Research of relevance to histamine poisoning in New Zealand
A review

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This report has been prepared by The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), which has its Head Office at 120 Mt Albert Rd, Mt Albert, Auckland.

This report has been approved by:
Graham C Fletcher
Scientist/Researcher, Food Processing and Preservation
Date: 30 July 2010

Jocelyn Eason
Science Group Leader, Postharvest Fresh Foods
Date: 30 July 2010

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Executive summary
Research of relevance to histamine poisoning in New Zealand – A review

Graham C Fletcher, July 2010, SPTS No. 4366

The context

Histamine poisoning from pelagic (predominantly scombroid) fish is a worldwide problem and in many countries is the most common cause of food poisoning from finfish. It occurs when bacteria convert high levels of naturally occurring histidine into histamine and, although the full aetiology is not resolved, when people eat fish containing high levels of histamine they suffer allergic type reactions. In New Zealand, histamine poisoning usually occurs after the consumption of hot-smoked fish, and many of the outbreaks and recalls arise from the consumption of local kahawai species. The author of the current report was part of a New Zealand team that published a series of studies on this issue in the late 1990s including a set of guidelines for the safe preparation of hot-smoked fish which were promulgated to industry. However, there have been ongoing subsequent histamine poisoning outbreaks due to New Zealand seafood, particularly hot smoked fish. The New Zealand Food Safety Authority therefore requested that the findings of the New Zealand research be summarised and subsequent international research be reviewed in order to identify future research or regulatory actions that could help ensure the safety of New Zealand seafood.

Aim and research method

This report reviews published New Zealand work related to histamine production in New Zealand fish carried out up to the year 2000. The results of unpublished work carried out by The New Zealand Institute for Crop & Food Research on identifying the bacteria involved and quantifying their growth and histamine production rates at different temperatures (0–20°C) are also presented. Over 200 international research studies of relevance to the problem of histamine have been published between 2000 and 2010 and this literature is reviewed from a New Zealand perspective.

Review results

Previous New Zealand studies have determined the levels of histamine and its precursor, histidine in retailed fresh and smoked fish products sold on the New Zealand retail market. Studies have also assessed the growth of histamine producing bacteria and the development of histamine in fresh and smoked kahawai. Subsequent international studies have broadened the range of products identified as hazards for histamine production and particular advances include understanding the role of histamine-producing bacteria able to grow at refrigeration temperatures (psychrotrophic organisms) and the development of mathematical models to describe the growth and histamine production of histamine-producing bacteria. Methods for detection of histamine and for identifying histamine producing bacteria have been advanced and a number of potential controls have also been investigated although some of these such as irradiation are not applicable to New Zealand.

Recommendations

Recommendations from the review include:
1. Agencies investigating food-borne outbreaks of histamine 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 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 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 poisoning.

4. Currently unpublished work on growth and histamine production by individual strains of histamine producing 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 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.

For further information please contact:
Graham C Fletcher
The New Zealand Institute for Plant & Food Research Ltd
Plant & Food Research Mt Albert
Private Bag 92 169
Auckland Mail Centre
Auckland 1142
NEW ZEALAND
Tel: +64-9-926 3512
Fax: +64-9-925 7001
Email: Graham.Fletcher@plantandfood.co.nz
1 Introduction

Histamine (or scombroid) poisoning in humans is an intoxication that results from the consumption of spoiled fish. The poisoning is only associated with a few fish species that naturally contain high levels of histidine, the best known internationally being members of the Scombridae family, particularly tuna and mackerel. Although the full aetiology of the intoxication has not been resolved it involves high levels of histamine generated by spoilage bacteria through the decarboxylation of histidine that naturally occurs at high levels in such species. The toxin involved in the poisoning is known as scombrotxin even though non-scombroid fish species can cause the intoxication. A range of bacteria are capable of converting histidine to histamine in fish. It has usually been attributed to bacteria which will only grow poorly if at all below 10°C (mesophilic bacteria) such as Morganella morganii although some psychrotrophic organisms which grow well at refrigeration temperatures such as Morganella psychrotolerans have also been implicated. The symptoms resemble an allergic attack and, although usually short-lived, are very unpleasant for those affected. In New Zealand, the illness has usually been associated with hot-smoked fish of various species, particularly kahawai (Arripis trutta), not a Scombridae fish.

Product recalls arising from histamine poisoning create a negative image for seafood products. Although, histamine poisoning is usually caused by fish containing over 1000 mg/kg histamine, incidents have been reported when levels as low as 200 mg/kg were detected (Bartholomew et al. 1987). Outbreaks usually involve fewer than 10 people (Hughes et al. 2007) and often single cases occur. The latter do not appear in statistics for food-borne outbreaks as, by definition, an outbreak requires at least two people to be affected. Food safety is regulated by limiting the amount of histamine permitted in fish flesh with the Australia/New Zealand food standard requiring less than 200 mg/kg histamine (Australian and New Zealand Food Authority (ANZFA) 2010) while the US FDA has a hazard action level of 500 mg/kg and a decomposition level of 50 mg/kg (Food and Drug Administration 1995). The EC has a sampling plan based on 9 samples requiring that no more than 2 exceed 100 mg/kg and none exceed 200 mg/kg although single samples are allowed at the retail level (Commission of the European Communities 2005). The draft Codex Alimentarius decomposition standard for smoked fish requires that product of susceptible species not contain more than 100 mg of histamine per kg fish flesh although when considering hygiene and handling the level is 200 mg/kg (Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission 2010). Despite the New Zealand standard being 200 mg/kg for food safety, New Zealand exported product must be able to meet the most stringent standards of the world which is the FDA decomposition standard of 50 mg/kg so we usually use this as the target in our research. The main way to ensure that susceptible product remains within the prescribed limits is to store it at temperatures below the minimum for growth of the mesophilic bacteria responsible for the histamine production or for durations such that psychrotrophic bacteria do not have time to proliferate and produce unacceptable levels of histamine.

From 1988 to 2000, the author was involved in a series of research projects designed to identify practices that might mitigate the risks from New Zealand seafood. Although industry guidelines were produced (Fletcher 1998a), New Zealand seafood has continued to cause outbreaks of histamine poisoning up to the present. The New Zealand Food Safety Authority therefore asked the author to review existing knowledge and make recommendations for future research.

The current report briefly reviews the New Zealand research that was carried out up to 2000, presents some work that was hitherto unpublished and then reviews international research on
histamine poisoning between 2000 and 2010. The report makes some recommendations about information that should be gathered in the event of any further outbreaks. It also presents the case for a range of research projects designed to enhance our understanding of the factors that lead to histamine poisoning and ways to reduce food safety risks. Information generated would inform future management practices by the New Zealand seafood industry and guide policy and planning activities in relevant regulatory agencies.
2  Histamine poisoning in New Zealand

Before 2000, there were at least 27 published outbreaks of histamine poisoning in New Zealand from New Zealand seafood. Early New Zealand publications detailing implicated products, season and histamine levels included those by Foo (1975b) (canned mackerel and smoked kahawai, summer, 8000 mg/kg), Foo (1975a) (kingfish, 7580 mg/kg), Mitchell (1984) (smoked kahawai, 2000 mg/kg; kingfish, 6000 mg/kg), Mitchell & O’Brien (1992) (smoked mackerel, summer, 3000 mg/kg) and Thornton & Calder (1993) (smoked Spanish mackerel, December, 3000 mg/kg). Mitchell (1993) reported a cluster of 19 samples suspected of causing histamine poisoning between January 1990 and June 1993 with the following implicated products (and sample numbers): smoked kahawai (6), smoked marlin (3), smoked mackerel (4), smoked trevally (1), unspecified smoked fish (5). There was another well publicised outbreak from smoked kahawai resulting in a recall of a batch of product produced from one smokehouse in 1997 (Anon. 1997). The dominance of smoked fish as the main product causing New Zealand cases of histamine poisoning was unusual in the world and led to our research group, then part of the DSIR, to initiate a research programme on controlling histamine poisoning from hot-smoked kahawai as outlined in Section 3 of this report. Based on the work on kahawai, a generic set of guidelines for the safe production of hot-smoked fish was produced (Fletcher et al. 1998a) and distributed to every registered facility producing hot-smoked fish in New Zealand but outbreaks have continued to occur (Table 1).

Information about New Zealand cases and outbreaks for the years 2006–09 is available on the NZFSA website (Pirie et al. 2008; Williman et al. 2008; Williman et al. 2009; Lim et al. 2010). This information has been supplemented with personal communications from Esther Lim, ESR (2010) to produce a summary of the 31 reported outbreaks from 2000 to 2009 presented in Table 1. During this period there have also been two major recalls in New Zealand, both from hot-smoked kahawai produced by the same company (New Zealand Food Safety Authority 2002, 2003). This company has since ceased to trade.

In contrast to the rest of the world where fresh fish are the main cause of histamine poisoning outbreaks, New Zealand outbreaks and product recalls are dominated by hot-smoked fish, mostly kahawai (the only New Zealand product for which significant product recalls have been required), but also marlin, trevally, kingfish, Spanish mackerel and tuna. Fresh or marinated kingfish have also caused outbreaks of histamine poisoning in New Zealand as have fresh tuna and marlin, canned mackerels and fish cakes (Table 1). The ongoing occurrence of outbreaks from hot smoked fish points to the need for more research and/or tighter control of production practices for hot-smoked fish by regulatory agents.
Table 1. New Zealand food-associated histamine poisoning outbreaks, 2006-09 (data extracted from Pirie et al. 2008; Williman et al. 2008; Williman et al. 2009; Lim 2010; Lim et al. 2010).

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Location</th>
<th>Product</th>
<th>Setting</th>
<th>Number ill *</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>November</td>
<td>Auckland</td>
<td>Smoked fish</td>
<td>Home, Seafood outlet</td>
<td>2P</td>
</tr>
<tr>
<td>2001</td>
<td>February</td>
<td>Auckland</td>
<td>Smoked fish</td>
<td>Home</td>
<td>3P</td>
</tr>
<tr>
<td>2001</td>
<td>March</td>
<td>Auckland</td>
<td>Smoked fish</td>
<td>Home</td>
<td>2P</td>
</tr>
<tr>
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<td>August</td>
<td>Auckland</td>
<td>Smoked fish</td>
<td>Home</td>
<td>2P</td>
</tr>
<tr>
<td>2002</td>
<td>January</td>
<td>Auckland</td>
<td>Smoked kahawai</td>
<td>Home, Supermarket, Fish</td>
<td>2P</td>
</tr>
<tr>
<td>2002</td>
<td>January</td>
<td>Auckland</td>
<td>Smoked fish</td>
<td>Home, Supermarket, Fish</td>
<td>5P</td>
</tr>
<tr>
<td>2002</td>
<td>January</td>
<td>Auckland</td>
<td>Smoked trevally</td>
<td>Home, Fish processor</td>
<td>2P</td>
</tr>
<tr>
<td>2002</td>
<td>March</td>
<td>Auckland</td>
<td>Smoked kahawai</td>
<td>Home, Supermarket, Fish</td>
<td>3P</td>
</tr>
<tr>
<td>2002</td>
<td>December</td>
<td>Auckland</td>
<td>Smoked kahawai</td>
<td>Fish processor</td>
<td>16C, 4P</td>
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<tr>
<td>2002</td>
<td>March</td>
<td>Auckland</td>
<td>Smoked kingfish</td>
<td>Fish shop</td>
<td>2C</td>
</tr>
<tr>
<td>2002</td>
<td>May</td>
<td>Auckland, Waikato, Tauranga, Manawatu</td>
<td>Smoked kahawai</td>
<td>Other food outlet</td>
<td>6C, 7P</td>
</tr>
<tr>
<td>2002</td>
<td>December</td>
<td>Auckland</td>
<td>Smoked kahawai</td>
<td>Other food outlet</td>
<td>2C</td>
</tr>
<tr>
<td>2002</td>
<td>December</td>
<td>Northland, Tauranga, Wanganui</td>
<td>Smoked kahawai</td>
<td>Home, other food outlet</td>
<td>1C, 6P</td>
</tr>
<tr>
<td>2003</td>
<td>December</td>
<td>Auckland</td>
<td>Smoked kahawai</td>
<td>Home, Seafood outlet</td>
<td>2C</td>
</tr>
<tr>
<td>2004</td>
<td>February</td>
<td>Auckland</td>
<td>Smoked kingfish</td>
<td>Takeaway</td>
<td>2P</td>
</tr>
<tr>
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<td>March</td>
<td>Auckland</td>
<td>Smoked kahawai</td>
<td>Home, other food outlet</td>
<td>7C, 4P</td>
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<td>Takeaway</td>
<td>2P</td>
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<tr>
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<td>April</td>
<td>Auckland</td>
<td>Smoked trevally</td>
<td>Takeaway</td>
<td>2C</td>
</tr>
<tr>
<td>2004</td>
<td>May</td>
<td>Auckland</td>
<td>Marinated kingfish</td>
<td>Home, other food outlet</td>
<td>2P</td>
</tr>
<tr>
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<td>March</td>
<td>Auckland</td>
<td>Smoked fish</td>
<td>Home</td>
<td>2P</td>
</tr>
<tr>
<td>2005</td>
<td>May</td>
<td>Auckland</td>
<td>Smoked tuna</td>
<td>Fish market</td>
<td>2C</td>
</tr>
<tr>
<td>2005</td>
<td>July</td>
<td>Auckland</td>
<td>Tuna steaks</td>
<td>Restaurant/cafés</td>
<td>3P</td>
</tr>
<tr>
<td>2006</td>
<td>January</td>
<td>Auckland</td>
<td>Smoked tuna</td>
<td>Home, Takeaway</td>
<td>2P</td>
</tr>
<tr>
<td>2006</td>
<td>February</td>
<td>Auckland</td>
<td>Fish</td>
<td>Supermarket</td>
<td>4C, 2P</td>
</tr>
<tr>
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<td>November</td>
<td>Auckland</td>
<td>Smoked kahawai</td>
<td>Fisk smoking plant, supermarket</td>
<td>2C 2, 5</td>
</tr>
<tr>
<td>2006</td>
<td>February</td>
<td>Rotorua</td>
<td>Kingfish</td>
<td>Restaurant/cafés</td>
<td>4P</td>
</tr>
<tr>
<td>2007</td>
<td>January</td>
<td>Northland</td>
<td>Fish cakes</td>
<td>Takeaway</td>
<td>5C</td>
</tr>
<tr>
<td>2007</td>
<td>September</td>
<td>Auckland</td>
<td>Tuna and marlin</td>
<td>Fish processing premise</td>
<td>3P</td>
</tr>
<tr>
<td>2008</td>
<td>January</td>
<td>Auckland</td>
<td>Smoked kahawai</td>
<td>Home, open air market</td>
<td>2P</td>
</tr>
<tr>
<td>2008</td>
<td>November</td>
<td>Auckland</td>
<td>Smoked kahawai</td>
<td>Home</td>
<td>4C</td>
</tr>
<tr>
<td>2009</td>
<td>February</td>
<td>Auckland</td>
<td>King fish</td>
<td>Restaurant/Cafe</td>
<td>3P</td>
</tr>
</tbody>
</table>

