Growth and survival in seafoods: *Aeromonas* is psychrotrophic with a 1–3 log increase in numbers observed in fish stored at 5 °C for 1 week. The minimum pH for growth is < 4.5, and the maximum sodium chloride concentration is 5–6 percent. *Aeromonas* is sensitive to elevated temperature with a $D_{51^\circ C}$ of 2.3 min (ICMSF, 1996).

For *P. shigelloides*, the minimum growth temperature is 8 °C and the pH range is 4–9. Maximum sodium chloride for growth is 5. The organism is sensitive to heat, with pasteurization at 60 °C for 30 min being effective in inactivating it.

### 3.2.2 Histamine and other biogenic amines (Lahsen Ababouch, Jette Emborg and Paw Dalgaard)

In small physiological doses, histamine is a necessary and desirable substance involved in the regulation of critical functions in the human body, e.g. the release of stomach acid. However, large amounts of histamine and other biogenic amines in food can be toxic.

In fish products, histamine and other biogenic amines are produced by enzymatic decarboxylation of the corresponding free amino acid (Table 26). The decarboxylases are produced by specific bacteria.

<table>
<thead>
<tr>
<th>Amino acid precursor</th>
<th>Biogenic amine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>Histamine</td>
</tr>
<tr>
<td>Ornithine</td>
<td>Putrescine</td>
</tr>
<tr>
<td>Putrescine¹</td>
<td>Spermidine</td>
</tr>
<tr>
<td>Lysine</td>
<td>Cadaverine</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyramine</td>
</tr>
<tr>
<td>Arginine</td>
<td>Agmatine</td>
</tr>
</tbody>
</table>

¹ Not an amino acid.

In order to cause histamine fish poisoning (HFP), it is necessary that:

- the fish muscle contains free histidine as substrate for histamine formation;
- the fish contains and/or becomes contaminated with bacteria capable of decarboxylating histidine and possibly other amino acids;
- the product characteristics and storage conditions allow growth of histamine-producing bacteria (HPB) to high concentrations of about 10 million cells per gram or more;
- consumers actually eat fish that contain high concentrations of histamine and possibly also other biogenic amines.

Consequently, control of HFP can be achieved by eliminating one or more of these steps.

With respect to toxicity of fish products, histamine is more important than other biogenic amines. High concentrations of biogenic amines other than histamine can cause disease and discomfort, but for healthy people the concentrations of biogenic amines found in fish products are usually not toxic (Taylor, 1990; Glória, 2006). However, for sensitive individuals, a very small dose of tyramine can cause migraine headaches. For these persons, an intake of no more than 5 mg tyramine per meal has been recommended (Caston *et al.*, 2002; McCabe, 1986; Walker *et al.*, 1996). Typically, fish products contain less than 5 mg of tyramine per kilogram and therefore represent no problem even for sensitive individuals. However, products involved in some incidents of HFP have contained 150 mg of tyramine per kilogram; thus, the content of a typical 100 g fish portion can be critical. Much higher concentrations of tyramine can be found in certain cheeses, sausages and yeast extract. Chocolate can cause migraine for individuals susceptible to phenylethylamine (Glória, 2006).
3.2.2.1 Histamine fish poisoning – disease, epidemiology and implicated products

Histamine fish poisoning is an intoxication that can be caused by consumption of many different types of marine finfish, but neither freshwater fish, crustaceans or molluscan shellfish seems to cause this disease. Histamine fish poisoning is common and occurs worldwide (Table 27).

