European Food Safety Authority

EFSA Journal 2015;13(7):4162

SCIENTIFIC REPORT OF EFSA

Scientific and technical assistance on the evaluation of the temperature to be applied to pre-packed fishery products at retail level[1]

European Food Safety Authority[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Relevant hazards associated with pre-packed fresh fishery products were identified through a literature search. The main temperature-dependent hazards identified are histamine formation, *Listeria monocytogenes*, *Clostridium botulinum*, and *Yersinia enterocolitica*. To assess bacterial growth and histamine production during storage and transport at retail level and to evaluate different storage scenarios, published predictive microbiology growth models were used assuming favourable growth conditions. Compliance with the legislative temperature requirement can only be assessed by translating the requirement into an objective measure, which, in this instance, is assumed to be 0 °C. Any assessment of temperature and its effect on histamine production or bacterial growth can be meaningful only in the context of a time period. The modelling results showed that packaged fresh fishery products can be stored at refrigeration temperatures above 0 °C (e.g. 3–5 °C) and be compliant with the current EU and international rules. For histamine, the modelling results showed that, for a fishery product with certain characteristics subject to the current temperature requirement, histamine formation would be 100 ppm (lower limit m of the safety criterion in EU Regulation (EC) No 2073/2005) at the end of its shelf-life. Thus, an equivalent condition to the above baseline scenario is any combination of storage temperature, shelf-life and CO_2 concentration in the package that leads to histamine formation of 100 ppm at the end of shelf-life. For example, for a retail temperature of 3 °C, 100 ppm would be reached under the following conditions: (1) shelf-life of 6 days and 0 % CO_2 in the packaging headspace, (2) shelf-life of 7 days and 20 % CO_2 in the packaging headspace or (3) shelf-life of 8 days and 40 % CO_2 in the packaging headspace. Similar estimates are provided for the other hazards identified.

KEY WORDS

fishery products, chilled storage, time-temperature, histamine

[1] On request from the European Commission, Question No EFSA-Q-2014-00528, approved on 22 June 2015.

[2] Correspondence: biohaz@efsa.europa.eu

[3] Acknowledgement: EFSA wishes to thank the members of the Working Group on evaluation of the temperature to be applied to pre-packed fishery products at retail level: Declan Bolton, Kostas Koutsoumanis and Micheál O'Mahony for the preparatory work on this scientific output and EFSA staff: Pablo Romero Barrios, Emmanouil Chantzis and Michaela Hempen for the support provided to this scientific output.

Suggested citation: EFSA (European Food Safety Authority), 2015. Scientific and technical assistance on the evaluation of the temperature to be applied to pre-packed fishery products at retail level. EFSA Journal 2015;13(7):4162, 48 pp. doi:10.2903/j.efsa.2015.4162

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

Following a request from the European Commission, EFSA was asked to deliver a scientific report providing scientific and technical assistance on the evaluation of the temperature to be applied to pre-packed fishery products at retail level.

The current EU rules for transport or storage conditions of fishery products do not define a specific temperature to be respected but refer to 'temperature approaching that of melting ice'. EFSA was asked to assess, in the light of the current EU and international rules, which temperature conditions, including a possible tolerance, could be applied for storage and transport of packed fresh fishery products, gutted or entire, including some parts of them, at retail level where icing is not possible.

Regulation (EC) No 853/2004, Annex I, defines fresh fishery products as unprocessed fishery products, whether whole or prepared, including products packaged under vacuum or in a modified atmosphere that have not undergone any treatment to ensure preservation other than chilling. EFSA was asked to expand the group of fresh fishery products for this assessment to include all fishery products when they are fresh (never frozen or processed); when they have been frozen and then thawed; and when molluscs or crustaceans have been cooked and then chilled. These categories are essentially those currently subject to the requirement to be maintained at a temperature approaching that of melting ice.

Relevant hazards associated with pre-packed fresh fishery products were identified through a literature search. The main temperature-dependent hazards identified are histamine formation, *Listeria monocytogenes*, *Clostridium botulinum*, and *Yersinia enterocolitica*.

To assess bacterial growth and histamine production and to evaluate different storage scenarios, the growth potential of relevant bacteria on fishery products during storage and transport at retail level was estimated using published predictive microbiology growth models assuming favourable growth conditions. The requirement to maintain products at a temperature approaching that of melting ice is an empirical requirement. It is generally interpreted to mean the contact of the fishery product with ice. Compliance is therefore difficult to demonstrate or verify in the case of packaged fishery products. Compliance with the requirement can be assessed only by translating the requirement into an objective measure, which, in this instance, is assumed to be 0 °C. While there is a temperature requirement for storage and transport of fishery products, this requirement does not exist for other refrigerated products of animal origin. Any assessment of temperature and its effect on bacterial growth can be meaningful only in the context of a time period. As the current legal requirement extends for long, potentially interrupted, and variable durations, the potential outcomes of different temperature scenarios are best considered on the basis of potential shelf-life durations. It is worth noting that the group of products in the terms of reference includes foods with likely short shelf-life such as packaged fresh fishery products, as well as products which have been processed to have relatively long shelf-life, e.g. cooked and chilled products from crustaceans.

For products for which criteria are laid out in legislation, such criteria are used to evaluate the impact of storage at a temperature different to that set out in legislation. Growth of pathogens at storage temperatures above 0 °C may also occur in any packaged fishery products for which criteria are not laid out in legislation. In this case the temperature that could be applied to storage and transport can be estimated as the temperature that will provide equal or less growth of the pathogens. The primary working assumption for those products for which there exist food safety criteria was that all storage temperatures used should continue to provide compliance with the relevant food safety criteria.

The results from the modelling showed that pre-packed fresh fishery products can be stored at refrigeration temperatures above 0 °C (e.g. 3–5 °C) and be compliant with the current EU and international rules. Examples of combinations of product durability (maximum shelf-life) and packaging atmosphere that should enable compliance with the safety criteria for various storage temperatures at retail are provided.

For histamine, the results of the modelling approach showed that, for a fishery product with certain characteristics subject to the current temperature requirement, histamine formation would be 100 ppm at the end of its shelf-life. Thus, an equivalent condition to this baseline scenario is any combination of storage temperature, shelf-life and CO_2 concentration in the package that leads to histamine formation of 100 ppm at the end of shelf-life. For example, for a retail temperature of 3 °C, 100 ppm would be reached under the following conditions: (1) a shelf-life of 6 days and 0 % CO_2 in the packaging headspace, (2) a shelf-life of 7 days and 20 % CO_2 in the packaging headspace, or (3) a shelf-life of 8 days and 40 % CO_2 in the packaging headspace.

For *L. monocytogenes* EU Regulations set a limit of 100 colony-forming units (CFU)/g in ready-to-eat products. In this case the food business operator can ensure compliance with such limit for products stored in retail at temperatures above 0 °C by adjusting the maximum shelf-life and/or by modifying the packaging atmosphere to ensure that the growth of the pathogen during distribution and storage will not exceed the limit of 100 CFU/g at the end of shelf-life. For this, the available predictive models show that, for a retail temperature of 3 °C for example, this limit would be respected with: (1) a shelf-life of 11 days and 0 % CO_2 in the packaging headspace, (2) a shelf-life of 14 days and 20 % CO_2, and (3) a shelf-life of 18 days and 40 % CO_2.

Apart from the non-compliance with the safety criteria, an increase of the storage temperature at retail above 0 °C may lead to growth of pathogenic microorganisms for which food safety criteria do not apply and are capable of growth at low temperatures. In this case the temperature that could be applied to storage and transport of pre-packed fresh fishery products at retail level can be estimated as the temperature that will provide equal or less growth of those pathogens.

Y. enterocolitica is the only hazard for which models predicting growth at 0 °C are available and time–temperature scenarios during storage and transport at retail level equivalent to the currently mandated storage requirements can be estimated. The results of the modelling showed that, for a fishery product which is stored in ice (0 °C) and a shelf-life of 14 days, the conditions that lead to equivalent growth of *Y. enterocolitica* to that at 0 °C are, for example: (1) a shelf-life of 10 days at 2 °C, (2) a shelf-life of 7 days at 4 °C, or (3) a shelf-life of 5 days at 6 °C.

It is recommended that, if a temperature limit is deemed necessary, then future legislation should include a clear temperature value instead of statements such as a ‘temperature approaching that of melting ice’.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The current EU rules for transport or storage conditions of fishery products do not define a specific temperature to be respected but refer to 'temperature of melting ice'. Point A of Chapter III of Section VIII of Annex III to Regulation 853/2004 states: 'Where *chilled, unpackaged products are not distributed, dispatched, prepared or processed immediately after reaching an establishment on land, they must be stored under ice in appropriate facilities. Re-icing must be carried out as often as necessary. Packaged fresh fishery products must be chilled to a temperature approaching that of melting ice*'.

The main problem for implementing the above rule by Member States competent authorities is related to the storage of pre-packed fishery products (entire fish or parts of them) in supermarkets, where they are normally not maintained under ice.

International Rules

The documents mentioned in the Annex to the mandate describe the temperature of melting ice as always identified '*as close as possible to* 0 °C' and the tolerance applied is '*just above that of melting ice, 0 °C (32 °F)*'.

Tolerance between – 2 °C to +4 °C is applied only to abalone. This could indicate that this tolerance is specific for this product and it should be considered as an exception not applicable to all fishery products.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In the context of Article 31 of Regulation (EC) No 178/2002, EFSA is asked to provide the following scientific and technical assistance to the Commission:

• *To assess, in the light of the current EU and international rules, which temperature conditions, including a possible tolerance, could be applied for storage and transport of pre-packed fresh fishery products, gutted or entire, including some parts of them, at retail level where icing is not possible.*

The TORs were subsequently modified by the EC to include:

a) fresh fishery products,

b) thawed unprocessed fishery products, and

c) cooked and chilled products from crustaceans and molluscs.

EFSA is requested to finalise its scientific assessment by 30 June 2015.

ASSESSMENT

1. Introduction

1.1. Interpretation of the term of reference

The term of reference (TOR) centres around the various situations when the European Union (EU) legislation requires certain fishery products at, or after, particular points of their food chain, to be maintained at a temperature approaching that of melting ice. The TOR specifically pertains to scenarios when this temperature requirement exists for packaged fishery products.

Arising from the various definitions set out in the EU legislation, the group of packed fishery products to which the TOR applies is arguably a relatively broad and diverse group.

The term 'fishery product' encompasses a much broader group of products than the apparently analogous term 'meat product' as the latter is limited to processed products only and excludes all raw unprocessed meats. The specific groups of fishery products to which the TOR applies are all fishery products when they are fresh (never frozen or processed); when they have been frozen and then thawed; and when molluscs or crustaceans have been cooked and then chilled. These categories are essentially those currently subject to the requirement to be maintained at a temperature approaching melting ice.