*C=confirmed, P = probable
3 New Zealand research

As mentioned above, Plant & Food Research (then Crop & Food Research and DSIR) carried out work on the issue of histamine poisoning from hot-smoked fish in the late 1980s and 90s with a focus on identifying the levels, development and inactivation of histamine-producing bacteria and histamine in smoked fish, particularly kahawai. These published studies are reviewed here. As we also completed a significant body of research on the growth and histamine production of individual strains of bacteria that has not yet been formally published these results are also presented in Section 3.4.

3.1 Retail surveys

3.1.1 Histidine levels in whole New Zealand fish of various species

To identify New Zealand species that might present a health risk to consumers, we determined the levels of free histidine present in the flesh of species on the local retail market (Fletcher et al. 1995). Fresh whole fish (47) from 28 species were purchased from Auckland retailers and levels of histidine and histamine were determined. The species could be divided into three groups on the basis of their histidine levels. Species with histidine levels above 10,000 mg/kg (albacore, kingfish and kahawai) had all previously been implicated in histamine poisoning. The other pelagic species (grey mullet, trevally, sprat, piper, jack mackerel, yellowtail, blue mackerel and barracouta) had histidine levels between 2000 and 10,000 mg/kg. Of these species, only mackerels had at that time formally been implicated in histamine poisoning in New Zealand (Foo 1975b; Mitchell & O’Brien 1992; Thornton & Calder 1993). However, we recorded histamine levels above 100 mg/kg for several other species in the Auckland retail market (Fletcher et al. 1998c and unpublished data). We therefore concluded that these species all have the potential to cause histamine poisoning. Subsequently, trevally has been implicated in outbreaks (Table 1). The remaining 17 species tested were all demersal species with histidine levels of less than 2000 mg/kg and are not expected to pose any risk from histamine poisoning.

3.1.2 Levels of histamine and histamine-producing bacteria in hot-smoked fish

In the retail survey described above, five of the 47 whole fish examined had histamine levels above 100 mg/kg (an albacore tuna, a kingfish, a kahawai and two jack mackerels) (Fletcher et al. 1995). However, as all New Zealand histamine poisoning incidents in the 10 years to 1997 involved hot-smoked fish, our subsequent work focused on this product. We purchased samples of smoked fish (107) from 9 Auckland retailers over a 9-month period and determined aerobic plate counts at 20°C (APC), histamine and moisture levels (Fletcher et al. 1998c). Eight samples, obtained from five different retailers, had histamine levels above 50 mg/kg and levels in two samples (346.4 and 681.8 mg/kg) exceeded 200 mg/kg. We stored 33 of the smoked fish samples at 20°C for 2 days and 8 of these developed histamine levels above 50 mg/kg with 4 exceeding 200 mg/kg (maximum 1659.4 mg/kg). The four stored samples exceeding 200 mg/kg all came from two outlets. Bacterial strains (3525) were isolated by selective plating on to Niven’s agar or by picking colonies from trypticase soy agar (TSA) incubated at 5, 20 or 35°C. Strains were tested for their ability to produce histamine in a histidine-saline solution containing just histidine (2 g/L) and NaCl (5 g/L). Although isolated at a range of different temperatures from refrigerated product, 97.6% of the 3525 tested cultures grew at 20°C so we conclude that an isolation temperature of 20°C is suitable for most psychrotrophic as well as mesophilic organisms. This is in line with other work that we have done on psychrotrophic bacteria from
seafood (Weirda et al. 2006). Other studies suggest 15°C or less as suitable isolation temperatures for psychrotrophic bacteria, e.g. (Dalgaard et al. 1998), but this typically necessitates separately incubating samples under higher temperatures to isolate mesophilic bacteria, which would increase both time and cost of isolation.

After re-inoculating into the histidine-saline solution, only 168 of the tested strains produced histamine from histidine when log phase cultures were inoculated into the histidine-saline solution and held for 8 days at 20°C (Fletcher et al. 1998). This low proportion of the tested strains highlights the fact that few of the bacteria present on smoked fish are capable of producing histamine. That many organisms from the selective Niven’s agar did not produce histamine in the broth pointed to the inadequacy of this agar. Many strains that were presumptive histamine producers in Niven’s agar turned out to be false positives.

### 3.2 Bacterial growth and histamine production in whole kahawai

We also carried out two trials in which un-inoculated kahawai were stored under 17 temperature regimes at temperatures between 0 and 35°C (Fletcher et al. 1995). In 10 regimes the fish were transferred from storage at various elevated temperatures to storage at 5°C in order to mimic likely commercial practice. Levels of histamine, APCs and sensory quality were monitored. Elevated histamine levels were first recorded in fish stored for 0.9, 0.9, 1, 2, 2.7, and 8 days at 35, 30, 25, 20, 15 and 10°C respectively (Figure 1b). Storage at 5°C after storage at higher temperatures did not result in elevated levels. Of the 59 samples with elevated histamine levels, examination of the raw fish suggested that 9 had acceptable sensory characteristics. However, all had aerobic plate counts exceeding 10^6 colony forming units (cfu)/g (Figure 1a).

### 3.3 Thermal death

Histamine is heat stable and thus, if formed before processing, is not destroyed by hot-smoking but histamine production subsequent to smoking could be prevented by the thermal elimination of histamine-producing bacteria during hot-smoking. We therefore determined the thermal death characteristics of two histamine-producing bacterial species. Under simple linear models, the thermal death characteristics of bacteria can be fully described by calculating two standard values: The D value refers to decimal reduction time - the time required at a particular temperature to cause a 10-fold inactivation (90% reduction) of the organism being studied. The z value is the change in temperature required to achieve a 10 fold reduction in D value for the organism. Within the experimental boundaries, once D and z values are known, it is possible to calculate what level of bacteria will be inactivated at any particular combination of time and temperature.

#### 3.3.1 Hafnia alvei

*H. alvei* strains have been implicated in incidents of histamine poisoning and *H. alvei* was the histamine-producing bacteria isolated from our sample with the highest histamine level (1659.4 mg/kg). Thermal death trials on our experimental strain of this organism were carried out in a model suspension (0.1% peptone). Samples containing high levels of *H. alvei* were subjected to different temperatures for different times and numbers of surviving bacteria used to calculate observed D values at the different temperatures. From the linear regression line fitted to the observed D values plotted against temperature (Figure 5), calculated D values for 54, 55, 56, 57 and 58°C were estimated to be 0.63, 0.36, 0.20, 0.11 and 0.06 min, respectively and a z value
of 4.14°C was calculated. Thermal death trials were also carried out for *H. alvei* that had been associated with hot-smoked kahawai. Here the calculated D values for 54, 55, 56, and 57°C were estimated to be 1.42, 0.74, 0.38, 0.20 min respectively, giving a z value of 3.57°C. Although the kahawai protected the bacteria from heat compared to when broth was heated, we still concluded that hot-smoking could be used to eliminate *H. alvei* from seafood products (Bremer et al. 1998).

3.3.2 *Morganella morganii*

As growth trials (Section 3.3.2) indicated that *M. morganii* was the most important bacterium for histamine production in hot-smoked kahawai, we investigated the thermal death characteristics of the nine strains (including a culture collection strain) that we had of this organism (Osborne & Bremer 2000). A mixed inoculum was added to kahawai mince and a variation of the Bigelow or z-value model was used to generate a thermal death time graph. The production of histamine, in a heat-treated and subsequently temperature-abused sample, was scored as a positive value (growth) and the absence of histamine was scored as a negative value (no growth). Temperatures tested were 58, 59, 60, 61 and 62°C. From the straight line fitted to the data (Figure 6), calculated times for eliminating histamine-forming bacteria were estimated to be 15.27, 4.79, 1.46 and 0.04 min respectively, giving a z value of 3.85°C. This approach to thermal death determination based on the presence/absence of a bacterial metabolite was an efficient way to determine the thermal regime required to eliminate histamine-producing bacteria from kahawai. Again, we concluded that *M. morganii* could be eliminated by the hot-smoking process.
3.4 Unpublished New Zealand research

As well as the published results presented above, we (Graham Fletcher, Scott Speed, Graeme Summers and Tricia Lee) also carried out a considerable amount of work on the identification and growth of histamine-producing bacteria isolated from smoked fish. We made several oral presentations (Fletcher et al. 1998b; Fletcher et al. 1999; Fletcher & Bremer 1999), but this work had not been published at the conclusion of the project. Key findings are summarised below.

3.4.1 Identification of histamine-producing bacteria in hot-smoked fish

The 168 strains of histamine-producing bacteria isolated from hot-smoked fish (Fletcher et al. 1998c) were identified using API 32, API 20NE and API 20E strips (BioMérieux, Lyon, France) and/or by using fatty acid methyl ester analysis (Microbial Identification Inc (MIDI), Newark, Delaware USA). One hundred and thirty-five strains were successfully identified, at least to the genus level, and these consisted of: Pseudomonas (81), Serratia (10), Morganella morganii (9), Hafnia alvei (9), Chryseomonas (8), Staphylococcus (4), Moellerella (4), Acinetobacter (2), Enterobacter (2), Alcaligenes (1), Bacillus (1), Brochothrix (1), Flavobacterium (1), Cellulomonas (1) and Moraxella (1). At the time this seemingly simple task required considerable effort because many of the test methods gave unacceptable or unknown profiles. Further phenotypic testing might have provided better clarification, but these were not completed due to constraints
of budget and time and it is likely that many of these organisms would still have been difficult to speciate with the phenotypic methods available at the time. Modern genotypic identification methods are more likely to yield more definitive results, and the cost of doing these are now comparable or cheaper than using phenotypic testing.