### TABLE 27
Incidents and cases of histamine fish poisoning in various countries

<table>
<thead>
<tr>
<th>Country, Region</th>
<th>Year</th>
<th>Incidents or outbreaks</th>
<th>Cases</th>
<th>Annual no. of cases per million people¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hawaii, U.S.A.</td>
<td>1990–2003</td>
<td>111</td>
<td>526</td>
<td>31.0</td>
</tr>
<tr>
<td>Denmark</td>
<td>1986–2005</td>
<td>64</td>
<td>489</td>
<td>4.9</td>
</tr>
<tr>
<td>New Zealand</td>
<td>2001–2005</td>
<td>11</td>
<td>62</td>
<td>3.1</td>
</tr>
<tr>
<td>Japan</td>
<td>1970–1980</td>
<td>42</td>
<td>1122</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>1994–2005</td>
<td>68</td>
<td>1523</td>
<td>1.1</td>
</tr>
<tr>
<td>France</td>
<td>1987–2005</td>
<td>123</td>
<td>2635</td>
<td>2.5</td>
</tr>
<tr>
<td>Finland</td>
<td>1983–2005</td>
<td>41</td>
<td>162</td>
<td>1.3–2.1</td>
</tr>
<tr>
<td>Taiwan Province</td>
<td>1986–2001</td>
<td>8</td>
<td>535</td>
<td>1.5</td>
</tr>
<tr>
<td>Norway, U.K.</td>
<td>1966–2004</td>
<td>608</td>
<td>1460</td>
<td>0.4–0.8</td>
</tr>
<tr>
<td>Australia, Canada, Netherlands, Philippines, Sweden and United States of America</td>
<td>1973–2005</td>
<td>603</td>
<td>3214</td>
<td>0.2–0.4</td>
</tr>
</tbody>
</table>

¹ To compare data between regions of different population size and for different recording periods, the annual number of cases per million people was calculated.

Source: Dalgaard and Emborg (2009).

The incubation time for HFP is short (from a few minutes up to 2 h) and people often develop symptoms while they are still eating. This facilitates attribution of disease to the fish consumed, but the occurrence of HFP is under-reported because:

- many countries do not collect data on incidents of HFP;
- symptoms can be mild and of short duration, so a physician may not be contacted;
- HFP symptoms can be incorrectly identified and recorded, e.g. as a food allergy;
- some statistics exclusively include cases that are reported as a part of an outbreak (where two or more people become ill), but for HFP single cases are common.

The primary symptoms of HFP are cutaneous (rash, urticaria, oedema, and localized inflammation), gastrointestinal (nausea, vomiting and diarrhoea), haemodynamic (hypotension) and neurological (headache, tingling, oral burning and blistering sensation, flushing and perspiration, and itching). More serious complications such as cardiac palpitations occur but are rare (Taylor, 1986; Lehane and Olley, 2000). Symptoms can be resolved by antihistaminic drugs (antihistamines). These drugs block the binding of histamine to specific receptors and thereby its effect and HFP symptoms (Parsons and Ganellin, 2006; Glória, 2006).

Shalaby (1996) reviewed the oral toxicity to humans of histamine and other biogenic amines. Based on this analysis, the following guideline levels for histamine content of fish were suggested:

- < 50 mg/kg  safe for consumption
- 50–200 mg/kg possibly toxic
- 200–1 000 mg/kg probably toxic
- > 1 000 mg/kg toxic and unsafe for human consumption
More recently, an extensive study found 90 percent of 1998 HFP cases were due to fish products with more than 500 mg of histamine per kilogram (Table 28). Data from fish products implicated in 30 different HFP incidents also showed that the concentration of histamine was ten times higher than the sum of the concentrations of other biogenic amines (Dalgaard et al., 2008). With a meal size of 100 g, these data suggest that HFP is caused by an intake of more than 50 mg of histamine (100–500 mg being most common) together with more than 5 mg of other biogenic amines (10–50 mg being typical).

A recent Joint FAO/WHO Expert Meeting on Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products identified 50 mg of histamine as the “no observable adverse effect level” (NOAEL) derived from outbreak studies. The benchmark dose assessment methodology also identified 50 mg of histamine per meal as the dose where adverse effects are not observed. Using available fish and fishery product consumption data combined with expert opinion, the meeting agreed that a serving size of 250 g captured the maximum amount eaten in most countries at a single eating event. Based on the hazard level of 50 mg of histamine and serving size of 250 g, the maximum concentration of histamine in that serving was calculated to be 200 mg/kg.

<table>
<thead>
<tr>
<th>Histamine (mg/kg)</th>
<th>Outbreaks</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>&gt; 5 000</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>1 000–5 000</td>
<td>66</td>
<td>47</td>
</tr>
<tr>
<td>500–1 000</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>&lt; 500</td>
<td>36</td>
<td>25</td>
</tr>
</tbody>
</table>

Source: Dalgaard et al. (2008).