From a risk perspective, particularly those risks which might be usefully managed through temperature control, there exists an extremely broad range of different hazard considerations across this group of products. Moreover, there are some notable exclusions from the TOR, for example live bivalve molluscs.

1.1.1. Definitions in the European Union legislation relevant to interpreting the terms of reference

Regulation EC 853/2004,[4] Annex I, sets out the following Definitions:

> 'Bivalve molluscs' means filter-feeding lamellibranch molluscs. Typical products in an EU context include mussels (*Mytilus edulis*), oysters (*Crassostrea gigas*) and also scallops (*Pectenus maximus*).
>
> 'Fishery products' means all seawater or freshwater animals (except for live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods, and all mammals, reptiles and frogs) whether wild or farmed and including all edible forms, parts and products of such animals.
>
> 'Fresh fishery products' means unprocessed fishery products, whether whole or prepared, including products packaged under vacuum or in a modified atmosphere, that have not undergone any treatment to ensure preservation other than chilling.
>
> 'Prepared fishery products' means unprocessed fishery products that have undergone an operation affecting their anatomical wholeness, such as gutting, heading, slicing, filleting, and chopping.

Regulation 852/2004,[5] Article 1, sets out the following relevant definitions, which are further described below:

[4] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p. 55–205.

[5] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–54.

'Primary products' means products of primary production including products of the soil, of stock farming, of hunting and of fishing.

'Wrapping' means the placing of a foodstuff in a wrapper or container in direct contact with the foodstuff concerned, and the wrapper or container itself.

'Packaging' means the placing of one or more wrapped foodstuffs in a second container, and the latter container itself; the packaging material is not in direct contact with the food product.

'Processing' means any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes.

'Unprocessed products' means foodstuffs that have not undergone processing, and includes products that have been divided, parted, severed, sliced, boned, minced, skinned, ground, cut, cleaned, trimmed, husked, milled, chilled, frozen, deep-frozen or thawed.

'Processed products' means foodstuffs resulting from the processing of unprocessed products. These products may contain ingredients that are necessary for their manufacture or to give them specific characteristics.

1.1.2. Interpretation of 'packaged/pre-packed'

The TOR applies only to fishery products that have been packaged. The interpretation taken regarding this requirement was to include fishery products that have been wrapped, in accordance with the definitions of Regulation (EC) No 852/2004, and fishery products that have been both wrapped and packaged in accordance with those definitions. Thus, any packaging operation that prevents contact with the surface of the fishery product has been included in the interpretation. Fishery products that have never been packaged or that have been unwrapped/unpackaged do not come within the scope of the TOR. Typical operations bringing products within the scope of the TOR might include hermetical sealing in plastic wrapping, or vacuum packaging. Processes such as canning would result in such products no longer being fresh fishery products, therefore these products are outside the scope of the TOR.

1.1.3. Interpretation of 'fresh fishery products'

This is a large group of products that have not been subject to any preservation technique other than chilling. Products of finfish are an important component of the TOR, including whole or gutted finfish or prepared fishery products such as cuts or fillets of such finfish. Both farmed and wild-caught finfish and both freshwater and seawater finfish products are included. The TOR includes these after they have been packaged, but do not include these products until they have been packaged.

Processed fishery products, such as smoked fish or canned fish, fall outside the definition of fresh fishery products and are therefore excluded from the TOR. Packaged unprocessed prepared fishery products, such as breaded unfrozen fillets, would be included.

Live bivalve molluscs may be sold and stored at retail level destined for direct human consumption. As the Regulation (EC) No 853/2004 definition of fishery products explicitly excludes live bivalve molluscs, these are excluded from the TOR. However, some molluscs may be marketed not as live but as fresh fishery products, e.g. shucked scallops, and so fall within the scope of the TOR.

Fresh fishery products comprise a potentially large group of chilled foods. As non-exhaustive typical examples in an European context, this group would include dead finfish such as cod, mackerel or salmon presented as whole eviscerated fish or as parts of fish, e.g. prepared to fillets or cutlets. It would also include crustacean products such as whole live crabs or oysters. Finally, this group of products might include parts of bivalve molluscs such as scallop adductor muscles.

1.1.4. Interpretation of 'thawed unprocessed fishery products'

Frozen fishery products fall outside the legal definition of fresh fishery products, both while they are frozen and after thawing. Frozen fishery products are subject to a specific temperature requirement (−18 °C) while frozen. Thawed unprocessed fishery products derived from previously frozen fishery products are required by EU legislation to meet the same temperature requirement of fresh fishery products, namely to be maintained at a temperature approaching that of melting ice and are therefore included in the TOR.

Typical examples include fishery products which have been frozen as part of the preservation techniques applied to facilitate long fishing trips of freezer or factory vessels, or elongated distribution chains such as imports to the EU. Such frozen products may be thawed to facilitate further processing, or to be presented as thawed unprocessed fishery products for retail sale, e.g. previously frozen imports of seawater cod or freshwater pangasius catfish. A further subgroup of products within this group arises from EU legislation, which states that fishery products to be consumed raw must have been frozen in order to manage certain parasitic risks (e.g. *Anisakis*). Such products are then prepared as thawed products for retail sale, for example products such as sushi or 'maatjesharing' (soused or marinated herring).

1.1.5. Interpretation of 'cooked and chilled products from crustaceans and molluscs'

This component of the TOR is interpreted as fishery products derived from crustaceans and molluscs that have been cooked and then chilled and wrapped, or wrapped and packaged. It does not include packed chilled live bivalve molluscs. These products are included in the assessment given the legal requirement to store them at a temperature approaching that of melting ice.

Whilst molluscs and crustaceans may be sold as live animals, they may also be processed by cooking and chilling at approved production establishments. In addition to servicing the increasing consumer demand for pre-prepared foods, processing to cook and kill the animals addresses the short shelf-life of live animals after removal from the sea, and it is also one of the methods for dealing with molluscs from low microbiological quality water. Typical examples in an EU context include vacuum-packed mussels presented in half-shells, whole cooked crab, or picked vacuum-packed crab meat.

1.1.6. Interpretation of 'temperature approaching that of melting ice'

The temperature of melting ice may reasonably be interpreted as 0 °C, and a temperature approaching that may be interpreted to be as close to 0 °C as is practically possible.

It is difficult to ascribe absolutely any particular temperature as the definitive temperature required to meet this stipulation in the legislation, but, for the purposes of this assessment, the baseline temperature for storage of fishery products is assumed to be 0 °C. As the assessment deals only with fishery products that have been pre-packed, it is understood that this temperature is achieved in the whole product.

1.2. Approach to addressing the terms of reference

This assessment involved the following steps:

1. Identifying the most relevant hazards in the scientific literature associated with the fishery products as defined in the terms of reference.

2. The effect on the relevant hazards of temperature and time during transportation and storage at retail of pre-packed fishery products was quantitatively assessed, using available predictive models, taking into account other factors affecting the growth and concentration of organisms constituting the hazards, such as pH and water activity (a_w), as well as the impact of uncertainty on the estimates. The impact of storage conditions on spoilage organisms was not assessed.

3. The current legislative requirement for fishery products is a temperature approaching that of melting ice. For this reason a temperature of 0 °C in the packaged product was selected as a baseline temperature, which was compared with various alternative temperature scenarios, at different storage times typical of retail storage of packaged fishery products in terms of histamine production and pathogen growth. In practice, the temperature of products placed on ice can be greater than 0 °C, depending on factors such as how the ice is applied, the air temperature and the size of the fish. Nevertheless, 0 °C was selected due to lack of data that could provide a possibly more realistic temperature.

4. Modified atmosphere conditions were also evaluated as they are an option for packaged fishery products.

It has to be noted that the quantitative estimates will reflect changes in hazard growth or concentration, which may not be proportional to the changes in public health risk.

A standard approach to assessing the effect of temperature on product safety is to examine the growth of pathogens in that food. The approach taken in addressing the TOR was to use published models and apply those at different temperatures to predict the quantity of those organisms (and in the case of biogenic amines their metabolites) for which there exist food safety microbiological criteria in EU legislation. For those products the outcomes are directly relevant for those foods within the TOR subject to those specific food safety microbiological criteria. However, within the TOR there are also many types of product for which there exist no food safety criteria in the EU legislation. For those products, some consideration was given to growth of organisms for which there does not exist legislative microbiological criteria (e.g. *Yersinia* spp.).

2. Regulations on fish and fishery products

2.1. General requirements

Fish and fishery products are covered by the Food Hygiene Regulations, which make provisions for their production and handling. Such foodstuffs must meet the relevant hygiene requirements and come from establishments, including vessels, registered or approved pursuant to the Hygiene Regulations. In addition to general hygiene requirements as laid down in Regulations (EC) No 852/2004 and (EC) No 854/2004[6] and their amendments, specific requirements for the hygiene of fish can be found in Regulation (EC) No 853/2004 (Section VIII of Annex III) and Regulation (EC) No 2073/2005 (Chapter 1 of Annex I).[7]

Regulation (EC) No 852/2004 on the hygiene of foodstuffs establishes general hygiene requirements concerning food and a common framework to ensure food safety. It includes the general obligations of the Food Business Operator (FBO) concerning food hygiene, the requirements for Hazard Analysis and Critical Control Point (HACCP)-based food safety management procedures, hygiene requirements for facilities, equipment and processes, staff training and compliance with microbiological criteria for foodstuffs. Regulation (EC) No 854/2004 sets out specific requirements for organising official controls on products of animal origin intended for human consumption.

2.2. Requirements for establishments (including vessels) handling fresh fishery products

Regulation (EC) No 853/2004 on hygiene for food of animal origin lays down specific rules for FBOs and supplements Regulation (EC) No 852/2004 by adding specific hygiene requirements for products of animal origin such as fish and fishery products. Requirements are set in regard to the temperature of premises on land as well as of on-board storage vessels. In the case of establishments (including

[6] Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. OJ L 139, 30.4.2004, p. 206–320.

[7] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1–26.

vessels) handling fishery products, it is required that '*where chilled, unpackaged products are not distributed, dispatched, prepared or processed immediately after reaching an establishment on land, they must be stored under ice in appropriate facilities. Re-icing must be carried out as often as necessary. Packaged fresh fishery products must be chilled to a temperature approaching that of melting ice*'.