### 3.4.2 Growth of individual bacterial strains in broth

Experiments were conducted to assess the growth and histamine production ability of different strains of histamine-producing bacteria isolated from hot-smoked fish. Thirty-nine of the strains collected from different samples with elevated levels of histamine (>50 mg/kg) were selected for growth studies: (*Morganella morganii* (8), *Hafnia alvei* (2), *Pseudomonas* (16), *Serratia* (7), *Acinetobacter* (2), *Alcaligenes* (1), *Moraxella* (1), *Moellerella* (1), and *Cellulomonas* (1)). Because it has been most implicated in international outbreaks of histamine poisoning, we thought it valuable to see whether the New Zealand isolated stains of *M. morganii* showed major differences to international strains: we therefore added a culture collection strain of this organism, giving a total of 40 strains to be used in growth experiments. We prepared kahawai broth from fresh kahawai muscle after the method of Omura et al. (1978) and determined the growth characteristics of each organism and the production of histamine in broth. Experiments on each culture were conducted at 0, 5, 10, 15 and 20°C with 5 sampling times spread over periods of 27, 12, 7, 4 and 3 days at the respective temperatures (see sampling points in Figure 2b). On each occasion bacterial counts were determined on plate count agar (Difco) with 1% added NaCl using the drop plate method and histamine levels were determined using the AOAC method (Association of Official Analytical Chemists 1990).

Initial bacterial counts varied with the different experiments, ranging from 2.354 to 5.288 log_{10} cfu/g. Predictably, some strains showed characteristics of psychrotrophic genera (*Pseudomonas*, *Alcaligenes*, *Moellerella*, *Moraxella* and *Serratia*) growing to high levels (10^5–10^10 cfu/g) at all tested temperatures while others (*Cellulomonas* and *M. morganii*) only grew at mesophilic temperatures (i.e.10°C or higher) and under the conditions of these experiments only reached high levels at 15 and 20°C. The two *Acinetobacter* strains grew at temperatures of 5°C and above but not 0°C. One of the *H. alvei* strains also grew at 5°C and above but not 0°C, while the other grew to high levels at all temperatures. Initial histamine levels in the kahawai broth averaged 1.87 mg/L. All 9 strains of *M. morganii* produced elevated histamine levels (>50 mg/L), but only one strain of each of four other species did: *H. alvei* (the strain that didn’t grow at 0°C), *Alcaligenes*, *Pseudomonas* and *Serratia*. Thus, a total of 27 of 40 strains that produced histamine in the histidine-salt solution did not do so in the more complex kahawai broth.

As *M. morganella* was the most prolific histamine-producing species found in smoked fish and the one most able to produce histamine over a range of conditions, we focused our analysis of results on this species. Curves were fitted to the growth results for each of the nine strains. As we only had one replicate growth curve for each of the strains and only a few points during the logarithmic growth phase, the accuracy of the growth rates is relatively low. However, analyses showed that none of the strains was significantly different to the others (e.g. see Figure 2a) so the bacterial growth rates at the different temperatures were averaged across the strains to give mean growth rates for the species as shown in Figure 2b, upper plot. Production of histamine by *M. morganii* was related to its growth (Figure 2b, lower plot), never producing elevated histamine levels at 0 or 5°C and only after more than 5 days at 10°C, 1 day at 15°C and 12 h at 20°C. When it occurred, histamine production invariably began as the bacteria entered the stationary growth phase (>10^9 cfu/mL) (Figure 2b). This result is in general agreement with...
those found in the whole fish and the temperature response and storage times required before histamine production occurred were also similar (compare Figures 1b and 2b). However, the actual bacterial counts at which histamine production was initiated were higher and levels of histamine produced were lower in the broths than in the kahawai (compare Figures 1a and 2c). A possible explanation for the higher counts might be that nutrients are more readily available to the bacteria in the broth than in the intact kahawai flesh so they reached higher levels before entering into the stationary phase, which may be initiated by nutrient limitation. Histamine production was still only initiated when bacterial growth entered the stationary phase, but the stationary phase in the broth occurred at a higher concentration of bacteria than in the fish. This supports the hypothesis that, although bacteria may have the capability to produce histamine to gain some metabolic benefit, they tend to only do so when other nutrients are limited. The fact that 25 of 40 bacterial strains produced histamine in a very low nutrient environment (histidine plus NaCl) but not in a nutrient-rich kahawai broth supports this hypothesis. The approximately 6-fold higher levels of histamine per kilogram reached in whole fish than in the broths per litre may simply reflect reduced levels of histidine in the broths, providing less substrate for histidine decarboxylase enzyme activity that produces histamine. The process of producing broth from kahawai flesh effectively involved a 1:4 dilution; measured histidine levels in the broth suggested that reductions in histidine levels were even greater than 1:4.

Figure 2. Results from unpublished studies on growth and histamine production in kahawai broth by Morganella morganii.
We then determined the logarithmic growth rates of *M. morganii* at the different temperatures and square roots of growth rates were plotted against temperature (Belehrádek plots) for each strain. The strains did not differ significantly from each other (Figure 2a) so mean growth rates at the different temperatures were plotted for the species (Figure 2d) and these showed a linear relationship with temperature. From this the maximum growth rate at any particular temperature can be calculated, and in a dynamic real-time situation, the time spent at each temperature can be used to cumulatively calculate the level of *M. morganii* that would be present in the broth. As the growth in broth was faster than in fish, this should provide an overestimation (fail-safe prediction) for predicting histamine producing bacteria in fish. If our hypothesis that histamine production begins when bacteria enter the stationary phase is correct, then to predict histamine production in fish, one would need to know the maximum bacterial count present in fish before the bacteria go into stationary phase. The Belehrádek plot also gives a theoretical minimal growth temperature (*T*$_{\text{min}}$, the temperature when the growth rate = 0). In the case of *M. morganella*, using regression analysis the *T*$_{\text{min}}$ was 4.4°C. In practice, bacteria can seldom be demonstrated to grow at temperatures very close to *T*$_{\text{min}}$, and Belehrádek plots tend to overestimate growth near *T*$_{\text{min}}$ (perhaps due to the stress the organism is under at these temperatures). Thus, we conclude that *M. morganella* will not grow below 4.4°C and that effective refrigerated storage will prevent the growth of this organism.

Similar growth rate and Belehrádek plots were prepared for the other four species of bacteria that produced histamine at levels above 50 mg/kg (Figures 3–6). These only produced histamine at 20°C (or 15°C for *H. alvei*) even though they grew to high levels and usually reached stationary phase at the other temperatures (Figures 3–6). As none of these species produced histamine at temperatures below 10°C, we concluded that effective refrigerated storage will prevent the growth of these organisms. If confirmed in practice this mean that effective refrigeration (4°C of colder) will prevent histamine production in hot smoked fish.
Figure 3. Growth and histamine production of a *Serratia* sp. in kahawai broth.
Figure 4. Growth and histamine production of an *Alcaligenes* sp. in kahawai broth
Figure 5. Growth and histamine production of a strain of *H. alvei* in kahawai broth.
Take as an example, *Serratia* where the $T_{min}$ was -5.04°C (Figure 4c): this histamine-producing organism is well adapted to grow at refrigerated temperatures. However, we found that only one of the seven strains of *Serratia* actually produced any histamine in the kahawai broths and then only when it had reached the stationary phase at 20°C. Thus, although the organism grew well at refrigeration temperatures, it did not appear to produce any histamine at those temperatures. Thus, if *Serratia* produced hazardous levels of histamine in refrigerated fish, these would need to be stored at refrigerated temperatures long enough for the bacterial colonies to reach the stationary phase but still no histamine would be produced. They would still have to be held at ambient temperatures for the bacteria to produce the histamine.
Although carried out more than 10 years ago, no subsequent piece of research has studied the growth and histamine production of such a wide range of bacteria in such depth. The study brief did not require publication so a number of the conclusions of the work are not readily available to the research community. It would, therefore, be beneficial to publish it in an international peer-reviewed journal. To achieve this it would be necessary to repeat the identification of at least the 40 organisms used in the study (if not the whole pool of 168 organisms from which they were taken) using modern genotypic methods.

This unpublished research was carried out with funding by the New Zealand Foundation for Research Science and Technology. Statistical analyses of the results were carried out by John Koolard.

3.5 Conclusions and industry recommendations

From our studies we concluded that, under normal circumstances, prevention of histamine poisoning from hot-smoked fish could be readily achieved by adequate refrigeration (4°C) before smoking and by ensuring that time-temperature combinations during smoking were sufficient to eliminate histamine-producing bacteria. We published a set of guidelines for the safe preparation of hot-smoked fish (Fletcher et al. 1998a) recommending specific refrigeration regimes for the storage of fish that may be susceptible to histamine poisoning and defining thermal processing regimes required for the hot-smoke process. Because New Zealand has also had incidents of listeriosis from hot-smoked seafood, and *Listeria monocytogenes* is more thermally resistant than histamine-producing bacteria (Bremer & Osborne 1995), the recommended thermal regimes were based on *L. monocytogenes*. As this was one of the first publications recommending time temperature regimes for the thermal inactivation of vegetative pathogens in seafood we took a very conservative approach, similar to that used in the canning industry to control *Clostridium botulinum*. Thus, we recommended temperature regimes to achieve an inactivation of $10^{12}$ cells of *L. monocytogenes* which would require a time equivalent to 12 times the D value (a 12 D reduction). However, in subsequent risk-based recommendations, if *C. botulinum* was not the target organism, 6 or 7 D reductions of *Listeria* were recommended (Price & Tom 2002a, 2002b). Our guidelines were distributed to all New Zealand registered producers of hot-smoked fish and relevant regulatory bodies.

As identified in Table 1, the publication and promulgation of these guidelines has not prevented outbreaks of histamine poisoning from New Zealand hot smoked fish. This may be due to producers of smoked fish not complying with the guidelines or it may be due to gaps in the knowledge on which the guidelines were based. One particular such knowledge gap arises because the research on histamine producers was only carried out on isolates obtained from hot smoked fish. These organisms had all survived the hot smoking process or were the result of subsequent contamination. They do not represent the histamine producing bacterial population that may be present on fish before smoking and the possibility remains that a different bacterial flora might be responsible for producing high levels of histamine before smoking. Although these organisms may have been inactivated during the smoking process the histamine produced before smoking may be that associated with histamine poisoning outbreaks from smoked fish.
4 Literature update 2000–10

4.1 Other reviews

There have been over 200 publications related to the problem of histamine in fish published in the scientific literature between 2000 and 2010, including at least five reviews (Lehane & Olley 2000; Mavromatis & Quantick 2002; Shin-Hee et al. 2004; Dalgaard et al. 2008; Al-Bulushi et al. 2009) plus brief summaries in English (Lehane 2000), Spanish (Gonzalez-Dominguez & Cardona-Galvez 2005) and Serbo-Croat (Cvrtila & Kozacinski 2003). The review by Lehane & Olley (2000) (for which I served as one of the journal referees) was a reasonably extensive review of literature (37 pp, 233 references) from a risk perspective covering literature up to 2009. Of particular value was their summary of knowledge on one of the more vexing issues of histamine fish poisoning – why fish that cause histamine poisoning invariably contain high levels of histamine but when histamine is fed to people at similar levels in volunteer studies histamine poisoning does not result. The reviewers hypothesised that cis-urolcanic acid, a different breakdown product of histidine and a recognised mast cell degranulator might play a role in augmenting the exogenous histamine. Mavromatis & Quantick (2002) also only reviewed literature up to 2000 apart from mentioning some surveillance data from post-2000. Reviewing the mechanism of histamine poisoning they 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.” The review by Shin-Hee et al. (2004) also only covered literature up to 2000 (i.e. similar to the review by Lehane & Olley) with the exception of some regulatory information, surveillance data, and some of their own work up to 2003. They conclude that histamine is mostly formed by a few prolific enteric bacteria such as M. morganella and these are best controlled with proper chilling and freezing of fish starting at the point of harvesting. Of the two more recent reviews, that of Al-Bulushi et al. (2009) was quite general, covering a number of biogenic amines and particularly the possibility of producing nitrosamines from them. Their review was based on the view that other amines (putrescine and cadaverine) were required to potentiate histamine 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. The review by Dalgaard et al. (2008) was more detailed and reasonably comprehensive although it was somewhat focused on the work carried out in the European BIOCOM project, which ran from 2004 to 2007. The review by Dalgaard et al. is well worth reading in conjunction with the current review. Their recommendations include:

1. More investigations of outbreaks of histamine 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. If ever 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 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 production by important bacterial species other than the model for *M. psychrotolerans* that was developed under BIOCOM. They have a model for *M. morganella* but this only accounted for temperature, not atmosphere, salt and pH, as included in their model for *M. psychrotolerans*. Section 4.3 of the current review shows that there are many other important histamine-producing bacteria whose growth and histamine production should be mathematically modelled.