Information from an HFP outbreak caused by escolar showed that persons consuming less than 113–215 mg of histamine experienced fewer symptoms that were of shorter duration than persons consuming more of the fish and thereby higher amounts of histamine (Feldman et al., 2005). In some challenge studies with human volunteers, 67.5–300 mg of histamine administered in water, grapefruit juice or fish resulted in no or mild symptoms only. However, it has also been found that 180 mg of histamine resulted in severe headache and flushing (Motil and Scrimshaw, 1979; Van Gelderen et al., 1992). Thus, available data from challenge studies with human volunteers suggest pure histamine cannot always explain the toxicity of histamine-containing seafood. This apparently low toxicity of pure histamine may, to some extent, be explained by variability in the sensitivity among the few volunteers used in these studies and the relatively low amounts of histamine (< 100–500 mg) sometimes evaluated. In addition, two different hypotheses to explain the apparently low toxicity of histamine have been extensively discussed in the scientific literature.

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The histamine-potentiator hypothesis is based on numerous experiments with laboratory animals where various compounds (agmatine, cadaverine, ß-phenylethylamine, putrescine, trimethylamine, tyramine, combinations of these compounds and ethanol) inhibited normal histamine-metabolizing enzymes (histamine-N-methyltransferase, monoamine oxidase and diamine oxidase [or histaminase]) and thereby increased the oral toxicity of histamine (Taylor and Lieber, 1979; Hui and Taylor, 1985; Lyons et al., 1983; Satter and Lorenz, 1990). However, data for humans are limited and do not clearly confirm the histamine-potentiator hypothesis (Taylor, 1986; Lehan and Olley, 2000; Van Gelderen et al., 1992). Van Gelderen et al. (1992), for example, found that 22 mg of cadaverine and 18 mg of putrescine were unable to potentiate the oral toxicity 88–90 mg of histamine when tested on eight volunteers.

The mast-cell-degranulation hypothesis suggests seafood that causes HFP should contain compounds that trigger a release of histamine from mast cells in the human intestinal tissue. The HFP symptoms would then be due to indigenous histamine rather than to histamine in seafood (Taylor, 1986; Ijomah et al., 1991; Clifford et al., 1991; Lehan and Olley, 2000; Arnold and Brown, 1978). Evidence to support this hypothesis is very limited. In fact, compounds including tryptase and prostaglandin D₂ are released from mast cells when degranulated. However, these compounds have not been detected in serum or urine from patients with HFP (Morrow et al., 1991; Sanchez-Guerrero, Vidal and Escudero, 1997).

Many species of marine finfish have caused HFP (Table 28) and the intoxication is often referred to as scombroid or scombrotoxin poisoning because of the frequent association of the illness with the consumption of scombroid fish such as tuna (Thunnus spp.), skipjack (Katsuwonus pelamis), saury (Kololabis saira) and mackerel (Scomber spp.). However, non-scombroid fish such as anchovies (Engraulis spp.), bluefish (Pomatomus spp.) escolar (Lepidocybium flavobrunneum), garfish (Belone belone), herring (Clupea spp.), kahawai (Arripis trutta), mahi-mahi (Coryphaena spp.), marlin (Makaira spp.), pilchards (Sardina pilchardus), sardines (Sardinella spp.) and swordfish (Xiphiidae) have also been implicated in outbreaks of this illness.

Considering that information on fish species that could be involved in HFP should be easily accessible to support risk management, the recently held Joint FAO/WHO Expert Meeting on the Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products developed the most comprehensive list of fish available to date, and this list can be accessed on the FAO website.

These fish species have significant amounts of histidine in their muscle tissue, where it serves as a substrate for bacterial histidine decarboxylase and formation of histamine. It seems that HFP is caused primarily by histamine rather than by other biogenic amines. Consequently, to reduce HFP, efforts to reduce growth and activity of HPB should be the main objective.

3.2.2.2 Histamine-producing bacteria

The kinetics of histamine formation during storage of seafood are sometimes characterized by a long phase with little or no histamine production, followed by a second phase where the concentration can increase rapidly (an example is shown in Figure 13). The first phase corresponds to the time needed for the specific HPB to reach high concentrations, and the length of this phase depends primarily on the initial concentration of these bacteria, their growth rate and temperature. The rate of histamine formation during the second phase corresponds to the activity of high concentrations of the HPB and it is influenced by storage conditions and product characteristics (Figure 13). Information about the bacteria that produce histamine in seafood is important. First, to reduce histamine formation, it is essential to
inhibit growth of the specific bacteria that actually produce this compound. Second, microbiological methods for seafood inspection must target the bacteria of importance and, therefore, the characteristics of these bacteria need to be known.