2.3. Requirements for mollusc production

Molluscs as filter feeders pose particular foodborne risks arising from the accumulation of microbes or phycotoxins. The latter hazard is managed through monitoring of toxins and toxigenic plankton in production areas, with the ability to close production areas to prevent harvesting when limits are exceeded. One component of the management of microbial risks in EU legislation is a classification of shellfish harvesting areas according to the concentration of *Escherichia coli*. Only shellfish from the lowest risk areas may be placed on the market for direct consumption as live raw entire animals. Another strategy sometimes used to manage microbial risks is 'relaying'. This involves moving the shellfish, towards the end of the production phase, to designated seawater areas of relatively low microbial content with the explicit purpose of reducing the microbial hazards in the molluscs through the normal filtration processes. The relaying phase lasts at least two months. A further possibility is immediate post-harvest depuration at an approved establishment. Depuration involves the storage of the molluscs in tanks through which sufficiently clean or, typically, disinfected seawater is circulated to facilitate depletion of shellfish microbial load. Depuration typically takes several days. Providing the necessary *E. coli* standard is met following relaying or depuration, such shellfish may be placed on the market for direct human consumption as raw live entire animals. Legislation further allows the cooking of such animals to manage microbial risk after they are no longer live bivalve molluscs but regarded as fishery products requiring maintenance at a temperature approaching that of melting ice. Shucking or cooking would substantially decrease the levels of pathogens found in these products.

2.4. Requirements for fishery products that are subject to freezing

According to Regulation (EC) No 853/2004 (modified by the Commission Regulation 558/2010[8]), all parts of frozen fishery products must be stored at a temperature of not more than – 18 °C. With the exception of whole fish initially frozen in brine and intended for the manufacture of canned food, all parts of fishery products must be maintained during transport at a constant temperature of not more than – 18 °C, possibly with short upwards fluctuations of not more than 3 °C. However, FBOs need not comply with this requirement when frozen fishery products are transported from a cold store to an approved establishment to be thawed on arrival for the purposes of preparation and/or processing, if the journey is considered short and the competent authority so permits.

2.5. Temperature requirements for storage and transport of fishery products

During storage and transport, fishery products (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. Requirements are also set for vessels designed and equipped to preserve fresh fishery products for more than 24 hours. They must be equipped with holds, tanks or containers for the storage of fishery products at a temperature laid down in Regulation (EC) No 853/2004 (i.e. a temperature approaching that of melting ice). In vessels equipped for chilling fishery products in cooled clean seawater, tanks must incorporate devices for achieving a uniform temperature throughout the tanks. Such devices must achieve a chilling rate that ensures that the mix of fish and clean seawater reaches not more than 3 °C 6 hours after loading and not more than 0 °C after 16 hours, and allows the monitoring and recording of temperatures.

[8] Commission Regulation (EU) No 558/2010 of 24 June 2010 amending Annex III to Regulation (EC) No 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin. OJ L 159, 25.6.2010, p. 18–21.

2.6. Microbiological criteria

Article 4 of Regulation (EC) No 852/2004 requires that FBOs comply with microbiological limits for foodstuffs, which requires the FBOs to obtain and analyse samples, to compare the results with values set for the criteria and to implement corrective actions if necessary. Chapter 1 of Annex I to Regulation (EC) No 2073/2005 (as well as its amendments, such as Regulation (EC) No 1019/2013[9]) sets out food safety criteria and process hygiene criteria.

Food safety criteria are set for histamine in two different types of fishery products placed on the market during their shelf-life. First, in the case of fishery products from fish species associated with relatively high concentrations of histidine (families *Scombridae*, *Clupeidae*, *Engraulidae*, *Coryphaenidae*, *Pomatomidae*, *Scomberesocidae*), the sampling plan comprises nine units. In two of the units, the histamine concentration may be between 100 and 200 mg/kg; in none may it be above the limit of 200 mg/kg, and the average should be less than 100 mg/kg. The second type comprises fishery products from the same fish species (see above) that have undergone enzyme maturation treatment in brine. Again, a sampling plan of nine units is required; in two of the units the histamine concentration may be between 200 and 400 mg/kg; in none of the units may it be above the limit of 400 mg/kg. For both of these types of products, the analytical method specified is high-pressure liquid chromatography (Malle et al., 1996; Duflos et al., 1999).

Table 1: Occurrence of histamine in fresh, frozen or canned fish meat not undergoing a fermentation process (EFSA BIOHAZ Panel, 2011a)

Number of samples (n)	Percentage of non-detected (ND)	Mean	Percentile P5	Median P 50	Percentile P95	Maximum
6 545	73 %	26.8–31.2	< 0.1	< 2.5	60.5–100	8 910

Other biogenic amines (tyramine, putrescine, cadaverine, etc.) produced from amino acid decarboxylation during bacterial growth in fish may potentiate histamine's effect (FAO/WHO, 2013), but the contribution of these biogenic amines to scombroid fish poisoning is not clear. According to EFSA (EFSA BIOHAZ Panel, 2011a), data from outbreaks show big variation in histidine concentrations leading to adverse effects in the consumer. Variations in sensitivity may also be the result of interaction with other biogenic amines, other diet constituents such as alcohol or medication with diamine oxidase inhibitors.

In addition, there are food safety criteria for cooked crustaceans and molluscan shellfish (as well as for live bivalve molluscs and live echinoderms, tunicates and gastropods) placed on the market during their shelf-life. The criteria specify the absence of *Salmonella* in five samples of 25 g of those products.

Regulation (EC) No 2073/2005 also contains food safety criteria for ready-to-eat (RTE) foods able to support the growth of *Listeria monocytogenes*. Products with $pH \leq 4.4$ or $a_w \leq 0.92$, products with $pH \leq 5.0$ and $a_w \leq 0.94$ and products with a shelf-life shorter than 5 days are considered to be unable to support *L. monocytogenes* growth. The relevance of these criteria is probably greatest in the context of the present mandate regarding RTE fishery products to be consumed raw or almost raw (e.g. sushi or maatjesharing).

Process hygiene criteria are set out in Regulation (EC) No 2073/2005 for shelled and shucked products of cooked crustaceans and molluscan shellfish: *E. coli* and coagulase-positive staphylococci are the microbiological groups to be tested for at the end of the manufacturing process.

[9] Commission Regulation (EU) No 1019/2013 of 23 October 2013 amending Annex I to Regulation (EC) No 2073/2005 as regards histamine in fishery products. OJ L 282, 24.10.2013, p. 46–47.

2.7. Other requirements

The total volatile basic nitrogen limits for fish products of certain categories (EU Regulation (EC) No 2074/2005[10]) are 25, 30 or 35 mg of nitrogen per 100 g of flesh, depending on the species.

3. General description of the fishery products and their production chains

3.1. Overview

Fishery products are food products derived from aquatic animals. The broad range of animals commonly consumed in part or whole as fishery products broadly fit into three categories, namely: vertebrate finfish, invertebrate arthropod crustaceans with an exoskeleton, and invertebrate molluscan species. The source of such animals can be either the marine environment (seawater) or in-land waterways (freshwater). The fish may be captured by fishing for wild fish or may be farmed. Aquaculture production involves ownership while growing before harvest, and the fish are subjected to husbandry techniques designed to encourage growth or productivity in excess of that which might exist in the wild, or it may involve the live capture of mature wild species followed by final finishing period in captivity.

The a_w of fresh fish and shellfish is close to 1.00 (range 0.99–1.00), while the pH range of different fresh finfish species varies greatly (range 5.2–7.0), with most species in the range 6.6–6.8. The carbohydrate content of finfish and crustaceans is very low, and this fact limits the pH depression due to lactic acid production during *rigor mortis*. Tuna and halibut can reach a pH as low as 5.2–5.4, whereas cod has a final pH between 6.0 and 7.0 (ICMSF, 2005). Molluscs, which contain 2–5 % glycogen, can reach a pH as low as 4.8 (oysters) as the carbohydrate is metabolised by the muscle tissue.

3.2. Wild capture

3.2.1. Capture

Finfish and smaller crustaceans are normally killed through the capture process, while larger crustaceans and molluscs may be captured as live animals. Fishing processes may involve the use of active fishing gear, with the propulsive effort of the fishing vessels being an inherent part of the capture process, or passive gear, in which case the gear is capable of capturing fish without any vessel propulsion. Typical active gears used to target finfish include bottom-trawling, in which a net is dragged along the seabed, targeting bottom dwellers, or mid-water trawling, in which the net targets shoaling species. Further active gears for targeting finfish include seining, in which a net is laid in a circle and then pulled tighter into a smaller purse to capture fish. Typical active gears used to target wild molluscs include dredging, which involves actively disturbing the seabed to liberate fish. Typical passive gears include baited pots or cages targeting wild crustaceans, or baited hooks on lines or floating gillnets/tangle nets to target wild finfish.

3.2.2. On-board storage

Fishing vessels vary significantly in scale and size, with resultant differences in fishing activities and on-board storage. Traditional fishing boats will spend a day at sea, eviscerating catches of finfish and storing them on-board in boxes covered with ice. Larger vessels can venture further from shore and stay longer at sea, with the resultant requirement to preserve fishery products caught throughout the trip. Although on-board storage in ice remains common, some vessels, targeting shoaling pelagic fish,

[10] Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004. OJ L 338, 22.12.2005, p. 27–59.

store those fishery products on-board in refrigerated seawater tanks, normally without evisceration. This active refrigeration of the storage water ensures compliance with the requirements described in Section 2. Some vessels go beyond primary production and freeze fishery products as 'whole round' animals, whilst some vessels are factory vessels capable of filleting fishery products before freezing at sea. The temperature requirements for frozen fishery products described in Section 2 apply to freezer establishments on land and at sea.

3.2.3. Landing

A common method of landing fish from traditional vessels is the lifting of iced boxes of eviscerated finfish onto shore and then onto transport vehicles. In the case of on-board bulk storage in refrigerated seawater tanks, the fish are pumped ashore to bulk transport vehicles or directly to processing plants for grading and sorting. Frozen cardboard boxes of fishery products are generally unloaded in a bulk palletised format. Live crustaceans may be transferred to aerated and refrigerated seawater tanks ('Vivier tanks') for transport.

3.3. Aquaculture

Farming of finfish may take various forms, generally mimicking natural life cycle conditions of a species while maximising productivity. Typical systems include on-shore tanks continuously aerated and replenished with freshwater or seawater, purpose-dug in-land ponds or cages suspended from the surface in freshwater rivers or at sea. Many important commercial species are diadromous, meaning that their life cycle involves both freshwater and seawater phases, typically resulting in farming systems whereby freshwater juveniles are grown and finished at sea. Fish are fed commercially formulated feed produced from processed fish protein, typically derived either as by-products of processing fishery products for human consumption or as species caught but not commercially used for human consumption.

Farmed finfish are typically slaughtered with percussive stunning followed by gill bleeding. Finfish finished at sea may be pumped alive onto a well-boat for slaughter on that vessel at sea, refrigerated, landed, then eviscerated and further processed at an approved establishment ashore. An alternative is for fish to be brought to shore alive either in a well-boat or pumped, for subsequent slaughter at an establishment approved for fish slaughter.

Molluscs may be farmed through 'bottom growing', whereby wild juvenile animals are brought to in-shore seabed areas to enhance production. Molluscs may also be attached to rope systems or placed in cages, and these are then suspended from the sea surface. A further system involves bags of molluscs grown on trestles placed on the intertidal foreshore.