4. More studies on the potential of modified atmosphere packaging (MAP) to control histamine poisoning. The BIOCOM project suggested combinations of high CO₂ and high O₂ 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.

4.2 Methods

4.2.1 Extracting biogenic amines from seafood

Standard methods (e.g. AOAC Official Methods 996.07 and 977.13) for extraction of biogenic amines are based on methanol extraction, but this has sometimes resulted in low recoveries from fresh fish. Addition of 25% 0.4N HCl to the 75% methanol-water extraction solvent resulted in histamine recoveries increasing from 54 to 89% in fresh and frozen fish (Richard et al. 2008). Such acidification did not improve the recovery rate in canned tuna, suggesting that protein denaturation might also eliminate matrix interference. Supapun & Wararat (2001) used solid phase extraction to extract histamine from fish sauces. When combined with a high-performance liquid chromatography-fluorescence (HPLC) detection method (see next section), recoveries from spiked samples ranged from 90 to 96%.

4.2.2 Measuring histamine and other biogenic amines

Fluorescence detection of histamine has long been the main analytical technique and currently the most commonly reported method for analysis of histamine is by HPLC detection. For example, the New Zealand Crown Research Institute, ESR uses an HPLC method based on that of van Boekel & Arentsen-Stasse (1987). This allows simultaneous detection of histamine and histidine, which is useful. However, as currently implemented (ESR Auckland Food Group 1996), the ESR method does not allow quantification of other biogenic amines, a procedure that some researchers consider essential to determine whether products cause histamine poisoning (Al-Bulushi et al. 2009). There are a number of methods available to simultaneously quantify a range of biogenic amines in seafood, and a critical review of all of these with a view to
implementing a suitable method is warranted. The EC (Commission of the European Communities 2005) specifies the method of Malle et al. (Malle et al. 1996) with the standard additions method of Duflos et al. (Duflos et al. 1999). Reports on detection and quantification in the last 10 years are reported briefly below.

Various refinements of HPLC technology have been published in the last 10 years, including development of an automated on-line pre-column derivatisation procedure (Jin-Feng et al. 2008) and the addition of nucleophilic agents and surfactant micelles to the system (Larionova et al. 2008). Özogul et al (2002a) used benzoyl chloride in acetonitrile as a derivatisation system and optimised times and conditions with a gradient elution system until it took only 7 min to determine 9 biogenic amines and trimethylamine (TMA). Niu et al. (2008) developed an HPLC method with pre-column derivatisation using a newly synthesised fluorogenic reagent 3-(4-chlorobenzoyl)-quinoline-2-carboxaldehyde with recoveries of 95–107%. Qing-Xi et al (2007) used reverse-phase HPLC with automatic o-phthalaldehyde post-column derivatisation and fluorescence detection. Post-column derivatisation capillary electrochromatography with o-phthalaldehyde/N-acetylcysteine has also been developed to determine biogenic amines (Oguri et al. 2008). A good correlation ($R^2 = 0.99$) was found between capillary electrophoresis with UV detection and gas chromatography methods for detecting histamine in dophinfish (Wen-Xian et al. 2001). A capillary electrophoresis method gave 102% recovery in canned tuna (Paturzo & Bizzozero 2000). A comparison of capillary electrophoresis, the AOAC fluorometric method and GC, showed a very good correlation ($R^2 > 0.99$) for histamine quantification in tuna samples (Du et al. 2002).

Micellular liquid chromatography was reported to give better detection limits than conventional techniques (Paleologos et al. 2003). An alternative method was developed using micellar electrokinetic chromatography along with laser induced fluorescence detection using the amino-reactive chameleon stain Py-1 (Steiner et al. 2009). Derivatisation took 30 min, but was visible to the naked eye because of a colour change from blue to red.

Simple thin layer chromatography methods using ninhiydrin to visualise the biogenic amines are suitable for identification and semi-quantification of histamine, cadaverine, putrescine, tryptamine and tyramine (Valls et al. 2002). Simple colorimetric methods have been developed (Patange et al. 2005; Kuda et al. 2007) and a colorimetric method was modified to detect histamine in fish meal (Kose & Hall 2000).

For laboratories that have the technology, gas chromatography/mass spectrometry (GC-MS) using ethylchloroformate derivative is an option for quantifying histamine and other biogenic amines (Marks & Anderson 2006). Another gas chromatography (GC) method was developed to simultaneously detect histamine and cadaverine, but recovery was only 67% for histamine, compared with 90% for the AOAC fluorometric method (Antoine et al. 2002a). In contrast, a biosensor based on immobilised diamine oxidase gave comparable results to a HPLC method ($R^2 = 0.9612$) when used on spiked prawns with a detection limit of 0.65 mg/kg (Ching Mai et al. 2007). The GC method of Bao-Shyung et al.(2003) was also effective, recovering 99–111% of histamine spiked into shrimp or tuna meat.

There have been a number of rapid methods developed for the successful detection of histamine based on ELISA and immunochromatography techniques (Berges 2008), biosensor enzymatic techniques using histamine oxidase for histamine (Frebort et al. 2000; Hibi & Senda...
2000) or amine oxidase for total biogenic amines (Muresan et al. 2008). ELISA results were in good agreement with HPLC for histamine levels below 50 mg/kg but large differences occurred above this (Vosikis et al. 2008). Japanese researchers have used an oxygen-sensor method that allows detection of both histamine and K-value (a measure of quality) with the advantage of being able to report times when histamine levels exceed histamine defect action levels (500 mg/kg) before spoilage occurred (Ohashi 2002). Some of the rapid methods have been commercialised and Rogers & Staruszkievicz (2000) compared six of these. All six kits tested were found to be acceptable for use as screening tests for histamine and were able to distinguish between products that contained <50 mg/kg and those that contained >50 mg/kg histamine. A recently developed presence/absence rapid method able to detect 15 mg/kg histamine in fish and seafood involved adsorption on to a paper disc, electrophoresis for 10 min, drying and colour development using Pauly's reagent (Sato et al. 2006).

4.2.3  Bacterial methods

Methods for detection of histamine-producing bacteria remain cumbersome with Niven's agar being the main selective agar available. However, more than 80% of presumptive positive cultures on Niven's agar prove to be negative for histamine production (i.e. false positives) (Shin-Hee et al. 2001b; Allen et al. 2005). Shin-Hee et al. (2001b) tried several media designed for other purposes and concluded that the best isolation of prolific histamine producers was obtained when using Pseudomonas isolation agar for Pseudomonads and eosin methylene blue (EMB) agar for enteric bacteria. Histamine production had to be confirmed on histidine decarboxylase differential media (Shin-Hee et al. 2001b). Literature up to 2002 on the detection of histamine-producing bacteria was reviewed by Shin-Hee et al. (2003a) who recommended moving to molecular PCR methods.

HPLC was found to be better than Niven’s agar to determine histamine-producing activity in bacteria, but this required isolating strains and testing them individually (Merialdi et al. 2001). Some isolated Pseudomonas and Acinetobacter spp. were not identified as histamine producers by histidine agar, but were shown by HPLC to produce considerable amounts of histamine in a broth medium (Silva et al. 2002).

Kim et al. (2003) and Shin-Hee et al. (2003b) successfully developed PCR methods to detect one of the main histamine-producing bacteria, M. Morganii, in albacore tuna, mackerel and sardines. The method could detect 10⁵–10⁶ cfu/g (levels reported to be required for histamine production) but with 9 h enrichment at 37°C, levels as low as 9 cfu/g could also be detected (Shin-Hee et al. 2003b). These are promising results but methods would need to be developed to detect histamine producers other than M. morganii. Takahashi et al. (2003) and Torres Alves et al. (2002) each developed PCR methods that amplified histidine decarboxylase genes. All 37 tested strains of histamine-producing Gram negative bacteria produced a PCR product, except Citrobacter braakii, while none of the 470 non histamine-producing strains produced a product (Takahashi et al. 2007). The researchers were then able to identify prolific histamine producers by subsequent single-strand conformation polymorphism (SSCP) analysis (Takahashi et al. 2007). The method of Torres Alves et al. (2002) amplified some unexpected fragments but when sequenced these were found to match the histidine decarboxylase enzyme of the Gram positive bacterium Clostridium perfringens. Thus, their method was not producing false positives but rather successfully identifying bacteria that otherwise might not be expected to be implicated as histamine producers.
Bjornsdottir et al. (2009) compared modified Niven’s agar with a potentiometric and a PCR method and although the latter two gave few false positives, they were not able to detect bacteria that only produced low levels of histamine. These authors concluded that there remains a need for a simple and straightforward yet sensitive method for detecting histamine-producing bacteria in seafood and environmental samples.

4.2.4 Methods to determine fish species involved

In order to know where controls need to be applied, it is important to know which fish are causing outbreaks of histamine poisoning. However, in many outbreaks (e.g. 7 of the 31 in Table 1) the actual species of fish involved remains totally unidentified and in other cases a generic term is applied rather than a specific one. For example, 4 of the 31 outbreaks in Table 1 just referred to tuna, which might include any of at least 9 species present in New Zealand waters (Ayling & Cox 1982) as well as numerous imported species. Lago et al. (2009) developed a genotypic test (FINS – Forensically Informative Nucleotide Sequencing) that could be used to determine the species of fish involved in histamine outbreaks. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was also used to identify the species of the suspected billfish samples in two Taiwanese outbreaks of histamine poisoning (Yung-Hsiang et al. 2007b).

4.3 Bacteria responsible

Production of histamine and other biogenic amines correlates with increasing bacterial numbers (e.g. Wen-Xian et al. 2001), but only a small proportion of the total bacterial flora that grows on fish can produce histamine (e.g. 12% in iced anchovies (Pons-Sanchez-Cascado et al. 2005a)). For bacteria to produce histamine, they must produce the exogenous enzyme, histidine decarboxylase, which converts histidine into histamine. In a review of literature up to 2002 Shin-Hee et al. (2003a) summarised the bacteria considered responsible for histamine production in fish. They identified two main groups, enteric bacteria and marine bacteria, but they considered that enteric *M. morganii* was the main contributor because of its ability to produce high levels of histamine.

Recent years have seen great advances in the ready identification of bacterial isolates by genotypic methods, particularly using PCR amplification for subsequent sequencing of 16S rDNA. This has led to the recognition of new bacterial species that can cause histamine production. Until 2000 most bacteria reported as being responsible for histamine poisoning were mesophiles, mostly of the Enterobacteriaceae family with minor contributions from other groups such as *Pseudomonas*. Recent work supporting this finding includes that of Du et al. (2002) who identified *Morganella morganii, Enterobacter agglomerans, Enterobacter intermedium, Serratia liquefaciens, Proteus vulgaris*, as well as *Pseudomonas fluorescens* as the decarboxylase-positive bacterial species responsible for histamine production in test tuna fillets.

*M. morganii* was the most commonly implicated organism, for example, 28% of bacterial isolates from spoiled Indian anchovies were identified as histamine producers with *Proteus vulgaris, Enterobacter aerogenes* and *Morganella morganii* identified as prolific histamine producers (Sureelak et al. 2005). Kim et al. (2002b) only found what they described as weak histamine formers (*Hafnia alvei, Photobacterium damselae, Acinetobacter lwoffii, Enterobacter cloacae, Enterobacter aerogenes*, all producing less than 350 mg/kg histamine) on fresh albacore tuna although the prolific histamine formers (*M. morganii* producing more than 3000 mg/kg histamine) were found following 3 days at 25°C when counts reached $10^7$ cfu/g.
M. morganii was more common in mackerel and sardines than in tuna (Kim et al. 2003). The most prolific and prevalent histamine former in Pacific mackerel was M. morganii, followed by Proteus vulgaris (Shin-Hee et al. 2000; Shin-Hee et al. 2001a). Histamine production by M. morganii was reported to be maximal for stationary phase organisms (≥10⁶ cfu/g) at 25°C and the organism did not grow or produce histamine at 4°C (Shin-Hee et al. 2000; Kim et al. 2002a), results comparable to those found in our work (see Section 3.4). M. morganii can also produce cadaverine, putrescine and phenylethylamine in tuna infusion broth, biogenic amines that have been proposed to act as potentiators for histamine poisoning (Shin-Hee et al. 2000). However, working with tuna flesh, Veciana-Nogues et al. (2004) observed that M. morganella produced cadaverine but not putrescine, although both were observed in bluefish (Pomatomas saltatrix). M. morganella was most frequently present in the Gill followed by the skin but rarely in the intestine and body cavity. The bacterium was not found in the processing plant, but was detected on the surfaces of conveyor belts and plastic totes during processing (Kim et al. 2003). It was considered endogenous to fish (Kim et al. 2003). Although S. liquefaciens showed lower histamine production than M. morganii in synthetic medium, the reverse was true in a medium formulated from tuna muscle protein extracts (Guillén-Velasco et al. 2004). This is the reverse of what we found in our medium derived from kahawai extracts where M. morganii was more prolific than Serratia (Section 3.4.2). However, as in our work, the authors of that study did suggest that high histamine production might be related to bacterial stress (Guillén-Velasco et al. 2004).