The bacteria responsible for histamine formation in seafood that actually caused HFP have been identified, but only in a very limited number of studies (Table 29). Prior to 2004, many were of the opinion that HFP was caused exclusively by the activity of mesophilic HPB in temperature-abused products (Kim et al., 2004). However, toxic concentrations of histamine are frequently formed in naturally contaminated fish products when these are stored in ice and at chill temperatures between –1 °C and +5 °C. A comprehensive study of 124 storage trials with naturally contaminated seafood at various temperatures found toxic concentrations of histamine (above 500 mg/kg) in 26 of 59 products stored at between –1 °C and +5 °C (Ababouch et al., 1991; Emborg, 2007; Dalgaard et al., 2008).

The importance of psychrotolerant HPB has now been recognized, and a new psychrotolerant, strongly histamine-producing species within the genus Morganella has been identified (Emborg, Dalgaard and Ahrens, 2006). Today, both mesophilic bacteria (Morganella morganii, Hafnia alvei and Raoultella planticola) and the psychrotolerant bacteria (Morganella psychrotolerans and Photobacterium phosphoreum) have been identified as responsible for histamine formation in seafood that actually caused HFP (Table 29). Several other species of bacteria are most likely to be important for histamine formation in fish products but these have not yet been related to illness, owing to the very limited number of HFP incidents where the bacteria responsible for histamine formation have been studied (Table 29).

### TABLE 29
Incidents of histamine fish poisoning where the bacteria responsible for histamine formation have been identified

<table>
<thead>
<tr>
<th>Implicated seafood</th>
<th>Bacterium</th>
<th>Year reported and region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesophilic bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh tuna</td>
<td>Morganella morganii</td>
<td>1956 Japan</td>
</tr>
<tr>
<td>Fresh tuna</td>
<td>Hafnia alvei</td>
<td>1967 Czechoslovakia</td>
</tr>
<tr>
<td>Fresh tuna</td>
<td>Morganella morganii</td>
<td>1973 Japan</td>
</tr>
<tr>
<td>Fresh tuna</td>
<td>Raoultella planticola</td>
<td>1978 United States of America</td>
</tr>
<tr>
<td>Tuna heated in flexible film</td>
<td>Morganella morganii</td>
<td>2006 Denmark</td>
</tr>
<tr>
<td>Psychrotolerant bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried sardines</td>
<td>Photobacterium phosphoreum</td>
<td>2004 Japan</td>
</tr>
<tr>
<td>Tuna in chilli-sauce</td>
<td>Morganella psychrotolerans and/or</td>
<td>2005 Denmark</td>
</tr>
<tr>
<td></td>
<td>Photobacterium phosphoreum</td>
<td></td>
</tr>
<tr>
<td>Cold-smoked tuna</td>
<td>Photobacterium phosphoreum</td>
<td>2006 Denmark</td>
</tr>
<tr>
<td>Cold-smoked tuna</td>
<td>Morganella psychrotolerans</td>
<td>2006 Denmark</td>
</tr>
<tr>
<td>Fresh tuna</td>
<td>Photobacterium phosphoreum</td>
<td>2006 Denmark</td>
</tr>
</tbody>
</table>

Source: Dalgaard et al. (2008).

In living bacteria, histidine decarboxylase (HDC) functions in cooperation with a membrane exchanger that allows histidine to be transported into the cell and histamine to be transported out of the cell (Molenaar et al., 1993). The function of histamine formation in bacterial metabolism is not clear. Excretion of histamine may generate metabolic energy or be involved in an acid stress response (Lucas et al., 2005; Van Poelje and Snell, 1990; Molenaar et al., 1993).