3.4. Processing and distribution

Fishery products may be distributed to the retailer as primary products (eviscerated with fins on). During distribution, fresh fishery products should be maintained at a temperature approaching that of melting ice. Live crustaceans may be transported in chilled aerated tanks of seawater, or alternatively may be further chilled for transport approaching 0 °C with dry ice. Fishery products may be further processed at establishments approved for that process. Typical processing of finfish includes filleting (separation of high-value muscle from the skeleton) followed by freezing. Further processing may include smoking or fermentation. Processing of crustaceans usually includes cooking and killing followed by removal of meat or freezing of entire carcasses. Typical processing of larger bivalve molluscs involves 'shucking' to remove undesirable body parts. Other bivalve molluscs may be cooked and packaged at processing establishments.

3.5. Production, trade and consumption of fishery products in the European Union

The following data are based primarily on the annual overview provided by the Directorate-General for Maritime Affairs and Fisheries (DG-Mare) and collated through the European Market Observatory for Fisheries and Aquaculture (EUMOFA) (EU, 2014a, b).

3.5.1. European Union production

Worldwide production of fishery products is dominated by China, which is by far the largest producer of fishery products (wild and farmed, marine and freshwater), with other countries accounting for the individually relatively small contributions when compared to that major player (FAO, 2014). Considering capture fisheries and aquaculture together, the EU is the fifth largest producer worldwide, producing approximately 6 million tonnes per year or approximately 3.5 % of worldwide production. Spain, the UK and Denmark are the three largest EU producers in terms of volume of production.

EU fish production remains predominantly (80 %) composed of capture fisheries, in contrast to other major world producers of fishery products, where aquaculture dominates. Wild-caught species may generally fall into two broad categories: demersal fish, which swim towards the bottom of the sea on continental shelf close to land; and pelagic fish, which swim as shoals higher up in the water. Pelagic species, namely herring, sprat, mackerel, sand eels, sardines and horse-mackerel, are the dominant wild-caught species. Demersal species of importance at an EU level include cod, hake and plaice. The EU fishing fleet comprises some 87 000 fishing vessels with approximately 15 % trawlers. The fleet is made up of large numbers of small vessels and a smaller number of large vessels, with the latter group contributing to the vast bulk of fish capture in the EU.

EU aquaculture production accounts for approximately 20 % of the total EU production of fishery products. Approximately 50 % of EU aquaculture volume comprises molluscs, with mussels, oysters and clams dominating, while 27 % is seawater farming of finfish, notably salmon, trout, sea bream and sea bass; the remaining 23 % of EU aquaculture volume comprises freshwater finfish such as carp. This contrasts with the picture in world aquaculture production, where freshwater fish dominate.

3.5.2. Trade to and from the European Union

The EU is a major world trader in fishery products with substantial imports and exports. The EU is a net importer, with 45 % of the total estimated EU consumption covered by EU production. Whilst the USA, Japan and China are the largest individual importing countries, the EU is the largest import market for fishery products in the world (FAO, 2014) In simplistic terms, the EU exports substantial quantities of wild-capture fishery products (approximately 1.9 million tonnes per year) and imports substantially higher quantities of fishery products (approximately 5.5 million tonnes per year), particularly farmed products. The main suppliers to the EU are Norway, China, Iceland, Ecuador, the USA and Vietnam. The main customers for EU fishery products are the USA, Norway, Switzerland, China, Nigeria, Japan and Russia. Of direct relevance to the present work is the fact that approximately 20 % of both imports to, and exports from, the EU are in the form of fresh/chilled fishery products.

3.5.3. Consumption in the European Union

Data from 2010 show that average EU consumption of fish is 23.1 kg per capita per year, ranging from 5.3 kg in Hungary to 56.7 kg in Portugal. For comparison, the worldwide average is 18.9 kg per capita, with figures of over 30 kg per capita in China, over 50 kg in Norway and Japan, and over 90 kg in Iceland. EU consumption comprises approximately 75 % wild-caught and 25 % farmed species. The top 13 species consumed by EU consumers are tuna, cod, salmon, pollack, herring, mussels, hake, mackerel, pangasius, shrimps, sardines, squid and scallops (EU, 2014a). Several of these (for example tuna, mackerel and herring) are amongst the species which might potentially be associated with the formation of biogenic amines (see Section 4.2.2).

3.6. Description of current chilling practices (temperature/time) at transport and retail for fishery products

Fish, as cold-blooded animals, exist at the ambient temperature of their environment, so the initial temperature for fishery products is generally substantially above 0 °C. Seawater and freshwater temperatures vary significantly depending on many factors, including latitude, depth, freshwater sources or oceanographic currents. In approximate terms, EU waters and resultant initial fishery

product temperatures range from 4 °C in northern EU waters to 25 °C or higher in southern EU waters. It is worth noting that, under the terms of various international agreements, EU fishing vessels may fish in extra-EU waters and over half of the fishery products consumed in the EU are imported from outside the EU. Fishery products may therefore have initial temperatures varying from polar (– 4 °C) to equatorial (35 °C).

The general approach to achieving a temperature approaching that of melting ice immediately following harvest is to eviscerate the fish and place the carcasses on or in ice. The resultant temperature gradient brings those fish surfaces in contact with ice close to 0 °C relatively rapidly, with the deeper core temperature dropping more slowly. Factors which might affect the speed of reaching the desired temperature include the ratio of fish to ice, the completeness of cavity icing, the size of the fish, the temperature of the ice, the temperature of the fish and, on longer fishing trips, the frequency of re-icing. An inevitable consequence of the heat transfer from the fish to the ice is for some ice to melt and become a liquid. Whilst the temperature of slush-ice mixture arguably approaches that of melting ice, the efficacy of icing to maintain the cold chain is optimised by separating fish from melt water. After landing and throughout the subsequent distribution chain, the typical approach to maintaining the cold-chain is to maintain the carcasses on or in ice and away from melt water whilst in boxes.

Physical contact with ice is the most common chilling method used from the point of supply to the final consumer for fresh fishery products, but refrigeration in temperature-controlled compartments is also used, particularly at retail level. In the context of the current report, refrigerated cabinet storage is normally the case for packaged fishery products.

Data on actual temperatures of fishery product storage are relatively scarce for those fishery products in the current TOR. Processed fishery products are not subject to any legal temperature requirement outside the current TOR; however, some temperature information of indirect relevance is provided by the EU-wide baseline survey for *L. monocytogenes*, which found a mean temperature of 3.5 °C with standard deviation of 1.8 °C for retail storage of packaged smoked fishery products of (EFSA, 2013).

4. Hazard identification

4.1. Methodology

A food-borne hazard is defined by the Codex Alimentarius Commission as a 'biological, chemical or physical agent or property of food with the potential to cause an adverse health effect' (CAC, 1999). Fresh fishery products are associated with foodborne illness. In 2013, for example, 8.5 % of strong-evidence foodborne outbreaks in Europe were associated with fish and fishery products, while crustaceans, shellfish, molluscs and products thereof accounted for 7.3 % (EFSA and ECDC, 2015). This is in broad agreement with Huss et al. (2000), who suggested that up to 10 % of foodborne outbreaks in any given year are linked to the consumption of seafood. While pathogens such as *Listeria monocytogenes*, *Clostridium botulinum* type E, *Vibrio* spp., *Aeromonas* spp. and faecal bacteria and viruses may contaminate fish, causing human illness (Lampila and McMillin, 2012), the majority of seafood-borne outbreaks are associated with biogenic amines (Huss et al., 2000; Dalgaard et al., 2006).

A preliminary screen of the scientific and grey literature suggested there was very little information available of the biological hazards occurring in the specific products defined by the TOR (fresh fishery products, thawed unprocessed fishery products and/or cooked and chilled products from crustaceans and molluscs). The first step in the hazard identification process carried out in this risk ranking activity therefore focused on identifying biological hazards occurring in all fishery products that may cause disease in humans. A narrative review of the scientific and grey literature was undertaken based on the TOR and using relevant databases. The search terms included; bacteria, bacterial contamination, pathogen, foodborne pathogens, foodborne outbreaks, *Morganella morgana, Raultella planticola, Photobacterium phosphoreum,* fish, fishery products, fish cut, fish fillet, fish processing, fresh fish,

fresh fish product, histamine, histamine levels, histamine poisoning, histamine food poisoning, biogenic amines, temperature, transport, packaging, chilling and frozen-thawed. There were no restrictions based on the year of publication. The output of this exercise was a long list of biological hazards (Table 2).

Each of the biological hazards included in this preliminary (long) list was then examined as to whether there was evidence of an association between contaminated seafood and human illness and whether it was capable of growth under refrigeration conditions. Those hazards from the preliminary long list that were associated with human illness and capable of growth under chilled conditions were included in a final short list of hazards to be considered for inclusion in the modelling.

4.2. Results of hazard identification

4.2.1. Preliminary long list of biological hazards

Following the methodology explained in Section 4.1.1, the hazards included in the preliminary long list are presented in Table 2. Each of those hazards was examined for evidence of an association with human illness and an ability to grow under refrigeration conditions. Based on this analysis, *Morganella* spp., *Photobacterium* spp., *Clostridium botulinum*, *Listeria monocytogenes* and *Yersinia enterocolitica* were considered for inclusion in modelling storage conditions (Section 5). Further information on each of the hazards, supporting the analysis summarised in Table 2, is provided in the remainder of this section.