Other studies implicating members of the Enterobacteriaceae did not find M. morganii. Min-Ki et al. (2009) found Enterobacter aerogenes to be the main organism producing large amounts of histamine in Korean fish although other histamine-producing Enterobacter were also present. Enterobacteriaceae (Citrobacter freundii, Enterobacter agglomerans, Serratia liquefaciens) were also the bacteria with the highest histamine-producing activity in Atlantic mackerel (Scomber scomber) (Merialdi et al. 2001) and most histamine producers in Brazilian shrimp belonged to the Enterobacteriaceae family (Sousa Andrade et al. 2008). Proteus, Enterobacter, Klebsiella, Rahnella and Acinetobacter, were identified as histamine producers in Taiwanese sailfish (Yung-Hsiang et al. 2004). In contrast to other research, showing histamine producers to only contribute a small part of the microflora, Özogul & Özogul (2005) reported that the majority of bacterial strains isolated from fresh, spoiled and vacuum or MAP herring produced histamine in a medium supplemented with histidine. Highest histidine decarboxylase activities were observed from Klebsiella oxytoca, Hafnia alvei and Proteus vulgaris, which produced 396, 232 and 54 mg/kg histamine, respectively (Özogul & Özogul 2005).

There have also been reports of mesophilic bacteria other than the well recognised Enterobacteriaceae potentially causing histamine poisoning. Working with mahimahi (dophinfinh) and yellowfin tuna, Allen et al. (2005) isolated the Gram positive Staphylococcus kloosii as well as Enterobacter cloacae and three other Gram negative bacterial species capable of producing histamine. These bacteria only produced low levels of histamine and only at temperatures above 15°C. Despite the presence of histamine-producing bacteria, no detectable levels were found in the composite fish muscle samples analysed even though they report 60% of the yellowfin tuna harvested did not meet the US FDA’s regulatory HACCP guidelines for temperature reduction (Allen et al. 2005). In line with our work (Section 4.1) this suggests that, although these organisms are capable of producing histamine, in practice they may not do so in seafood. In contrast to the findings of Allen et al. (2005), Pons-Sanchez-Cascado et al. (2005a) found that although E. cloacae produced putrescine and cadaverine, it did not produce histamine. Similarly, none of the Gram-positive catalase-positive cocci that they isolated from iced anchovies showed aminogenic activity, although all enterococci isolates did
(Pons-Sanchez-Cascado et al. 2005a). Although most commonly associated with perfringens food poisoning, *Clostridium perfringens* can also produce histamine (Torres Alves et al. 2002). While confirming *M. morganii* as the most prolific histamine producer in tuna, Economou et al. (2007) also found that *Staphylococcus hominis*, *Enterococcus hirae* and *Klebsiella oxytoca* produced high levels of histamine. In dried milkfish using 16S rDNA sequencing with PCR amplification, histamine producers were identified as *Staphylococcus sciuri* subsp. *sciuri*, *Serratia grimesii*, *Bacillus cereus* and *Raoultella ornithinolytica* (Yung-Hsiang et al. 2006). *R. ornithinolytica* was a potent histamine-former capable of producing more than 800 mg/kg of histamine in broth in the presence of 1.5% or 3.5% NaCl and this was suggested as the causative agent of an outbreak in dried milkfish (Yung-Hsiang et al. 2007a). Although *Bacillus subtilis* was the only histamine producing bacteria isolated from marlin fillets implicated in an outbreak researchers suspected that other bacteria might have been involved because *B. subtilis* only produces low levels of histamine (Chen et al. 2010). *K. oxytoca* produced cadaverine over a wide range of temperatures but only produced histamine at room temperatures (Veciana-Nogues et al. 2004). Hsiu-Feng et al. (2008) identified *Staphylococcus carnosus* as a histamine producer in salted mullet roe while *Enterobacter* spp., *Pantoea agglomerans*, *Klebsiella variicola* and *Serratia marcescens* were implicated in tuna dumplings (Hwi-Chang et al. 2008). Hsiu-Hua et al. (2009) identified *Enterobacter aerogenes*, *Citrobacter* sp., *Staphylococcus xylosus*, *Staphylococcus sciuri*, *Bacillus thuringiensis*, *Citrobacter freundii*, *Klebsiella pneumoniae* and *Enterobacter cloacae* as histamine-producing bacteria found in dried monkfish. Ben-Gigirey et al. (2000) isolated *Stenotrophomonas maltophilia* strains from tuna that exhibited histidine carboxylase activity although only low levels (<25 mg/kg) of histamine were produced. *Stenotrophomonas maltophilia* was previously classified as a *Pseudomonas* species so could possibly be one of the organisms classified in our study as a Pseudomonad not likely to contribute significantly to histamine production in practical situations (Section 3.4). Silva et al. (Silva et al. 2002) found that most histamine-producing bacteria isolated from Portuguese vacuum-packed, cold smoked fish tended to produce histamine at 25°C but not at 5°C although histamine production by lactic acid bacteria was similar at both temperatures.

The source of the mesophilic bacteria responsible for histamine production has been shown not to be from fishing vessels or fish processing facilities (Gingerich et al. 2001). They are normally considered to be natural parts of the microflora of gills, and guts of saltwater fish that contaminate the flesh after death with contamination being aggravated if left in the water after death or during processing (e.g. butchering or filleting) (Food and Drug Administration 2001). Lokuruka et al. (2006) found that both skin and guts were potential sources of histamine producing bacteria and contamination from skin was more important than that from gutting in Atlantic mackerel.

As well as mesophilic bacteria, it has long been recognised that a few psychrotrophic organisms are implicated (Okuzumi et al. 1982). Recently Danish workers have identified some of these psychrophiles. Initially they reported the psychrotrophic *Photobacterium phosphoreum* and a psychrotrophic *Morganella*-like organism (later named *Morganella psychrotolerans* (Emborg et al. 2006) as being responsible for histamine production in Sri Lankan chilli-marinated tuna when stored at 1–3°C (Emborg et al. 2005). They also reported both of these organisms as being responsible for an outbreak from cold smoked tuna (Emborg & Dalgaard 2006) and *P. phosphoreum* as being responsible for producing high levels of histamine in garfish stored at 5°C (Dalgaard et al. 2006). *P. phosphoreum* is well known to be a major spoilage organism of fish packaged under MAP: although its growth is reduced by CO₂, histamine production was found to be higher in an atmosphere containing 60% CO₂/15% O₂/25% N₂ than in air (Lopez-
Caballero et al. 2002). Although most organisms that produce histamine only do so under certain conditions (e.g. see Figures 3–6), *M. morganii* and *M. psychrotolerans* appear to produce it whenever present in sufficient numbers and where histidine is present as a substrate. On this basis, Dalgaard and co-workers have modelled the growth and histamine production of *M. morganii* under different temperature regimes (Emborg & Dalgaard 2008a) and *M. psychrotolerans* under different temperatures, atmospheres, salt levels and pH (Emborg & Dalgaard 2008a): these two models are freely available in user friendly software (Dalgaard 2009). The combined temperature models for the two organisms show *M. psychrotolerans* to be the main histamine producer under refrigeration conditions while *M. morganella* is the main organism responsible under temperature abuse situations (Dalgaard 2009).

The histamine-producing capability of *P. phosphoreum* has been attributed to two histidine decarboxylases (one constitutive and one inducible enzyme) that have been isolated and separated from cell-free extracts using GC (Morii & Kasama 2004). Although cell-free extracts of the constitutive and inducible enzymes had higher activity in cultures grown at 7°C, 5% NaCl and pH 7.5 and 6.0 respectively, the activity of the enzymes were optimum at 0% NaCl, temperatures of 30°C and 40°C respectively and at pH of 6.5 and 6.0 respectively. The differences in these two enzymes extended the histidine decarboxylase activity range of *P. phosphoreum* to a wide range of conditions (Morii & Kasama 2004).

Zihhua et al. (2009) found that *M. morganii* and *P. phosphoreum* behaved differently with regard to diffusion of histamine in tuna. Tuna inoculated with *M. morganii* and incubated at 25°C produced high levels of histamine (4000 mg/kg) at the point of inoculation but lower levels (ca 2000 mg/kg) at points 0.5–2.5 cm away from the point of inoculation. In contrast *P. phosphoreum* produced 1000–2000 mg/kg in all positions. At 20°C *M. morganii* produced less than 2000 mg/kg at the point of inoculation and less than 100–1000 mg/kg at more distant points while *P. phosphoreum* produced at 2000 mg/kg at the point of inoculation and 200–1000 mg/kg at more distant points. The researchers postulated that histamine production was preceded by the spread of bacteria, but did not confirm whether diffusion of exogenous enzymes could have caused a similar result. Each bacterial species showed a pattern of increasing histamine levels with time followed by some decreases (Zihhua et al. 2009). This decrease may be due to the action of histamine oxidases that can break down histamine.

Bermejo et al. (2003) reported that the organisms producing the greatest amount of histamine in jack mackerel were *Proteus vulgaris*, *Aeromonas hydrophila* and *Photobacterium damselae*: at least the latter two are psychrotrophic bacteria. They report that histamine production was proportional to the growth rate of the whole bacterial population when measured at 25, 15 and 5°C (Bermejo et al. 2003, 2004). This reflects our New Zealand work where we found total bacterial counts were a reasonable indicator of risk of high histamine levels (Fletcher et al. 1995). Bermejo et al. found histamine levels to increase and then decrease after long periods of storage, presumably due the actions of amine oxidases (Bermejo et al. 2004).

The halophilic *Tetragenococcus muriaticus* was shown to be able to produce histamine under the low pH and high salt conditions found in fish sauce (Kimura et al. 2001). *Pantoea* spp., *Enterobacter cloacae* and *P. agglomerans* were also identified as histamine-producing bacteria in Taiwanese salted mackerel (Yung-Hsiang et al. 2006). Satomi at al. (2008) identified another *Tetragenococcus* species, *T. halophilus* as producing histamine in fish sauce and further showed that its histidine decarboxylase gene was located on a plasmid. They conclude that histidine decarboxylase genes could be encoded on transformable elements of lactic acid...
bacteria. In contrast Lakshmanan et al. (2002) did not find any histamine-producing bacteria among the halophilic bacteria that they isolated during salt-drying of sardines.

4.4 Products and handling practice effects

Products that are implicated in histamine poisoning usually contain levels of more than 500 mg/kg histamine in their flesh (Dalgaard et al. 2008). However, some incidents have been caused by fish with less than 200 mg/kg histamine, e.g. a recent outbreak in Japan was attributed to histamine where only 4–74 mg/kg histamine were recorded (Kan et al. 2000). Dalgaard et al. (2008) suggest that this apparent discrepancy might be explained through sampling protocol: in most cases the actual portion of fish being consumed is not tested for histamine but rather other fish or portions from the same batch. They cite the work of Frank et al. (1981) showing that whilst the anterior dorsal region of tuna might have a histamine level of 1020 mg/kg, a portion from the tail section might only contain 29 mg/kg. Work on other species has shown similar levels of variation (Dalgaard et al. 2008). The question as to why dietary histamine causes food borne illness when consuming pure histamine is not toxic was reviewed by Lehane & Olley (2000), Mavromatis & Quantick (2002) and Dalgaard et al. (2008) with different conclusions (see Section 4.1). One hypothesis is that seafood products contain a mast cell degranulator and that it is not the histamine in seafood per se that is directly contributing to the condition while another is that the presence of other biogenic amines found in seafood potentiates the toxicity of histamine by inhibiting the normal histamine metabolising enzymes. The latter hypothesis has been supported by the fact that patients taking the drug isoniazid, which is known to inhibit histamine-metabolising enzymes, had increased sensitivity to histamine in food (Miki et al. 2005).

