retail market. *Food Additives & Contaminants: Part B - Surveillance Communications*. Vol. 1:2, pp122-129.

Wei, C. I., Chen, C. M., Koburger, J. A., Otwell, W. S. & Marshall, M. R. (1990) Bacterial growth and histamine production on vacuum packaged tuna. *Journal of Food Science*. Vol. 55:1, pp59-63.

Xu, Y., Xia, W. & Kim, J. M. (2009) Biogenic and volatile amines in Chinese mitten crab (*Eriocheir sinensis*) stored at different temperatures. *International Journal of Food Science & Technology*. Vol. 44:8, pp1547-1552.

Yeh, C. Y., Lin, S. J. & Hwang, D. F. (2006) Biogenic amines, histamine and label of dressed fried fish meat products in taiwan. *Food Control*. Vol. 17:6, pp423-428.

Zaman, M. Z., Abu Bakar, F., Selamat, J. & Bakar, J. (2010) Occurrence of biogenic amines and amines degrading bacteria in fish sauce. *Czech Journal of Food Sciences*. Vol. 28:5, pp440-449.

Zarei, M., Najafzadeh, H., Eskandari, M. H., Pashmforoush, M., Enayati, A., Gharibi, D. & Fazlara, A. (2012) Chemical and microbial properties of mahyaveh, a traditional Iranian fish sauce. *Food Control*. Vol. 23:2, pp511-514.

Zotos, A., Petridis, D., Siskos, I. & Gougoulias, C. (2001) Production and quality assessment of a smoked tuna (*Euthynnus affinis*) product. *Journal of Food Science.* Vol. 66:8, pp1184-1190.

Disclaimer

The work associated with this contract / grant has been carried out in accordance with the highest academic standards and reasonable endeavours have been made to achieve the degree of reliability and accuracy appropriate to work of this kind.

However, the Institute does not have control over the use to which the results of this work may be put by the Funder and the Funder will therefore be deemed to have satisfied itself in every respect as to the suitability and fitness of the work for any particular purpose or application.

In no circumstances will the Institute, its servants or agents accept liability however caused arising from any error or inaccuracy in any operation, advice or report arising from this work, nor from any resulting damage, loss, expenses or claim.