Table 2: Preliminary long list of biological hazards and the result of the assessment against the two criteria (i.e. evidence of association with human illness and ability to grow under refrigeration conditions)

Biological hazard	Evidence that the organism may cause human illness associated with fishery products?[(a)]	Evidence that the organism is capable of growth and/or biogenic amine production under chill conditions	Included in short list for modelling consideration	Evidence (references)
Histamine-producing bacteria[(b)]				
Aeromonas spp.	No	Yes	No	Bermejo et al. (2003)
Citrobacter koseri	No	No	No	Bermejo et al. (2003)
Clostridium perfringens	No	No	No	
Enterobacter spp.	Yes	No	No	Allen et al. (2005), Chen et al. (2011), Lee et al. (2013)
Hafnia alvei	No	No	No	
Klebsiella pneumoniae	No	No	No	
Morganella spp.	Yes	Yes	Yes	Emborg et al. (2006) ; Lee et al. (2013)
Photobacterium spp.	Yes	Yes	Yes	Kanki et al. (2004) ; Dalgaard et al. (2006)
Proteus vulgaris,	No	No	No	
Pseudomonas spp.	No	Yes	No	Gui et al. (2014)
Vibrio spp.	No	Yes	No	Gui et al. (2014)
Bacteria				
Aeromonas spp.	Yes	No, (species associated with	No	Davies and Slade (1995); Davies et al. (2001);

Biological hazard	Evidence that the organism may cause human illness associated with fishery products?[(a)]	Evidence that the organism is capable of growth and/or biogenic amine production under chill conditions	Included in short list for modelling consideration	Evidence (references)
		human disease are mesophilic)		Thayumanavan et al. (2003); Di Pinto et al. (2012)
Bacillus cereus	Yes	No	No	Domenech-Sanchez et al. (2011)
Clostridium botulinum	Yes-Type E	Yes	Yes	Peck et al. (2006)
Clostridium perfringens	Yes	No	No	Sciarrone et al., (1997); Hewitt et al. (1986)
Listeria monocytogenes	Yes	Yes	Yes	Dorsa et al. (1993); Gawade et al. (2010); Pinto et al. (2006); Tham et al. (2000)
Salmonella spp.	Yes	No	No	Friesema et al. (2014); Wyatt et al. (1979)
Staphylococcus aureus	Yes	No	No	Colavita et al. (2000); Solano et al. (2013); Vazquez-Sanchez et al. (2012)
Vibrio spp.	Yes	Yes	No	McLaughlin et al. (2005); Gui et al. (2014)
Yersinia enterocolitica	No	Yes	Yes	Davies and Slade (1995); Davies et al. (2001); Papadopoulou et al. (2007)
Viruses				
Norovirus	Yes	No	No	Prato et al. (2004); David et al. (2007)
Hepatitis A	Yes	No	No	Potasman et al. (2002)
Parasites				
Cestodes	Yes	No	No	Iwamoto et al. (2010)
Giardia spp.	Yes	No	No	Iwamoto et al. (2010)
Nematodes	Yes	No	No	Iwamoto et al. (2010)
Trematodes	Yes	No	No	Iwamoto et al. (2010)

NA: not applicable as none of the criteria is met.
(a): May cause illness associated with fishery products: this includes both fishery products within the terms of reference (TOR) and fishery products outside the TOR.
(b): Bacteria that produce histidine decarboxylase.

4.2.2. Histamine-producing bacteria

The most important biogenic amine in fish is histamine, as it is the hazard most frequently implicated in outbreaks associated with fish and fishery products (Huss et al., 2000; Dalgaard et al., 2006). This causes an allergy-type of food poisoning associated with fish, primarily with scombroid fish such as tuna and mackerel. However, non-scombroid species such as sardines, pilchards, anchovies, herring, marlin, bluefish, salmon and swordfish have also been implicated in outbreaks. Symptoms include headache, rash, oral burning, a tingling sensation in the fingers and diarrhoea and usually occur within 60 minutes of ingestion (Dalgaard et al., 2006). The muscle tissues of scombroid fish are rich in

histidine and scombrotoxin is typically formed when histidine is converted to the biogenic amine histamine by the bacterial enzyme histidine decarboxylase (Ruiz-Capillas and Moral, 2004).

Histidine decarboxylase is produced by a range of bacteria including *Hafnia alvei*, *Morganella morganii*, *Morganella psychrotolerans*, *Photobacterium phosphoreum*, *Photobacterium psychrotolerans*, *Photobacterium damsel*, *Citrobacter koseri*, *Aeromonas hydrophila*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Clostridium perfringens*, *Vibrio* spp., *Pseudomonas* spp. and *Enterobacter* spp. (Kanki et al., 2004; Emborg et al., 2006; Ozogul and Ozogul, 2006; Dalgaard et al., 2008).

As most of these bacteria are mesophilic, it was initially thought that histamine formation could be prevented if fish were stored at correct refrigeration temperatures. However, more recent research has reported *M. psychrotolerans* and *P. phosphoreum* as significant producers of histamine at temperatures of 0 to 5 °C (Kanki et al., 2004; Emborg et al., 2006). Dalgaard et al. (2006), for example, reported histamine levels of up to 4 490 ppm produced by *P. phosphoreum* strains in garfish stored at 5 °C both in air and in modified atmospheric packaging (MAP). Histamine fish poisoning outbreaks have been associated with fish containing 750–900 ppm, 1 200 ppm and 1 000–1 200 ppm histamine (Dalgaard et al., 2006). A list of reported bacteria-product combinations is provided in Table 3 and information on some fish-associated histamine outbreaks is provided in Table 4.

Thus, whilst higher temperatures may increase the likelihood of growth of bacterial organisms capable of producing histidine decarboxylase, some relevant bacteria may also grow at low temperatures. Furthermore, apart from the effect of temperature on bacterial growth and enzyme production, the activity of the enzyme once formed is temperature independent. Large amounts of histamine are formed, for example, by *M. morganii*, even at low temperatures (0–5 °C), following storage at higher temperatures (10–25 °C), even though bacterial growth does not take place at 5 °C or below (Klausen and Huss, 1987). This occurs because the enzyme histidine decarboxylase, generated during storage at high temperatures, remains active at the lower temperatures and is responsible for histamine production at 5 °C or below. van Spreekens (1986) reported similar findings. Moreover, biogenic amines are very heat stable and, once formed, they will not be destroyed even by dramatic heat treatment such as autoclaving (121 °C at 103.4 KPa for 15–20 minutes).

Although there is a dearth of data on histidine and histamine levels in fish, a survey of the levels of free histidine present in retail fish in New Zealand found levels above 10 000 mg/kg in albacore, kingfish and kahawai and levels between 2 000 and 10 000 mg/kg in grey mullet, trevally, sprat, piper, jack mackerel, yellowtail, blue mackerel and barracuda (Fletcher et al., 1995). It was concluded that all of these species have the potential to cause histamine poisoning (Fletcher, 2010). As all New Zealand histamine poisoning incidents in the 10 years prior to 1997 involved hot-smoked fish, the study was extended to investigate the levels of histamine in these products. In eight samples, obtained from five different retailers, histamine levels were above 50 mg/kg, with levels in two of these samples exceeding 200 mg/kg (346.4 and 681.8 mg/kg). Moreover, when stored at 20 °C for 4 days, eight out of 33 samples developed histamine levels above 50 mg/kg, with levels in four out of the 33 samples exceeding 200 mg/kg (maximum 1 659.4 mg/kg).

Cold chain control is the primary risk management strategy used to manage the risk of histamine production in scombroid fish. Other strategies include limiting fishing gear soak time (the time fishing gear is left in the water, thereby the maximum time that fish remain in the water before being lifted onto the vessel) to minimise duration from death to initiating chilling; in addition, efficient and gentle handling of fish *post mortem* is a good strategy for minimising tissue damage and resultant extracellular histidine. EU regulations (EC No 2073/2005) specify a food safety criterion for histamine based on nine sample subunits (see Section 2.6).

It should be highlighted that data from outbreaks and individual studies show a large variation in concentrations of histamine leading to adverse effects in the consumer. Variations in sensitivity may also be the result of: synergistic interaction with other biogenic amines such as tyramine, cadaverine

and putrescine; unidentified chemicals in the fish; diet constituents such as alcohol; or medication with certain drugs, particularly with monoamine oxidase inhibitor drugs (EFSA BIOHAZ Panel, 2011a; FAO/WHO, 2013).

Table 3: Bacteria-product combinations associated with histamine production

Histamine-producing bacteria	Fishery product	Country	Reference
Morganella morganii	Salted semi-preserved anchovies (*Engraulis encrasicholus* var. *mediterraneus*)	Spain	Rodriguez-Jerez et al. (1994)
	Yellowfin tuna (*Thunnus albacares*)	India	Emborg et al. (2005)
	Tuna (*Thunnus obesus*)	Japan	Tao et al. (2009)
Photobacterium phosphoreum	Garfish fillets (*Belone belone belone*)	Denmark	Dalgaard et al. (2006)
	Yellowfin tuna (*Thunnus albacares*)	India	Emborg et al. (2005)
	Mackerel (*Scomber* or *Trachurus* spp.)	Japan	Morii and Kasama (2004)
	Tuna (*Thunnus obesus*)	Japan	Tao et al. (2009)
	Salmon (*Salmo salar*)	Norway	Emborg et al. (2002)
Photobacterium damsela	Jack mackerel (*Trachurus symmetricus*)	Chile	Bermejo et al. (2003)
Enterobacter cloacae	Mahi-mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*)	USA	Allen et al. (2005)
Enterobacter aerogenes	Sailfish (*Istiophorus platypterus*) and milkfish (*Chanos chanos*)	Taiwan	Tsai et al. (2005)
Staphylococcus kloosii	Mahi-mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*)	USA	Allen et al. (2005)
Staphylococcus xylosus	Salted semi-preserved anchovies (*Engraulis encrasicholus* var. *mediterraneus*)	Spain	Rodriguez-Jerez et al. (1994)
Proteus vulgaris	Jack mackerel (*Trachurus symmetricus*)	Chile	Bermejo et al. (2003)
Aeromonas hydrophila	Jack mackerel (*Trachurus symmetricus*)	Chile	Bermejo et al. (2003)

Table 4: Fish-associated biogenic amine outbreaks

Pathogen	Fishery product	Country	Reference
Photobacterium phosphoreum	Dried sardine (*Iwashi maruboshi*)	Japan	Kanki et al. (2004)
Specific histamine-producing bacteria strains not reported	Frozen raw swordfish fillets (*Xiphias gladius*)	Taiwan	Chang et al. (2007)
Enterobacter aerogenes, *Raoultella ornithinolytica* and *Morganella morganii*	Striped marlin (*Tetrapturus audax*)	Taiwan	Lee et al. (2013)
Bacillus subtilis and *Enterobacter aerogenes*	Mahi-mahi (*Coryphaena hippurus*)	Taiwan	Chen et al. (2011)
Specific histamine-producing bacteria strains not reported	Marlin fillets	Taiwan	Hwang et al. (1999)

4.3. Bacteria

4.3.1. *Aeromonas* species

Aeromonas species are Gram-negative bacteria widely distributed in aquatic environments (Janda and Abbott, 2010). Although some species are capable of growth at refrigerated temperatures (e.g. *A. salmonicida*, a fish pathogen), the species associated with human disease (*A. hydrophila*, *A. caviae* and *A. sobria*) are mesophilic. Associated symptoms include gastroenteritis, with more serious illnesses, such as septicaemia, occurring in immune-compromised individuals. These bacteria are

found in finfish and prawns. Thayumanavan et al. (2003) reported incidences of 37.3 % and 35.6 % in finfish and prawns, respectively. Davies et al. (2001) detected *A. hydrophila* in fish from the UK, France, Poland and Greece, with an overall incidence of 40 %. Moreover, an Italian study in 2012 detected *Aeromonas* spp. in 70.3 % of RTE seafood samples including sushi (90.5 %), sea salad (85.7 %), surimi (91.7 %) and peeled shrimp (75 %) (Di Pinto et al., 2012), although it was not known if any of these *Aeromonas* isolates were pathogenic to humans. Reducing the risk of human infections requires the implementation of a good management programme on fish farms, including infection-preventative strategies.