Tuna continues to be the main cause of histamine poisoning internationally, often as a result of imported product, for example tuna from Indonesia and Vietnam causing outbreaks in the USA (Davis et al. 2007). There was an unseasonal increase in outbreaks associated with tuna in the UK in the 6 months between December 2004 and June 2005 with 16 reported outbreaks compared to 56 for the 12-year period between 1992 and 2004 when outbreaks were more frequent in the summer (Anon. 2005). These outbreaks appeared to be due to wholesale tuna (often vacuum packed) being temperature-abused at catering premises. In a 1998 tuna associated outbreak in Pennsylvania, although the tuna may have had some temperature abuse before processing, indications were that the scombrotxin formed at the restaurant where it was consumed (Maher et al. 2000). Processed tuna has also been implicated a number of US poisoning cases involving tuna burgers as well as tuna salad and fillets (Becker et al. 2001). For the most part these cases are also related to temperature abuse by restaurants (Becker et al. 2001). Elevated levels of histamine were recorded in skipjack tuna burgers stored for 4 weeks at 5°C (Mahendradatta 2003).

Other well known scombroid and non-scombroid fish that accumulate high levels of histamine include bonito (Sarda sarda); mackerel (Scomber japonicus peruanus) and dophinfish (Coryphaena hippurus) (Gonzaga et al. 2009). Dophinfish are found in New Zealand as are species of the other two genera. Anchovies (Engraulis encrasicolus) and sardines (Sardina pilchardus) developed high levels of histamine (>1000 mg/kg) within 24 h at 25°C although only 38 mg/kg developed in mackerel (Visciano et al. 2004; Visciano et al. 2007). Canned anchovies were also reported to occasionally contain histamine levels above 1000 mg/kg with 20% of samples exceeding 500 mg/kg (Kim et al. 2004; Hyoungill et al. 2005).
A survey of 17 South African seafood species showed that *Thrysites atun* (snoek known as barracouta or snake mackerel in New Zealand) and *Seriola lalandi* (yellowtail) are the primary fish species in South Africa posing a histamine poisoning risk for consumers (Auerswald et al. 2006). Both of these species contained elevated levels of free histidine, and yellowtail was also identified in several histamine poisoning outbreaks in South Africa. With a measured histidine level of 250 mg/kg, we listed barracouta as of a lower risk when considering which species had potential to cause histamine poisoning in New Zealand (Fletcher et al. 1995). We note that a species related to South African yellowtail (*Seriola grandis* – kingfish) has been implicated in New Zealand cases (Foo 1975a) and another related species, *Seriola hippos* (Samson fish from Australia), occasionally occurs in New Zealand waters (Ayling & Cox 1982). The level of histamine exceeded the legal limit (50 mg/kg) in eel (*Anguilla anguilla*) stored without ice after 6–7 days and, in ice, after 13–14 days of storage, when eels were rejected by the sensory panel. Thus, native eels may be another potential source of histamine poisoning in New Zealand.

Two outbreaks of histamine poisoning were caused by species of billfish (*Makaira nigricans* and *Xiphias gladius*) in Taiwan with each product containing histamine levels greater than 1500 mg/kg (Yung-Hsiang et al. 2007b). These game fish species (known locally as blue marlin and broadbill swordfish respectively) also occur in New Zealand: presence of histamine-producing bacteria in these species may account for some of the outbreaks attributed to swordfish and marlin in New Zealand (Section 2). In another reported outbreak in Taiwan, the implicated blue marlin only contained about 450 mg/kg histamine (Chen et al. 2010). Histamine was recorded at levels of up to 288 mg/kg in sailfish (*Istiophorus platypterus*) in Taiwan (Yung-Hsiang et al. 2004). This game fish species occasionally occurs in northern New Zealand (Ayling & Cox 1982). Milkfish (*Chanos chanos*) also caused an outbreak in Taiwan (Yung-Hsiang et al. 2007a) and was a better substrate for histidine decarboxylase than sailfish (Yung-Hsiang et al. 2005c), but this genus is not known in New Zealand (Ayling & Cox 1982).

Histamine at levels greater than or equal to 50 mg/kg was detected in 10% of Japanese retail seafood with levels reaching 3400 mg/kg (Kan et al. 2005). Of the positive samples, 35% were of semi-dried round and split sardines. Venezuelan results showed that some sardine batches arriving at the processing plants contained up to 5 mg/kg histamine due to temperature abuse during transportation (Rosas & Reyes 2009). Control of time and temperature during transportation and processing was recommended to manage this. Only 9% of tested Austrian retailed fish products exceeded histamine levels of 50 mg/kg (Paulsen et al. 2000) and none exceeded their national limits of 200 mg/kg (Anon. 1993). In Greece 15% of tested retail samples exceeded 50 mg/kg (Vosikis et al. 2008) with highest levels found in herring and anchovy samples (Vosikis et al. 2008). Temperate Atlantic mackerel (*Scomber scombrus*) produced higher levels of histamine than tropical fish (Lokuruka & Regenstein 2004).

Escolar fish (*Lepidocybium flavobrunneum*) caused an outbreak of histamine poisoning in the USA: people consuming less than 215 mg histamine experienced fewer symptoms that were of shorter duration than those consuming more fish and thereby more histamine (Feldman et al. 2005). Escolar has also been recorded as the cause of outbreaks in Denmark along with garfish (*Belone belone* belone) and imported tuna products (Dalgaard et al. 2008). Dried milkfish (*Chanos chanos*) in Taiwan commonly exceeded acceptable histamine limits with 44% of retail samples exceeding 500 mg/kg (Hsiu-Hua et al. 2009). At ambient temperatures (32°C) tropical species of mackerel (*Rastrigillier kanagurta*), sardine (*Sardinella fimbriata*) and trevally (*Carangoides armatus*) exceeded histamine levels of 50 mg/kg after 12, 15 and 15 h respectively even though sensory properties remained acceptable (Jeya Shakila et al. 2003).
Tropical parrotfish (Callyodon gutatus), barracuda (Sphyraena japonica) and scavengerfish (Lethrinus nebulosus) showed no evidence for potential histamine poisoning while skipjack tuna (Euthynnus pelamis), rabbitfish (Siganus oramin) and little mackerel (Rastrelliger kanagurta) did (Lokuruka & Regenstein 2004). Histamine levels in sea bass (Dicentrarchus labrax) fillets remained below 30 mg/kg during 16 days’ storage in ice (Paleologos et al. 2004). None of these potentially scomberotoxic fish genera occur in New Zealand (Ayling & Cox 1982) but they could be imported.

The NZFSA (2009) explicitly identifies the following fish species imported into New Zealand as potentially problematic from a histamine poisoning perspective: tuna (all species), mackerel (Scomber scombrus, Scomber australasicus, Scomber japonicus), amberjack (yellowtail kingfish) (Seriola lalandei), mahimahi (Coryphaena hippurus), bluefish (Pomatomus saltatrix), sardine including pilchard (Sardinia pilchardus, Sardinops spp., Sardinella spp.) and herring (Clupea harengus, Clupea pallasii). From the review above at least escolar, garfish, swordfishes and billfishes should be added to this list.

Fish such as salmon, which contain low levels of histidine, do not develop high levels of histamine, even if they contain high levels of histamine-producing bacteria such as P. phosphoreum (Embrog et al. 2002). Histamine levels remained low during 33 days’ refrigerated storage of hake (Merluccius merluccius L.) under different controlled atmospheres (Ruiz-Capillas & Moral 2001) and when stored in air for up to 24 h at 25°C (Visciano et al. 2004). Sole also developed very low levels of histamine (Visciano et al. 2004). Seer fish (Scomberomorus spp.) are similarly reported to only develop low levels (<4000 mg/kg) of histamine (e.g. Scomberomorus commersonii developed less than 100 mg/kg in 10 days at 5°C (Jeya Shakila et al. 2005b)) and therefore to not cause histamine poisoning compared to skipjack, which develops high levels (>10,000 mg/kg) (Thadhani et al. 2002). Although histamine-producing bacteria have been found in a number of freshwater fish (Ferraz de Arruda Silveira et al. 2001), low levels of histidine substrate in these fish mean that it is considered unlikely that they will ever be the cause of histamine poisoning. In our work (Section 3.1.1) we came to similar conclusions: only New Zealand fish with histidine levels above 10,000 mg/kg had caused actual outbreaks of histamine poisoning. However, we suggested fish that contained histidine at between 2000 and 10,000 mg/kg may have potential to cause histamine poisoning and, subsequently, trevally in which we measured histidine at 6300 mg/kg has caused an outbreak (Table 1). In contrast to the above, Kim et al. (2002a) found that M. morganii inoculated on to salmon (species not specified) produced histamine at levels of up to 4500 mg/kg and Jeya Shakila & Vasundhara (2002) also found unacceptable levels of histamine to develop in Indian freshwater carp after 6–12 h at 30°C or 3–5 days at 5°C. Levels of histidine were higher in the white muscle of dophinfish and tuna than the red muscle (Antoine et al. 2001), which agrees with our findings in New Zealand fish (Fletcher et al. 1995)

Korean retail fish, squid and shellfish all contained low levels of histamine (<50 mg/kg) (Min-Ki et al. 2009). Qing-Xi et al. (2007) also found low histamine levels in squid and white prawn during refrigerated storage. However, Chinese mitten crabs were reported to contain up to 180 mg/kg histamine after 24 h at 20°C (Yanshun et al. 2009) so under some circumstances histamine can exceed regulatory levels and might become toxic to humans.

Processed specialty fish products can also develop increased levels of histamine. These include marinated sardines (Gokoglu 2003; Gokoglu et al. 2003) and anchovies (Olgunoglu et al. 2009), anchovy paste (Pirazzoli et al. 2006), brined Spanish mackerel (Orawan & Pantip 2000), salted mackerel (Yung-Hsiang et al. 2005a), salted roe (Hsien-Feng et al. 2008), missoltini (salted air
dried twaite shad, *Alosa argentea* (Pirani et al. 2010), budu (a Malaysian fermented mixture of anchovies and salt) (Rosma et al. 2009), (Egyptian salted-fermented fish) (Rabie et al. 2009), fermented smoked fish (Petaja et al. 2000), Myeolchi-jeot (a Korean salted fermented fish product) (Jae-Hyung et al. 2002), dried fish (Hsiu-Hua et al. 2009), fish dumplings (Hwi-Chang et al. 2008), fish sauce (Poonsap 2000; Kimura et al. 2001; Jiang et al. 2010), fish-nukazuke (a Japanese salted and fermented fish with rice-bran) and Indonesian lawa teri (fresh anchovy mixed with citrus juice or vinegar and fried coconut) (Mahendraadatta 2003). Korean smoked and seasoned-dried Pacific saury (*Cololabis saira*) developed histamine levels of 120 mg/kg after 80 days at 19±5°C (Yong-Jun et al. 2001). Although not usually commercially produced in New Zealand, any of these products may be imported for local ethnic communities and importers need to be aware of the potential for histamine poisoning from such products. Ethnic communities may also start producing these types of products from New Zealand raw materials with potential for histamine formation. Dalgaard et al. (2008) made the point that many of these products are strongly flavoured so are usually only consumed in small quantities, which may explain the low level of food poisoning history attributed to them. An outbreak attributed to salted milkfish did occur in 2006 (Yung-Hsien et al. 2006). Working in China, Jiang et al. (2010) found histamine levels ranging between 14 and 8400 mg/kg in fish sauces. This may be of concern as imported fish sauces are commonly used in Asian cooking in New Zealand but again, only small quantities are usually consumed.

In contrast to the products just mentioned, histamine levels were reported not to exceed 50 mg/kg in salted tuna roe (Periago et al. 2003). Although histamine-producing bacteria increased during the salting process and some histamine formed during the first week of storage at 30°C, properly stored salted tuna roe was not considered a serious health risk (Periago et al. 2003). Similarly, while levels of histamine increased during the ripening of salted anchovies they did not exceed 20 mg/kg (Pons-Sanchez-Cascado et al. 2005b). However, Australia recently had a product recall due to histamine in dried whole anchovies imported from Vietnam (Food Standards Australia New Zealand 2009).