Histidine is the only amino acid so far known for which decarboxylases of two different types have evolved (Van Poelje and Snell, 1990; Tanase, Guirard and Snell, 1985). One type is the pyridoxal 5’-phosphate-dependent HDC. This HDC has been isolated and characterized from Gram-negative bacteria (M. morganii [Tanase, Guirard and Snell, 1985], Raoultella planticola [Guirard and Snell, 1987; Kanki et al., 2007],
Enterobacter aerogenes [Guirard and Snell, 1987], P. phosphoreum [Morii and Kasama, 2004; Morii and Kasama, 1995; Kanki et al., 2007] and Photobacterium damselae JCM 8968 [Kanki et al., 2007]). The other type of HDC is the pyruvoyl-dependent HDC produced by Gram-positive bacteria. This enzyme has been isolated from the following bacteria: Lactobacillus 30a (Hackert et al., 1981), Lactobacillus hilgardii 0006 (Lucas et al., 2005), Leuconostoc oeni IOEB (Coton et al., 1998), Tetragenococcus muriaticus (Konagaya et al., 2002) and Clostridium perfringens (Huynh and Snell, 1985). No organism able to produce HDC of both types is yet known (Van Poelje and Snell, 1990).

Strains of some Gram-positive bacteria that can produce histamine have been isolated from salted, dried or fermented foods (Landete, Pardo and Ferrer, 2006). However, Gram-positive HPB have not yet been identified as responsible for histamine formation in fish products that actually caused HFP (Table 29). A wide range of Gram-negative bacteria isolated from seafood are able to produce histamine. However, only a minor part are able to produce histamine in high concentrations (> 1 000 mg/kg) even under optimal conditions. These bacteria have been designated prolific histamine producers (Behling and Taylor, 1982).

Some HPB such as P. phosphoreum are part of the natural microflora in seawater, and a part of the natural flora in the intestines, gills and on the skin of fresh fish (see review by Dalgaard, 2006). They invade fish flesh from these reservoirs. This is reflected by higher histamine concentrations in fish flesh adjacent to gills and intestines and higher histamine concentrations in undressed as compared with dressed fish (Frank, Yoshinaga and Nip, 1981; Kim, An and Price, 1999; Kim et al., 2001; López-Sabater et al., 1996; Taylor and Speckhard, 1983). Enterobacteriaceae in fish products often result from post-harvest contamination as these HPB are found in water, baskets and floors/equipment at fish processing plants and fish markets (Corlett, Jeffrey and Niven, 1978; Subburaj, Karunasagar and Karunasagar, 1984).

Niven’s agar (Niven, Jeffrey and Corlett, 1981) has been used for enumeration of HPB. However, this method relies on pour plating (with 45 °C warm agar) and incubation of plates at 37 °C. Consequently, Niven’s agar will detect neither P. phosphoreum nor M. psychrotolerans as these bacteria do not grow at 37 °C. In addition, Niven’s agar has been associated with false positive results. Therefore, results must be interpreted carefully (Lehane and Olley, 2000).

Various PCR methods to detect the gene encoding for histidine decarboxylase (hdc) have been developed (Landete et al., 2007). Primer sets for the detection of hdc in both Gram-negative and Gram-positive bacteria are available. In addition, a PCR assay to detect the four most important decarboxylase genes (histidine, tyramine, putrescine and cadaverine) from a wide range of Gram-positive and Gram-negative bacteria associated to food has been suggested (De las Rivas et al., 2006). The ability of PCR methods to differentiate between weakly and strongly HPB deserves further study. This is important if PCR methods are to be used in seafood inspection as detection of weak HPB might lead to unnecessary concerns.

3.2.2.3 Prevention and control
Despite decades of research, efforts by the seafood sector and efforts by national and international authorities, HFP remains common. This indicates available information is either incomplete or not used appropriately to manage this seafood safety issue (Dalgaard et al., 2008).

Growth of HPB, and the related formation of histamine, depends on several factors including their presence in a specific seafood, storage conditions (temperature, atmosphere) and various product characteristics (pH, lactic acid, salt, smoke components and added antimicrobial agents) (Figure 13). To control HFP, it is important to know the effect of these parameters on the most important HPB. The information can be
used, for example, to determine safe the shelf-life or to obtain a desired shelf-life by changing storage conditions or product characteristics.

Chilling of fish and fish products is highly important to increase the time to formation of critical histamine concentrations. Below 7–10 °C, mesophilic and strongly HPB do not form toxic concentrations of histamine in fish products. However, the psychrotolerant bacteria *M. psychrotolerans* and *P. phosphoreum* can produce toxic concentrations of histamine at 0–5 °C (Dalgaard *et al.*, 2006; Emborg, Laursen and Dalgaard, 2005; Kanki *et al.*, 2004; Okuzumi, Okuda and Awano, 1982). Simple empirical models to predict histamine formation have been suggested (Frank, 1985; Frank and Yoshinaga, 1987; Frank, Yoshinaga and Wu, 1983). The precision of these models is modest, and they have not been widely adopted by the seafood sector. The most accurate of the empirical models has been developed by Frank (1985) for histamine formation during high-temperature storage (21.1–37.8 °C) of skipjack tuna.