4.3.1. *Bacillus cereus*

B. cereus is also a Gram-positive spore former, and is widely distributed in the natural environment. It causes illness in consumers in two ways: an infection in which ingested cells produce the enterotoxin in the small intestine and intoxication due to the preformed toxin. While most foods can be contaminated, the majority of cases are associated with heat-treated foods that are then subject to temperature abuse. Seafood-associated illness is rare but, in 2007, an outbreak associated with *B. cereus*-contaminated tuna fish that had been inadequately cooked occurred at a Spanish resort (Domenech-Sanchez et al., 2011). Reducing contamination, proper cooking and chilled storage would prevent *B. cereus* illness associated with seafood.

4.3.2. *Clostridium botulinum*

C. botulinum is a Gram-positive spore-forming bacteria that produces a potent neurotoxin associated with severe illness in humans. Eight types of botulinum toxin are known (A to H), with consumption of types A, B and E usually associated with human illness. Type E-producing *C. botulinum* is commonly found in aquatic environments, while *C. botulinum* strains producing types A and B are present in the general environment. These bacteria may grow and produce toxin in food products, including seafood, that are stored under low-acid, anaerobic conditions. Under conditions that support clostridial growth, raw fish could be a high-risk product, with numerous challenge tests indicating the possibility of toxin formation in 10 days at 8 °C (Peck et al., 2006). Fishery products in MAP have been associated with botulism. In 1990, three people died in the USA after consuming barbequed (fresh) sturgeon fish (palani) which contained *C. botulinum* type B toxin. Further investigation suggested that this cooked and chilled product had been temperature abused (Peck et al., 2006). Implicated seafood also includes canned fish. Cooking for a minimum of 90 °C for 10 minutes is recommended for these products to achieve a 6 log reduction in *C. botulinum* spores. However, inadequate heat treatment or post-processing contamination may result in food poisoning, as was the case with the 1978 tinned salmon outbreak in the UK, in which two elderly consumers died after the product was contaminated post retorting (Peck et al., 2006). Although many fish processors prefer vacuum packaging, MAP is still used. Fresh fish in MAP (low-fat/non-oily fish) will have a 6- to 8-day shelf-life if stored at – 1 to 2 °C in packs containing 30 % O_2, 40 % CO_2 and 30 % N_2 (retail) or 70 % CO_2 and 30 % N_2 (bulk) or, in the case of high-fat/oily fish, 40 % CO_2 and 60 % N_2 (retail) and 70 % CO_2 and 30 % N_2 (bulk) (Peck et al., 2006). In Germany (1997), two people died after consuming hot-smoked vacuum-packed fish (Räucherfisch) and, in 2004, another German consumer died after eating vacuum-packed smoked salmon. Both products were contaminated with non-proteolytic *C. botulinum* type E.

4.3.3. *Clostridium perfringens*

C. perfringens is a Gram-positive, spore-forming organism that is ubiquitous in soil, decaying vegetation and marine sediment. Seafood-associated cases are rare and are usually associated with foods held at growth-supporting temperatures for extended periods. The storage temperature of fishery products affects both the rate of bacterial multiplication and the rate of enterotoxin elaboration by the bacteria present, and food poisoning can be prevented by effectively chilling raw seafood

4.3.4. *Listeria monocytogenes*

L. monocytogenes is found in the general environment and in fish processing plants and may also contaminate seafood products. While the levels are usually low and well below the legal safety limit of 100 cells per gram that is considered unsafe for healthy consumers, they may present a risk to susceptible people such as the elderly, immune-compromised people and pregnant women. RTE seafood products outside the current TOR are among the food samples regularly reported to exceed the legal safety limit. In 2013, for example, 4.6 % of single samples and 19.9 % of batches (mainly smoked fish) were found not to comply with the criterion of ≤ 100 colony-forming units (CFU)/g (EFSA and ECDC, 2015). In a different group of products also not included in the present TOR, Pinto et al. (2006) found *L. monocytogenes* in 4 % of raw bivalve molluscs, although at low contamination levels (less than 100 most probable number (MPN/g)). A similar study by Gawade et al. (2010) reported *L. monocytogenes* prevalence of 12.5 % in raw bivalve molluscs, 3.8 % in prawns and 2.9 % in finfish. Arumugaswamy et al. (1994) reported a prevalence of 44 % of *L. monocytogenes* in raw prawns and of 22 % in RTE samples.

Protecting consumers is therefore reliant on implementing measures to prevent cross-contamination, storing these products under strict chilled conditions, establishing an appropriate shelf-life and consuming within this shelf-life. Whilst *Listeria* is one of the few pathogenic organisms capable of growth at common refrigeration temperatures (see Table 5), its growth rate is considerably reduced at lower temperatures.

Table 5: Growth conditions for bacterial pathogens found in fishery products as reported in the literature

Bacterial hazard	Temperature growth range	Minimum a_w supporting growth	pH range for growth	Oxygen requirement
A. hydrophila	0–43 °C	0.94	4–10	facultative anaerobe
C. botulinum	Type A and B, 10–50 °C; Type E, 3–45 °C	0.95	4.6–8.9	anaerobe
C. perfringens	10–52 °C	0.93	5–9	anaerobe
L. monocytogenes	− 1–45 °C	0.91	4.5–9.6	facultative anaerobe
Salmonella spp.	5–46 °C	0.93	4.1–9.0	facultative anaerobe
S. aureus	4–46 °C	0.86	4.8–8.0	facultative anaerobe
V. parahaemolyticus	4–45 °C	0.94	4.8–11	facultative anaerobe
Y. enterocolitica	− 2–45°C	0.96	4.2–10	facultative anaerobe

Compiled using data from Huss (1993), ICMSF (International Commission on Microbial Specifications for Food) (1996), James and James (2014).

4.3.5. *Salmonella* species

Salmonella species are Gram-negative bacteria that cause acute gastroenteritis and are the most frequently reported bacterial cause of foodborne outbreaks in Europe. Different serotypes are associated with particular ecological niches and many animal hosts serve as reservoirs for non-typhoidal species. Humans are the only known source of *Salmonella* Typhi. Serovars such as *S.* Weltevreden are part of the indigenous microflora of fish farms and ponds used in aquaculture with reported prevalences of 21, 5 and 22 % in Japanese eel ponds (Saheki et al., 1989), North American catfish ponds (Wyatt et al., 1979) and prawn and shrimp ponds (Reilly and Twiddy, 1992), respectively. However, these species are not usually linked to human disease. Most non-typhoidal salmonellosis cases are associated with a range of foods including eggs, egg products, poultry and red meats. Seafood has also been implicated in foodborne outbreaks, including fish, shrimps, oysters and clams harvested from faecally-contaminated waters (Balasubramanian et al., 1992). Moreover, large shellfish-associated outbreaks of *S.* Typhi have been traced to harvest beds contaminated with sewage. Seafood may also be contaminated with *Salmonella* during processing, handling and storage. Prevention is reliant on maintaining uncontaminated waters, proper processing (including adequate cooking) and chilled storage. Products included in the category 'cooked and chilled products from

crustaceans and molluscs' are subject to *Salmonella* food safety criteria, as specified in Regulation (EC) No 2073/2005 (see Section 2.1).

4.3.6. *Staphylococcus aureus*

S. aureus is a Gram-positive coccus, normally found in the nasal passage, on the skin and in wounds in humans. Seafood contamination usually occurs as a result of cross-contamination from food handlers. Vazquez-Sanchez et al. (2012) reported an incidence of 27 % in salted fish, 26 % in smoked fish, 25 % in ready-to-cook products, 20 % in non-frozen surimi, 17 % in fish roe and 10 % in RTE products. This species of bacteria can grow in temperature-abused seafood and can produce enterotoxins which cause nausea and vomiting within one to six hours after ingestion. Whilst the toxin is relatively heat stable once formed, the storage temperatures of the fishery products affect both the rate of bacterial multiplication and the rate of enterotoxin elaboration by the bacteria present. Control is reliant on the strict implementation of GHP during handling and processing combined with effective chilling.

4.3.7. *Vibrio* species

Vibrio species are Gram-negative halophilic bacteria widely distributed in marine environments. At least 14 of the 30 known species are pathogenic, with *Vibrio parahaemolyticus* and *Vibrio vulnificus* most commonly associated with human illness. Moreover, *V. cholerae*, the causative agent of cholera, has been isolated from and associated with the consumption of fish (Senderovich et al., 2010). In general, *Vibrio* foodborne infections are almost exclusively associated with seafood, including raw oysters and bivalve molluscs. Higher temperature waters have a greater likelihood of supporting higher *Vibrio* contamination. Outbreaks are often seasonally distributed, with the majority occurring during the summer and early autumn when the water is warmer. Control is reliant on rapid cooling to inhibit post-harvest growth, thorough pasteurisation, high-pressure treatment, irradiation and/or quick freezing and the prevention of cross-contamination from raw seafood or contaminated water.

4.3.8. *Yersinia enterocolitica*

The bacterial genus *Yersinia* comprises a diverse group of environmental, human and fish (e.g. *Y. ruckeri*) pathogenic species. The predominant species associated with foodborne human disease is *Yersinia enterocolitica*. Most human infections are caused by a limited number of bioserotypes, including 4/O:3, 2/O:9, 2/O:5,27 and 1B/O:8. *Y. enterocolitica* can grow at refrigeration temperatures, including in foods in MAP and vacuum-packed foods. Although widely distributed in the natural environment, most foodborne cases are associated with porcine meat. However, *Y. enterocolitica* is not completely limited to this food source and has also been isolated from milk, vegetables and seafood. Papadopoulou et al. (2007), for example, found *Y. enterocolitica* in 40 % of mussels, although the organism was not detected in other seafood. Furthermore, in a study of fish from France, Greece, Portugal and the UK, *Y. enterocolitica* was detected in salmon (four out of five samples) and trout (three out of 20 samples) (Davies et al., 2001). Although there is no evidence *Y. enterocolitica* has caused a seafood associated illness or outbreak, any risk to human health associated with consuming seafood contaminated with *Y. enterocolitica* could be further reduced by harvesting from uncontaminated waters, applying Good Hygiene Practice (GHP) and effective chilling.

4.4. Viruses

4.4.1. Hepatitis A virus

Humans are the only known reservoir for the hepatitis A virus, and, like norovirus, transmission is also usually via the faecal–oral route. Seafood outbreaks have been associated with molluscs, oysters and clams grown in areas contaminated with human sewage. The largest outbreak to date occurred in China in 1988, when almost 300 000 people became infected after consuming clams harvested from sewage-polluted areas (Potasman et al., 2002). Raw and steamed shellfish are a particular problem, as mild cooking is not sufficient to destroy this virus. As is the case for norovirus, those seafood products most at risk of transmitting hepatitis A are live bivalve molluscs consumed raw and in their entirety

(EFSA BIOHAZ Panel, 2011b), and therefore are not within the scope of the fishery products covered by this report.