Although there is speculation that biogenic amines form precursors for carcinogenic N-nitrosamines in salted and dried fish (Al-Bulushi et al. 2009), these were not produced from histamine *in situ* during heating with nitrites in a traditional Korean fermented anchovy dish (Jae-Hyung et al. 2005). However, N-nitrosamines were produced from other biogenic amines (putrescine and spermidine) (Jae-Hyung et al. 2005).

4.5 Time temperature considerations

The development of histamine is dependent on both storage time and temperature, with shorter times being required at higher temperatures to reach a particular level of histamine. In experiments, the formation of unacceptable levels of histamine has traditionally been associated with storing product at unacceptably high temperatures for relatively long periods. For example, Korean amberjack, mackerel, saury and Spanish mackerel did not show increases of histamine until after 2 days’ storage at 7 or 10°C while little or no increases were observed during 7 or 9 days at 4°C (Min-Ki et al. 2009). Similarly tuna did not exceed 50 mg/kg histamine after a whole day at 10 or 22°C (Du et al. 2002) or 6 h at 30°C (Jeya Shaila et al. 2005a). Average histamine levels were recorded at 91 mg/kg after just 9 days at 4°C (Du et al. 2002), a more likely commercial scenario in New Zealand. Histamine levels in tuna chunks containing relatively high levels of histamine-producing bacteria were reported to increase from 4.5 to 46.6 mg/kg after just 48 h in ice (Jeyasekaran et al. 2006). In dophinfish at 26°C, more than12 h of incubation was required before a histamine concentration of 50 mg/kg was reached while at 35°C this level
was formed within 9 h (Staruszkiewicz et al. 2004). Histamine levels exceeded 500 mg/kg within an additional 3 h of incubation at 35°C and similar results were found for skipjack and yellowfin
tuna (Staruszkiewicz et al. 2004). Although histamine is formed by the decarboxylation of
histidine in fish, there was poor correlation between decreases of histidine in dolphinfish and
increases in histamine (Antoine et al. 2002b). Eight days’ storage at 5°C only resulted in 6
mg/kg histamine in tuna but at 20 and 30°C, histamine formation in mackerel and tuna
exceeded 500 mg/kg within 1 and 2 days respectively (Ohashi 2002). Similarly histamine levels
exceeded 100 mg/kg in Atlantic mackerel held at 25°C for 24 h but not when held at 4°C for 3
days (Merialdi et al. 2001). Histamine levels in Indian anchovies remained below 20 mg/kg
during 15 days’ storage in ice but were around 200 mg/kg after 32 h at 15°C and 8 h at 35°C
(Sureelak et al. 2005). Histamine levels in black skipjack tuna remained below 50 mg/kg during
24 days’ storage in ice (Mazorra-Manzano et al. 2000). Tropical barracuda (Sphyraena
barracuda) did not show major increases in histamine-producing bacteria during 6 h at an
ambient temperature of 32°C although sensory shelf-life was decreased by 6 days
(Jeyasekaran et al. 2004). Auerswald et al. (2006) reported that histamine levels in barracouta
exceeded 500 mg/kg after 4 days at 4°C (implicating psychrotrophic bacteria) and 1 day at
30°C. Shin-hee et al. (2001a) found that significant amounts of histamine were produced in
Pacific mackerel stored for up to 14 days at 1°C but not 0°C and the optimum temperature for
histamine production was 25°C.

Repeated short times at high temperatures can have can have a cumulative impact on
histamine production, somewhat similar to that predicted from continuous storage at high
temperatures for a similar time period. For example, tuna loins stored for 2 h periods at 20°C
each day gave histamine levels of 260 mg/kg after 12 days when stored the rest of the time at
0–2°C and gave levels of 430 mg/kg after 4 days’ storage at 6–7°C with daily 2 h periods at
30°C (Economou et al. 2007).

The rate of histamine production appeared to be higher in summer-caught mackerel compared
to winter-caught fish when stored under refrigerated temperatures (3 rather than 4 days to
exceed 100 mg/kg) (Vusilovic et al. 2008). Similar results were found when fish were stored at
ambient temperatures (2 rather than 3 days to exceed 100 mg/kg for summer- and winter-
caught mackerel), but presumably the ambient temperature was higher in summer (Vusilovic et
al. 2008). In Peru, fish purchased from retail markets later in the day (with longer storage times)
had higher histamine levels than those purchased earlier (Gonzaga et al. 2009).

Among processed products, Mahendradatta et al. (2003) found Lawa teri (fresh anchovy mixed
with citrus juice or vinegar and fried coconut) to give the highest levels of histamine (230 mg/kg)
during 2 weeks’ storage at just 5°C. Feseekh (Egyptian salted-fermented fish) was safe during
40 days’ storage but exceeded tolerance limits (200 mg/kg) by 60 days (Rabie et al. 2009).

Strong correlations have been made between sensory deterioration and the levels of histamines
and other biogenic amines, e.g. in dolphinfish (Antoine et al. 2004). Consequently, levels of
biogenic amines have been used as a useful objective quality indicator: for a number of fish
species they increase as sensory quality decreases (e.g. Jeya Shakilla et al. 2001; e.g. Antoine
et al. 2004). In bigeye tuna steaks (Thunnus obesus) and whole skipjack tuna (Katsuwonus
pelamis) cadaverine appeared prior to and/or accumulated at a faster rate than histamine so
was proposed as an index of decomposition either alone or in combination with histamine
(Rossi et al. 2002). Staruszkiewicz et al. (2004) also noted that cadaverine levels in dolphinfish
and tuna increased before histamine. However, K. oxytoca produced cadaverine over a wide
range of temperatures but only produced histamine at room temperatures (Veciana-Nogues et
al. 2004) so using cadaverine as a pre-emptive predictor of histamine would likely result in many false positive predictions. Guizani et al. (2005) reports that yellowfin tuna (Thunnus albacares) was rejected by sensory panellists before it reached toxic histamine levels. These authors recorded unacceptable levels of histamine after just 1 day at 20°C or 4 days at 8°C. At 0°C they recorded declines in histamine levels. Whole ungutted sardines (Sardina pilchardus) were reported to develop histamine levels above legal limits after 7 days’ storage in ice but the sardines were rejected by sensory panels by that time (Erkan & Ozden 2008). Tuna fillets stored for 9 days at 22°C were considered unacceptable from a sensory perspective. However, a sensory panel had not rejected tuna stored for 3 days when histamine averaged 1000 mg/kg (Du et al. 2002). Up to 75% of an experienced untrained panel rated the odour of bluefish (Pomatomus saltatrix) that was either inoculated or uninoculated with M. morganii as acceptable (Lorca et al. 2001). These results confirm our findings that while spoilage usually occurs as histamine levels increase, sensory evaluation is sometimes but not always sufficient to assure safety (Fletcher et al. 1995).

4.6 Controls

4.6.1 Rapid refrigeration

The most commonly recommended method for preventing histamine poisoning is to rapidly chill susceptible fish to slow down or prevent the growth of the bacteria that produce histamine. The most commonly applied recommendations in this regards 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 h of death, or placed in refrigerated seawater or brine at 10°C or less within 9 h 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 h 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 h of death.

It is quite possible that some of the products causing problems in New Zealand are through the first recommendation not being met. Based on research with tuna, Lampila & Tom (2009) indicate that the safe shelf-life can be as little as 5 to 7 days for product stored at 4.4°C and any exposure time above 4.4°C significantly reduces the expected safe shelf-life. In some instances New Zealand fish that have caused outbreaks may have been stored longer than this or at higher temperatures before being smoked. However, work on kahawai suggests that as long as temperatures do not exceed 4.4°C, this fish can be stored for longer periods without increases in histamine (Section 3.2).

Tuna left hooked on the line for up to 20 h were suspected to have contributed to an outbreak of histamine poisoning in Pennsylvania (Maher et al. 2000). It is quite possible that the time between capture and landing on a boat also contributes to the high number of poisonings from kahawai in New Zealand. This fish is usually caught by gillnetting from small fishing dories and the fish may be left in the net for considerable time before landing on the boat and being iced. Histamine (or at least high levels of histamine-producing bacteria) and perhaps histidine decarboxylase may be produced during this time. That many of the outbreaks occur in summer when water temperatures are higher would support this hypothesis. The effect on levels of
histamine and histamine producing bacteria of the time between capture and landing on the boat should be researched. The effect of fish struggling in the net should also be investigated.

4.6.2 Heading, gutting and skinning

As many of the naturally occurring histamine-producing bacteria are present on the gills, skin and in the guts of freshly harvested fish, early removal of any of these may delay histamine production. For example, delayed gutting increased histamine development during the ripening of European anchovies (Engraulis encrasicolus) (Pons-Sanchez-Cascado et al. 2003).

4.6.3 Heat processing

Histamine is relatively heat-stable and is not eliminated by canning: canned products are recorded to contain high levels of histamine on occasion (>1000 mg/kg) in some markets (Erkan et al. 2001; Hyoungill et al. 2005) and have caused outbreaks of food poisoning in New Zealand (Foo 1975b). Canned mackerel was responsible for a 2001 outbreak of histamine poisoning in Taiwan and incriminated product contained 1540 mg/kg histamine (Yung-Hsiang et al. 2005b). The histamine in canned products is presumably formed before canning in that histamine forming bacteria are not detected in the canned products (Hyoungill et al. 2005). However, although not eliminated, histamine levels are definitely reduced by canning (Baygar & Gokoglu 2004) so heat processing does mitigate the risk to some degree.

Heat processing below canning requirements can be used to inactivate histamine-producing bacteria before histamine is formed as described for hot smoked fish in Section 3.3. Emborg & Dalgaard (2008a) determined the inactivation dynamics of M. morganii and M. psychrotolerans in Luria Bertani broth with amino acids and lactic acid added to match the levels found in tuna. Predictably, the psychrotrophic M. psychrotolerans was more heat-sensitive than the mesophilic M. morganii (Emborg & Dalgaard 2008a) with D values of 5.3 min and 13.1 min and z-values of 6.8 and 7.2°C respectively. These M. morganii results suggest more heat resistance than we found working with M. morganii associated with hot-smoked kahawai (Osborne & Bremer 2000). Components of the hot-smoked kahawai might have sensitised the cells to heat inactivation.

4.6.4 Smoked and salted fish

Little work has been done internationally on hot-smoked fish. Zotos et al. (2001) concluded that histamine levels did not exceed 75 mg/kg immediately after smoking tuna under a number of different configurations, but they did not appear to test increases in histamine during storage despite storing vacuum packed product for 3 months at 5°C. Histamine levels in yellowfin tuna were reported to increase to 12 mg/kg during mild hot-smoking (70°C, 2 h) whilst numbers of amine-producing bacteria decreased but were not eliminated (Shakila et al. 2003). Predominant amine-forming bacteria identified were Micrococcus, Alcaligenes and Corynebacterium. While the temperature within the smoker was monitored, these researchers (Shakila et al. 2003) did not measure actual product temperatures, and it is likely that product temperatures did not reach the levels we 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, which is possibly the case in New Zealand as well.
Emborg & Dalgaard (2006) found that cold-smoked tuna that caused outbreaks had low (1.3–2.2%) water phase salt (WPS) compared to other commercially available product (4.1–12.7%). They found that although *M. psychrotolerans* grew at a WPS of 4.4%, at 6.9% the microflora was dominated by benign lactic acid bacteria and neither *M. psychrotolerans* nor *P. phosphoreum* grew. They therefore recommended WPS of >5% to prevent histamine formation. This is quite high for New Zealand smoked products and goes against dietary recommendations to reduce salt intake. For hot-smoked products we would generally recommend relying on the heat process to eliminate histamine-producing bacteria rather than adding high levels of salt to prevent their growth (Fletcher et al. 1998a). Hazard analysis and critical control point (HACCP) plans to control storage temperatures and fish quality controls to ensure that fish has not been stored for too long before smoking are also required to prevent histamine forming before smoking.

Polish salted herrings prepared in high salt (26%) brines did not produce any histamine when stored at 4 or 22°C, and those prepared in low salt (16%) brines only produced 35 mg/kg during 3 weeks of storage (Fonberg-Broczek et al. 2003). When salting Spanish mackerel at fish:salt levels of 1:1, 2:1 and 3:1 by weight, Orawan & Pantip (2000) found that histamine levels 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 to19%. High salt levels (20 or 23 g/L in the aqueous phase) in anchovy paste stored for 1 year prevented histamine production (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 preformed histidine decarboxylase in the anchovies.