![FIGURE 13](chart.png)

(A) Predictions for 2.0 °C (dashed lines) and 4.4 °C (solid lines). (B) Predictions for 5.0 °C with 3.5% NaCl (dashed lines) and 5.0% NaCl (dotted lines). Predictions were obtained by using the Seafood Spoilage and Safety Predictor (SSSP) software (http://sssp.dtuauqua.dk).
Regulation EC 853/2004 of the European Union (Member Organization) states that “Fresh fishery products, thawed unprocessed fishery products, and cooked and chilled products from crustaceans and molluscs, must be maintained at a temperature approaching that of melting ice” (EC, 2004a). In some countries of the European Union (Member Organization), this is interpreted as temperatures between 0 °C and +2 °C. Lightly preserved seafood with less than 6 percent salt and a pH above 5, e.g. smoked and marinated products, should be at 5 °C or less. Regulations in the United States of America specifically indicate maximum times to reach critical chill storage temperatures, but the allowed chill storage temperature of 4.4 °C is relatively high (FDA, 2011c). Storage at 2.0 °C or 4.4 °C has a markedly different effect on growth and histamine formation, as shown in Figure 13 for M. psychrotolerans. In the United States of America, seafood in reduced-oxygen packaging must be stored and distributed at less than 3.3 °C. This is a requirement owing to the risk of toxin formation by Clostridium botulinum type E (FDA, 2011d), but compared with storage at 4.4 °C the risk of histamine formation in high concentrations is also considerably lower at 3.3 °C.

Concentrations of salt above 1–2 percent NaCl reduce growth of the Gram-negative and strongly histamine-producing bacteria. For vacuum-packed cold-smoked tuna, the potential histamine formation by M. psychrotolerans and P. phosphoreum can be controlled using 5 percent water phase salt and a declared shelf-life of 3–4 weeks or less at 5 °C (Embarg and Dalgaard, 2006). As shown in Figure 13, growth and histamine formation by M. psychrotolerans is delayed much more by 5.0 percent water phase salt as compared with 3.5 percent water phase salt. Gram-positive bacteria including Staphylococcus epidermis and Tetragenococcus muriaticus can produce histamine at higher NaCl concentrations (Hernández-Herrero et al., 1999; Kimura, Konagaya and Fujii, 2001). This may be important for fish sauce, fermented fish and salted-ripened, fish but the relative importance of these bacteria and of the activity of histidine decarboxylase produced by other bacteria prior to the mixing of fish and salt remains to be quantified.

Vacuum packing and modified-atmosphere packaging (MAP) are increasingly being used by the seafood sector. Vacuum packing reduces lipid oxidation in seafood but does not seem to delay histamine formation in fresh fish. However, MAP with gas mixtures containing carbon dioxide (CO₂) and nitrogen (N₂) can slightly delay histamine formation when high CO₂-concentrations are used. Compared with fresh MAP fish, these atmospheres delay histamine formation more efficiently for frozen and thawed products where the highly CO₂-resistant bacterium P. phosphoreum has been inactivated by frozen storage (Dalgaard et al., 2006). For lean fish, atmospheres with high concentrations of both CO₂ and oxygen (O₂) inhibit histamine formation markedly, as shown e.g. for chilled MAP tuna (Emborg, Laursen and Dalgaard, 2005).

Growth and histamine formation by M. psychrotolerans can be predicted by using a new kinetic model that takes into account the effect of the initial cell concentration, storage temperature (0–25 °C), atmosphere (0–100 percent CO₂), NaCl (0–5 percent) and pH (5.4–6.5). Predictions are not highly accurate but validation studies have found an average deviation between measured and predicted times to formation of 500 mg histamine per kilogram of about 10 percent (Emborg and Dalgaard, 2008a, 2008b). To predict the effect of delayed icing/chilling of fish, and other scenarios with large variations in storage temperature, a predictive model for growth and histamine formation by both M. morganii and M. psychrotolerans has been developed (Emborg and Dalgaard, 2008b). These predictive models are included in the Seafood Spoilage and Safety Predictor (SSSP) software (available free of charge at http://sssp.dtuaqua.dk). Development of similar predictive microbiology models for other important HPB will improve options to manage histamine formation in various fish products.