4.4.2. Norovirus

Humans are the only known reservoir for norovirus, which is usually transmitted person to person via the faecal–oral route. As this virus can persist in the environment and the infectivity is high, norovirus is a leading cause of gastrointestinal illness and outbreaks are particularly common in closed populations such as in child care centres, on cruise ships and in homes for the elderly. Norovirus may also occur in marine environments and in seafood. Boxman et al. (2006) reported that 4.8 % of Dutch oyster farms and 12.5 % of oysters were norovirus-positive, as were 38.5 % of imported mussels. Large seafood-associated outbreaks have been linked to shellfish (especially bivalve filter feeders), oysters and clams harvested from sewage-contaminated waters (Simmons et al., 2007; Iwamoto et al., 2010; Wall et al., 2011). In general terms, the highest risk products in a seafood context are live bivalve molluscs consumed in their entirety and raw. In 2002, an oyster-associated outbreak in Italy and France resulted in 327 confirmed cases (Guyader et al., 2006). In the same year, the consumption of raw fish contaminated with norovirus was linked to an outbreak of gastroenteritis in Bari (south-east Italy). Of the 103 confirmed cases in Bari, 22 were defined as 'probable' secondary cases (Prato et al., 2004). Two years later, norovirus-contaminated oysters were responsible for another outbreak (53 cases) in British Columbia, Canada, over a three-month period (David et al., 2007). This report covers cooked and chilled molluscs and proper cooking should be sufficient to kill any norovirus present in the raw material.

4.5. Parasites

The EFSA BIOHAZ Panel (2010) concluded that human fishery product-borne parasitic diseases primarily include those caused by nematodes, trematodes and cestodes. These diseases are either caused by an infection following ingestion of viable parasites, or as an allergic (hypersensitivity) reaction against parasite antigens. The nematode species most commonly associated with human infection is *Anisakis simplex*, followed by *Pseudoterranova decipiens*. Trematodes belonging to the family Opisthorchiidae play an important role as fish-borne parasitic zoonoses in humans and cause serious disease such as cholangitis, choledocholithiasis, pancreatitis, and cholangiocarcinoma in certain areas of the world. *Diphyllobothrium* is a genus of cestode, and at least 13 species have been reported infecting humans. Consumption of raw or insufficiently cooked or marinated fish is the main source of infection with *Diphyllobothrium* for humans. Freezing or heat treatments remain the most effective processes to ensure killing of parasitic larvae, under well-defined conditions. As parasites do not reproduce outside of their hosts, these hazards were excluded when modelling different storage conditions.

4.6. Toxins

Ciguatera is a type of food poisoning associated with eating fish from tropical and subtropical waters contaminated with biotoxins produced by dinoflagellates such as *Gambierdiscus toxicus*. The dinoflagellates adhere to algae, seaweed and coral and are consumed by herbivorous fish, which are then consumed by carnivorous fish, resulting in biomagnification of the toxin up the food chain.

Shellfish poisoning, such as paralytic shellfish poisoning, diarrhoetic shellfish poisoning, neurotoxic shellfish poisoning and amnesic shellfish poisoning, can also be caused by dinoflagellates, which accumulate in filter feeders, producing a range of phycotoxins such as saxitoxin, brevetoxin, domoic acid and okadaic acid, all of which are heat stable and are not destroyed by cooking. Control is therefore reliant on harvesting from uncontaminated waters. Given that toxins are not influenced by post-harvest storage temperatures, they are excluded from the assessment.

4.7. Concluding remarks

- In the context of this report, which focuses on fresh or thawed unprocessed fishery products and cooked and chilled products from crustaceans and molluscs stored at temperatures

approaching that of melting ice, the main hazards to be addressed are biogenic amines, specifically histamine, for fresh fishery products.

- The storage temperature must be sufficiently low to inhibit the growth of bacteria, such as *M. psychrotolerans* and *P. phosphoreum*, which are capable of producing histamine in fishery products at refrigeration temperatures.
- The hazards that are capable of growth in seafood at refrigeration temperatures include histamine producing bacteria (e.g. *Morganella* spp. and/or *Photobacterium* spp.), *Clostridium botulinum*, *Listeria monocytogenes* and *Yersinia enterocolitica*, and these were included in the modelling studies.
- Viruses and parasites do not reproduce outside of their hosts and phycotoxins are not influenced by post-harvest storage temperatures. These hazards were therefore excluded when modelling different storage conditions.

5. Modelling

5.1. Modelling methodology

Fishery product-associated pathogens, as identified in Section 4, capable of growth at refrigerated temperatures were included in the modelling tasks. Predictive models available in predictive modelling software tools were used to predict the effect of storage temperature and time on the growth of these pathogens, including *M. psychrotolerans* as a histamine producer, *L. monocytogenes*, *Y. enterocolitica* and non-proteolytic *C. botulinum*. Growth of pathogens and histamine formation values were used, based on data from the literature (Emborg and Dalgaard, 2008). The assumptions for the factors that influence bacterial growth are shown in Table 6. Also, it has to be noted that the effect that the packaging could have on the temperature of the product was not evaluated. Temperatures used in this assessment are thus assumed to reflect those in the product.

Table 6: Assumptions used for the prediction of microbial growth in fishery products

Factor	Assumption
pH	6.5
Water phase salt	1%
Initial concentration of histidine	10 750 ppm
Initial concentration of *Morganella psychrotolerans*	1 000 CFU/g
Lag phase	No lag phase

CFU: colony-forming unit.

The uncertainty of the predictions related to the above assumptions was analysed for histamine production, which has been identified as the main hazard for fishery products. Uncertainty was evaluated by estimating the time required for 100 ppm histamine formation at a reference temperature of 5 °C for a range of representative values of pH, water phase salt, initial concentration of histidine and initial concentration and lag phase of *Morganella psychrotolerans*, based on data from the literature (Emborg and Dalgaard, 2008). The direction of the effect was quantitatively expressed as the percentage difference, from the time predicted based on the initial assumption.

5.2. Models used

A detailed description of the predictive models used is presented in the following sections.

5.2.1. *Morganella psychrotolerans*

To predict the effect of temperature on growth of and histamine formation by *Morganella psychrotolerans* in packaged fishery products at retail level, the Food Spoilage and Safety Predictor (FSSP™) v. 4.0 was used (www.fssp.dtu.dk). The model's characteristics are shown in Table 7.

Table 7: Characteristics of FSSP™ v. 4.0 for *Morganella psychrotolerans* growth and histamine formation and assumptions used for the prediction of microbial growth in fishery products

Model[(a)]	**Characteristic**
Primary growth model	Expanded logistic model with delay
Secondary growth model	Cardinal parameter type model
Environmental parameters in model	Temperature, atmosphere (CO_2), water phase salt (a_w) and pH
Product validation studies	Fresh garfish, fresh tuna, canned tuna and cold-smoked tuna (Emborg and Dalgaard, 2008)
Range of applicability	Temperature (0–20 °C), atmosphere (0–100 % CO_2), water phase salt (0–6 %), pH (5.4–6.5)
Applied values	
Temperature	1-7 °C
pH	6.5
Water phase salt	1 %
Initial concentration of histidine	10 750 ppm
Initial concentration of histamine	0 ppm
Initial concentration of *M. psychrotolerans*	1 000 CFU/g
CO_2 %	0 %, 20 %, 30 %, 40 %
Lag phase	No lag phase

CFU: colony-forming unit.
(a): FSSP™ v. 4.0 is available at www.fssp.dtu.dk

This model for *M. psychrotolerans* includes the effect of four environmental parameters (temperature, atmosphere (CO_2), water activity (water phase salt) and pH) on growth and histamine formation. FSSP™ can be used to evaluate how changes in storage conditions (e.g. the chill storage temperature) or product characteristics (e.g. salt concentration) influence growth and histamine formation by *M. psychrotolerans.* Information on the lag time of *M. psychrotolerans* in naturally contaminated marine finfish products is limited. Therefore, the growth and histamine formation model for *M. psychrotolerans* was used without lag time (fail safe predictions). FSSP™ uses a relative lag time of 2.55 for *M. psychrotolerans* (Emborg and Dalgaard, 2008).

5.2.2. *Listeria monocytogenes*

To predict the effect of temperature on the growth of *L. monocytogenes* in packaged fishery products at retail level, FSSP™ v. 4.0 was used (www.fssp.dtu.dk). The model's characteristics are shown in Table 8.

Table 8: Characteristics of FSSP™ v. 4.0 for growth of *Listeria monocytogenes* and assumptions used for the prediction of microbial growth in fishery products

Model[(a)]	**Characteristic**
Primary growth model	Logistic model with delay
Secondary growth model	Simplified cardinal parameter type model
Environmental parameters in model	Temperature, atmosphere (CO_2), water phase salt/a_w, pH, smoke components/phenol, nitrite and organic acids in water phase of product (acetic acids, benzoic acid, citric acid, diacetate, lactic acid and sorbic acid)
Product validation studies	The model has been extensively validated using data from ready-to-eat food products (Mejlholm and Dalgaard, 2007a, b, 2009, 2015; Mejlholm et al., 2010, 2014)

Model[(a)]	Characteristic
Range of applicability	Temperature (2–25 °C), atmosphere (0–100 % CO_2), water phase salt (0.7–9.0 %), pH (5.6–7.7), smoke components/phenol (0–20 ppm), nitrite (0–150 ppm in product), acetic acid (0–11 000 ppm in water phase), benzoic acid (0–1 800 ppm in water phase), citric acid (0–6 500 ppm in water phase), diacetate (0–3 800 ppm in water phase), lactic acid (0–60 000 ppm in water phase) and sorbic acid (0–1 300 in water phase)
Applied values	
Temperature	0–7 °C
pH	6.5
Water phase salt	1%
Initial concentration of *L. monocytogenes*	1 CFU/g
CO_2	0 %, 20 %, 30 %, 40 %
Nitrite mg/kg	0
Phenol, acetic acid, benzoic acid, citric acid, diacetate, lactic acid, sorbic acid (ppm)	0
Lag phase	No lag phase

CFU: colony-forming unit.
(a): FSSP™ v. 4.0 is available at www.fssp.dtu.dk

Information on the lag time of *L. monocytogenes* in naturally contaminated foods is still limited. Therefore, the growth model for *L. monocytogenes* can be used without lag time (fail-safe predictions) or with lag time (more realistic predictions for naturally contaminated products). The latter case was only used in the uncertainty assessment.

5.2.3. Clostridium botulinum

To predict the effect of temperature on the growth of non-proteolytic *Clostridium botulinum* in pre-packed fishery products at retail level, the ComBase model was used (www.combase.cc). The model's characteristics are shown in Table 9.