### 4.6.5 Modified atmosphere packaging

Some modified atmosphere packaging (MAP) conditions have been reported to reduce the formation of histamine in susceptible products. A CO₂:O₂:N₂ gas mix of 40:40:20 inhibited histamine production 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% CO₂ (preferably combined with 5% NaCl) inhibited growth and histamine production of *M. morganii*. MAP (60% CO₂:40% N₂) decreased histamine production in Atlantic herring compared to air storage at 2°C or in ice (Özogul et al. 2002a, 2002b). Similar results were recorded for sardines at 4°C (Özogul et al. 2004). Emborg et al. (2005) found that although vacuum packaging and MAP in an atmosphere of 60% CO₂:40% N₂ did not prevent the development of *M. psychrotolerans* in tuna, MAP storage in an atmosphere of 40% CO₂:60% O₂ did. As no histamine was produced in tuna storage under this gas mix for 28 days at 1°C, they suggested that it be used instead of vacuum packing for cold storage of fresh tuna. However, they did not report whether storing tuna under atmospheres with high O₂ had any sensory effects. Having identified *P. phosphoreum* as an important psychrotrophic histamine producer in garfish stored at 5°C, Dalgaard et al. (2006) found that MAP did not reduce histamine production by this organism. *P. phosphoreum* is well reported as a major spoilage organism of fish packaged under modified atmosphere packaging, and although the growth of *P. phosphoreum* was reduced by CO₂, histamine production was higher in an atmosphere containing 60% CO₂:15% O₂:25% N₂ than in air (Lopez-Caballero et al. 2002).

Vacuum packaging was not found to control histamine production in seer fish (*Scomberomorus commersonii*) although it did increase shelf-life, presumably reducing the margin of safety between spoilage and histamine production (Jeya Shakila et al. 2005b). Vacuum packaging was
reported to increase histamine production in herring compared to ice storage (Özogul et al. 2002b) although the reverse was true in sardines at 4°C. The use of an oxygen scavenger to remove oxygen from packs was also reported to significantly decrease histamine production in seer fish while also increasing shelf-life (Mohan et al. 2009).

4.6.6 Freezing and chilled storage

Freezing can reduce histamine formation both by preventing the growth of histamine-producing bacteria and by reducing the activity of pre-formed histidine decarboxylase. Thus, histamine production in anchovies was greatest at storage temperatures above 20°C but frozen storage decreased the rate of production (Rossano et al. 2006). Dalgaard et al. (2006) observed that because P. phosphoreum is resistant to CO2, it cannot be controlled by modified atmosphere packaging, but because it is very sensitive to freezing it can be controlled by freezing and thawing of the product. Thawed fish showed marked reductions in histamine formation when stored at 5°C. Economou et al. (2007) also noted reductions in histamine formation in thawed tuna compared to fresh tuna. In contrast, Kim et al. (2002a) found that histamine accumulated rapidly when thawed fish was stored at 25°C. This may relate to pre-formed histidine decarboxylase or to the survival of heat-resistant histamine-producing bacteria other than P. phosphoreum. Staruszkiewicz et al. (2004) also demonstrated that histidine decarboxylase activity could be retained in frozen fish and could cause increases in histamine levels on thawing. Yung-Hsiang et al. (2005c) found that although histamine production stopped when fish were frozen, once samples were thawed histamine accumulated rapidly exceeding 500 mg/kg within 36 h at 25°C. Thus, freezing may limit histamine production while product is frozen but will not necessarily prevent its occurrence in thawed fish.

4.6.7 Gamma irradiation

Irradiation has been shown to inhibit growth and histamine production of M. morganii in mackerel fillets (Ay tac et al. 2000) and to reduce histamine content in bonito in a dose-dependent fashion (Mbariki 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 production during that time (Mbariki 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). Thus, irradiation is an option to control histamine poisoning but the technology is not approved for use on fish in New Zealand and there could be consumer resistance.

4.6.8 Additives

When different food additives were tested, glycine was found to be more effective in reducing the production of histamine and other biogenic amines in Myeolchi-jeot (a Korean salted fermented fish product) than other additives (sodium chloride, sucrose, glucose, D-sorbitol, lactic acid, citric acid and sorbic acid) (Jae-Hyung & Han-Joon 2009). In culture, glycine reduced histamine production by 93% with similar reductions when 5% was included in the Myeolchi-jeot during the ripening process. Garlic was found to be more effective than other spices (ginger, green onion, red pepper, clove cinnamon) at reducing the production of histamine and other biogenic amines in Myeolchi-jeot (Jae-Hyung et al. 2009). However, histamine production was only reduced by 12% in culture and 9% when garlic was incorporated at 5% during the
fermentation process. The glycine and garlic acted by inhibiting the growth and histamine production by histamine-producing bacteria. Other research suggested that including nuka, a Japanese by-product of rice polishing, may be useful in reducing histamine in fermented fish products such as fish sauces (Kuda & Miyawaki). The spice *Garcinia cambogia* inhibited histidine decarboxylation in homogenised skipjack samples: this was attributed to its effect on pH (lowered to 3.6) while *Tamarindus indica* (tamarind) and fruits of *Avverhoea bilimbi* (bilin) did not prevent histamine formation (Thadhani et al. 2002).

NaCl (5%) and potassium sorbate (1%) also helped to control the growth and histamine production of *M. morganii* in mackerel fillets (Aytac et al. 2000).

Thus, certain additives may assist in preventing histamine poisoning but none are proposed as reliable methods of control by themselves.

4.6.9 Protective cultures of bacteria

Although histamine is produced by bacteria that produce histidine decarboxylase, some bacteria produce diamine oxidases capable of degrading histamine. Enes Dapkevicius et al. (2000) proposed two such lactic acid bacteria 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* was proposed as a protective organism that could be used as a starter culture to prevent histamine production in Korean fermented foods as well as other products (Jae-Hyung et al. 2008). *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).

4.7 Applying predictive models

Another way to control histamine production is to monitor temperatures during transport and storage and use mathematical models to predict when hazardous levels might occur and thus when to reject product.

Working on jack mackerel, Bermejo et al. (2004) fitted mathematical models for both bacterial growth and histamine production. From these 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 h to 2 days at 25°C before the quality of fishmeal produced from the mackerel would be affected.

Based on work on tuna, Emborg & Dalgaard (2008a) developed mathematical models for growth, inactivation and histamine production of *M. psychrotolerans* and *M. morganii* together in response to temperature alone and for the growth and histamine production of *M. psychrotolerans* in response to combinations of temperature, CO₂, A₆, and pH (Emborg & Dalgaard 2008b). Their models were validated by applying them to the results of their own data and various studies on histamine production in fish found in the literature. Both of these models have been made freely available in a relatively user-friendly format as part of the Seafood Safety and Spolilage predictor software (Dalgaard 2009).
5 Conclusions and recommendations

New Zealand seafood continues to cause outbreaks of histamine poisoning and hot-smoked fish and particularly hot-smoked kahawai are most commonly implicated. Kahawai is a valuable fish resource and smoked kahawai is a sought after product for consumers. However, fish companies are shying away from producing it because of fears of potential histamine poisoning. Although there is a very large body of international literature on histamine poisoning, outbreaks still occur and some research questions remain: answers to these could help protect consumers of New Zealand seafood. In previous work (Section 3.4.1) on the microflora, of hot-smoked products, we assumed that histamine production occurred during or after smoking and that histamine-forming bacteria had survived the smoking process. We therefore isolated and identified histamine-producing bacteria from a range of hot-smoked seafoods produced in New Zealand (Fletcher et al. 1998c), identified them and evaluated their ability to grow and produce histamine at different temperatures (Section 4.1). Continued failure of hot-smoking facilities to produce safe products may be because operators of these facilities fail to comply with the guidelines developed from our work or it may mean that the histamine had already formed before product arrived at the smokehouse. Recent literature suggests that psychrotrophic bacteria have a very significant role in histamine poisoning: further work on the role of these bacteria in the New Zealand environment is warranted. Taking the review commentary presented above into consideration, our recommendations for monitoring and research into histamine poisoning in New Zealand include:

1. Agencies investigating food-borne outbreaks of histamine poisoning should carry out more detailed investigations: if ever possible, the actual remains of the fish causing outbreaks of histamine poisoning should be tested for levels of histamine and other biogenic amines; the histamine-producing bacteria involved should be identified and characterised and results published. If the fish species is in doubt, it would be useful to identify this by genetic means, such as was done for Taiwanese billfish (Yung-Hsiang et al. 2007b). Results of such investigations would provide a clearer picture of the factors contributing to outbreaks of histamine poisoning in New Zealand and help guide the development of methods to prevent such outbreaks.

2. Some fish species that have been reported to cause histamine poisoning overseas should be added to the NZFSA list of imported fish species of histamine poisoning concern so that those handing these species are advised to take extra precautions with these species as well as those already listed. These include escolar, garfish, swordfishes and billfishes,

3. In order to quantify the levels of other biogenic amines in outbreak cases and research studies, HPLC or other methods to effect this need to be reviewed in detail and a suitable method implemented. Knowledge of other biogenic amines in products causing outbreaks will allow knowledge gained from New Zealand outbreaks to contribute to understanding the aetiology of histamine poisoning.

4. The unpublished work summarised in Section 3.4 should be formally published in a peer-reviewed journal in order to make findings available to the international research community and to increase understanding of the conditions under which high levels of histamine can occur. The evidence that histamine is only produced when bacteria enter the stationary phase should be particularly useful for those developing mathematical models of histamine production and developing software packages to predict risk of
histamine poisoning under different conditions. Publication of the research would necessitate revisiting the identification of the strains using genetic identification methods.

5. The contribution of psychrotrophic histamine-producing bacteria to the production of histamine in kahawai and other New Zealand fish should be evaluated. Histamine-producing bacteria should be isolated from freshly caught and temperature-abused fish with a focus on psychrotrophic histamine-producing bacteria. Their ability to produce histamine under different conditions and the conditions required to inactivate these bacteria should be determined. This information would guide the identification of hazardous handling practices that present a food safety risk and identify controls to protect against this.

6. Histamine production in kahawai before smoking should be more fully investigated, in particular, taking into account the effect of time spent in gill nets before harvest and the stress to the animals that will occur during this time. This could lead to identifying other hazardous practices and to future seafood management guidelines for safer harvesting methodologies and technologies.

7. Methods to evaluate the quality of kahawai (and other hazardous species) at the point of smoking should be defined so that these can accurately be performed by industry. A quality index method was used in earlier research (Fletcher et al. 1995), but this has not been validated. Practical quality thresholds that can be applied by industry are needed and this research would help industry to reject fish that have been stored for too long or at too high temperatures rather than smoking them.

8. Factors that increase histamine production without excessively increasing sensory spoilage should be more closely evaluated. Most studies suggest that by the time excessive levels of histamine are formed, the product would already be rejected by normal sensory evaluation. However, the number of food poisoning incidents from smoked kahawai where high levels of histamine have been found in product show that this may not be the case for this product. Research should determine why such incidents happen and what circumstances lead to products containing high levels of histamine having sensory qualities that are acceptable to consumers. Understanding this may lead to other measures that might indicate that fish are potentially hazardous.
6 References


ESR Auckland Food Group 1996. Histamine in fish. ESR Auckland Food Group methods
Manual. Auckland, ESR.

Feldman KA, Werner SB, Cronan S, Hernandez M, Horvath AR, Lea CS, Au AM, Vugia DJ
2005. A large outbreak of scombroid fish poisoning associated with eating escolar fish


Food Standards Australia New Zealand 2009. Seafood recall - dried whole anchovies, biotoxin - histamine.


Lampila LE, Tom PD 2009. Scombrotoxin (Histamine) Formation. Compendium of fish and fishery product processes, hazards, and controls. Davis, Sea Grant Extension Program, Food Science & Technology Department, University of California.


New Zealand Food Safety Authority 2009. Imported Food Requirements: Fish – species susceptible to production of histamine. Wellington, NZFSA.


Staruszkiewicz WF, Barnett JD, Rogers PL, Benner RA, Jr., Wong LL, Cook J 2004. Effects of on-board and dockside handling on the formation of biogenic amines in mahimahi (Coryphaena hippurus), skipjack tuna (Katsuwonus pelamis), and yellowfin tuna (Thunnus albacares). Journal of Food Protection 67(1): 134-141.