It has been shown that histamine, when formed in seafood, is relatively stable and not inactivated by freezing or heating such as normal cooking, hot-smoking or
even canning (Arnold and Brown, 1978; Taylor, 1986; Lehane and Olley, 2000; Flick, Oria and Douglas, 2001; FDA, 2011c; Kim et al., 2003b). Freezing of the fish can significantly reduce the bacterial load, and it will limit the activity of decarboxylase enzymes that may have been produced prior to freezing (Kanki et al., 2007).

The best ways to prevent the formation of histamine and biogenic amines in the fish industry are:

- Rapid chilling of fish immediately after death. This is particularly important for fish that are from warmer water or are exposed to warm air, and for large tuna that generate heat in the tissues of the fish following death.
- Good hygiene practices on board, at landing and during processing to avoid contamination or recontamination of the fish by bacteria capable of amino-acid decarboxylation.

Regulation EC 1441/2007 of the European Union (Member Organization) includes sampling plans \( (n = 9 \text{ and } c = 2) \) and limits for critical concentrations of histamine in “fishery products from fish species associated with a high amount of histidine” where \( m = 100 \text{ mg/kg and } M = 200 \text{ mg/kg (EC, 2007a)} \). Samples must be taken from each batch of fish species especially of the following families: Scombridae, Clupeidae, Engraulidae, Coryphaenidae, Pomatomidae and Scombresosidae.

For “fishery products which have undergone enzyme maturation treatment in brine, manufactured from fish species associated with a high amount of histidine” higher limits \( (m = 200 \text{ mg/kg and } M = 400 \text{ mg/kg}) \) are applied. The European Union (Member Organization) regulation does not include critical limits for other biogenic amines (EC, 2007a).

The United States of America uses a defect action level \( (m) \) of 50 mg of histamine per kilogram. A total of 18 fish per lot should be analysed individually or can be composited into, for example, 6 units, but then the critical limit is reduced accordingly from 50 mg/kg to 17 mg/kg (FDA, 2011c).

Examinations must be carried out in accordance with reliable, scientifically recognized methods, such as high-performance liquid chromatography (HPLC) (EC, 2007a) or fluorescent methods (AOAC, 1995).

Industry data made available to the Joint FAO/WHO Expert Meeting on Public Health Risks of Histamine and Other Biogenic Amines for Fish and Fishery Products indicated that where food business operators apply GHPs and HACCP, an achievable level of histamine in fish products is less than 15 mg/kg (based on a test method with a lower detection limit of 15 mg/kg).

3.2.3 **Viruses** (Iddy Karunasagar and David Lees)

Viruses are very small micro-organisms (15–400 nm) that consist of a nucleic acid (DNA or ribonucleic acid [RNA]) associated with proteins and, in some cases, they may also have a lipid bilayer membrane (or envelope). Viruses are obligatory intracellular pathogens and cannot multiply outside their host cells, although they may survive for long periods outside the host cells. Thus, viruses do not replicate in food or water. Viruses can infect all major groups of organisms from bacteria to mammals. Viruses are classified according to the nature of their genome (DNA or RNA, single stranded or double stranded, segmented or non-segmented, linear or circular and, in the case of single stranded RNA viruses, whether it can function as messenger RNA [mRNA]) and, in addition, their structure (symmetry, enveloped or not, number of capsomeres). Viruses cause a number of diseases in humans ranging from the common cold to serious illnesses such as rabies and HIV/AIDS. Viruses are abundant in nature and most are not pathogenic to humans. There are millions of virus-like particles in a millilitre of seawater, and they are a major cause of mortality in bacteria and plankton. Thus, viruses play a very important role in nutrient and energy cycles in the marine environment (Suttle, 2007). However, these viruses are not pathogenic to humans.
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Background: Fishing community in Aido Beach at work. ©FAO/D. Minkoh
Inset top: Workers in the NovaNam Ltd. Fish processing plant on the harbour in Luderitz. ©FAO/M. Namundjebo
Inset bottom: A variety of fish. ©FAO/FIPM
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