Table 9: Characteristics of the ComBase growth model for non-proteolytic *Clostridium botulinum* and assumptions used for the prediction of microbial growth in fishery products

Model[(a)]	Characteristic
Primary growth model	Baranyi and Roberts (1994)
Secondary growth model	Polynomial
Environmental parameters in model	Temperature, salt, pH
Product validation studies	Unknown
Range of applicability	Temperature (4–30 °C), salt (0–4.5 %), pH (5.1–7.5)
Applied values	
Temperature	0-7 °C
pH	6.5
Initial level of *C. botulinum*	1 CFU/g
NaCl (%)	1 %
Physiological state	1 (i.e. no lag phase)

CFU: colony-forming unit.
(a): ComBase model is available at www.combase.cc

5.2.4. *Yersinia enterocolitica*

For predicting the effect of temperature on the growth of *Yersinia enterocolitica* in pre-packed fishery products at retail level the ComBase model was used (www.combase.cc). The model's characteristics are shown in the Table 10. The concentration of CO_2 was not modelled in this case, but it is expected

that it would have a similar effect to that achieved with *L. monocytogenes* (see Section 5.3.2), i.e. prolonging the shelf-life of the products.

Table 10: Characteristics of the ComBase growth model for *Yersinia enterocolitica* and assumptions used for the prediction of microbial growth in fishery products

Model[(a)]	**Characteristic**
Primary growth model	Baranyi and Roberts (1994)
Secondary growth model	Polynomial
Environmental parameters in model	Temperature, salt, pH
Product validation studies	Unknown
Range of applicability	Temperature (–1 to 37 °C), salt (0–7 %), pH (4.4–7.2)
Applied values	
Temperature	0-7 °C
pH	6.5
Initial concentration of *Y. enterocolitica*	1 CFU/g
NaCl (%)	1%
Physiological state	1 (i.e. no lag phase)
CO_2	0%

CFU: colony-forming unit.
(a): ComBase model is available at www.combase.cc

5.3. Results

5.3.1. Effect of storage temperature on *Morganella psychrotolerans* growth and histamine formation

Histamine formation in fishery products at temperature conditions from 0 to 7 °C and storage periods from 1 to 14 days was predicted using FSSP™. Histamine formation prediction is based on the growth of histamine-producing *M. psychrotolerans.* The selection of the *M. psychrotolerans* model was based on the fact that it grows faster than *M. morganii* at refrigeration temperatures (Emborg and Dalgaard, 2008). Figure 1 shows a representative comparison: *M. psychrotolerans* growth and histamine formation in fishery products at 2 and 5 °C with 0 % CO_2 in the packaging headspace at equilibrium. As is shown in this figure, histamine is formed when the population of *M. psychrotolerans* reaches about 6.5 log CFU/g. The effect of storage temperature and time on the formation of histamine in fishery products with various levels of CO_2 in the package headspace is presented in Tables 11–14. Table 15 presents the effect of storage temperature and CO_2 in the package headspace on the time required for the production of 100 ppm histamine, which is the lower limit (m) of the safety criterion for fishery products from fish species associated with a large amount of histidine, including the families *Scombridae*, *Clupeidae*, *Engraulidae*, *Coryphaenidae*, *Pomatomidae* and *Scomberesocidae*. Thus, the time to production of 100 ppm histamine indicates a maximum storage time of the product, assuming the initial concentration of histamine is 0.

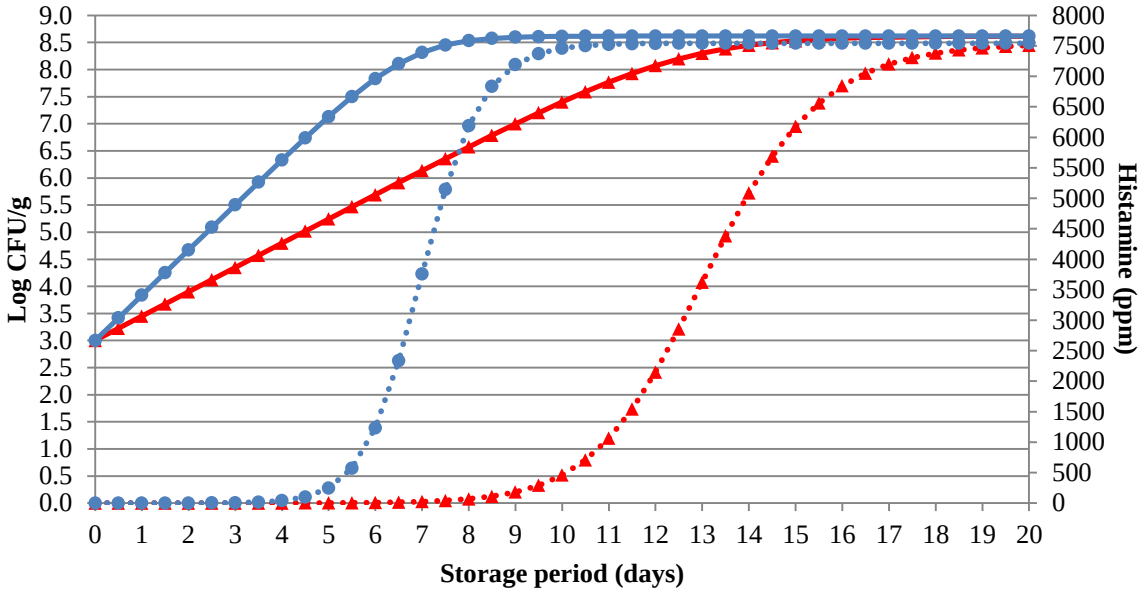

Figure 1: Predicted growth of *M. psychrotolerans* (—) and histamine formation (···) in fishery products (pH 6.5, water phase salt=1 %, CO_2=0 %) during the storage period of 20 days at 2 °C (▲) and 5 °C (●), assuming no lag phase

Results in Table 11 indicate that, assuming an initial concentration of histamine of 0 ppm, the formation of histamine remains under 100 ppm for up to 10 days depending on the temperature of storage. For example, this limit would be respected for 8 days and a storage temperature of 2 °C.

Table 11: Predicted histamine formation due to *Morganella psychrotolerans* growth in fishery products with 0 % CO_2 in the packaging headspace at equilibrium as affected by temperature during transport and storage at retail.

Time (days)	Temperature (°C)							
	0	1	2	3	4	5	6	7
	Histamine formation (ppm)							
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	1	2	4
3	0	0	0	1	2	6	17	53
4	0	0	1	3	11	39	155	629
5	0	1	3	12	52	245	1 122	3 647
6	1	2	9	43	235	1 234	4 136	6 727
7	1	5	25	150	928	3 758	6 659	7 439
8	2	11	68	486	2 688	6 191	7 382	7 532
9	4	23	181	1 364	5 028	7 191	7 517	7 543
10	7	51	459	2 994	6 586	7 461	7 540	7 545
11	12	110	1 064	4 896	7 232	7 526	7 544	7 545
12	22	231	2 148	6 289	7 449	7 540	7 545	7 545
13	39	470	3 621	7 019	7 516	7 544	7 545	7 545
14	69	907	5 085	7 337	7 536	7 544	7 545	7 545

ppm: parts per million.
Applied assumptions: pH (6.5), water phase salt (1 %), initial concentration of *M. phsychrotolerans* (1 000 colony-forming units (CFU)/g), initial concentration of histidine (10 750 ppm), initial concentration of histamine (0 ppm), no lag phase.
Grey shading indicates the time-temperature combinations leading to histamine production of less than 100 ppm.

Results in Tables 12 to 15 show the effect of CO_2 in the packaging headspace in addition to temperature and duration of storage, also assuming an initial concentration of histamine of 0 ppm. For example, for a temperature of 2 °C the formation of histamine remains less than 100 ppm for up to 10.6 days depending on the concentration of CO_2, compared to 8.4 days of storage without CO_2 (see Table 15).

Table 12: Predicted histamine formation due to *Morganella psychrotolerans* growth in fishery products with 20 % CO_2 in the packaging headspace at equilibrium as affected by temperature during transport and storage at retail.[(a)] Grey shading indicates the time-temperature combinations leading to histamine production of less than 100 ppm.

Time (days)	Temperature (° C)							
	0	1	2	3	4	5	6	7
	Histamine formation (ppm)							
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	1	1	3
3	0	0	0	1	2	4	9	25
4	0	0	1	2	6	19	63	221
5	0	1	2	7	24	94	387	1 466
6	1	2	5	20	90	426	1 770	4 550
7	1	3	13	61	322	1 551	4 458	6 561
8	1	6	30	175	1 006	3 728	6 342	7 066
9	2	12	72	475	2 467	5 725	6 974	7 158
10	4	24	166	1 156	4 385	6 696	7 130	7 173
11	7	46	370	2 370	5 874	7 032	7 166	7 176
12	11	89	778	3 924	6 652	7 134	7 174	7 176
13	19	170	1 496	5 313	6 979	7 164	7 176	7 176
14	31	317	2 548	6 232	7 104	7 173	7 176	7 176

(a): Applied assumptions: pH (6.5), water phase salt (1 %), initial concentration of *M. phsychrotolerans* (1 000 colony-forming units (CFU)/g), initial concentration of histidine (10 750 ppm), initial concentration of histamine (0 ppm), no lag phase.

Table 13: Predicted histamine formation due to *Morganella psychrotolerans* growth in fishery products with 30 % CO_2 in the packaging headspace at equilibrium as affected by temperature during transport and storage at retail.[(a)] Grey shading indicates the time–temperature combinations leading to histamine production of less than 100 ppm.

Time (days)	Temperature (° C)							
	0	1	2	3	4	5	6	7
	Histamine formation (ppm)							
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	1	1	2
3	0	0	0	1	1	3	7	17
4	0	0	1	2	5	13	41	133
5	0	1	2	5	17	60	227	849
6	0	1	4	15	58	249	1 039	3 194
7	1	3	9	40	190	904	3 116	5 733
8	1	5	21	107	579	2 467	5 392	6 712
9	2	9	46	276	1 506	4 535	6 515	6 941
10	3	17	102	668	3 066	5 989	6 870	6 987
11	5	31	218	1 442	4 743	6 645	6 966	6 996

Time (days)	Temperature (° C)							
	0	1	2	3	4	5	6	7
	Histamine formation (ppm)							
12	8	57	449	2 651	5 921	6 882	6 990	6 998
13	13	104	875	4 054	6 536	6 961	6 996	6 999
14	21	188	1570	5 259	6 810	6 987	6 998	6 999

(a): Applied assumptions: pH (6.5), water phase salt (1 %), initial concentration of *M. phsychrotolerans* (1 000 colony-forming units (CFU)/g), initial concentration of histidine (10 750 ppm), initial concentration of histamine (0 ppm), no lag phase.

Table 14: Predicted histamine formation due to *Morganella psychrotolerans* growth in fishery products with 40 % CO_2 in the packaging headspace at equilibrium as affected temperature during transport and storage at retail.[(a)] Grey shading indicates the time–temperature combinations leading to histamine production of less than 100 ppm.

Time (days)	Temperature (° C)							
	0	1	2	3	4	5	6	7
	Histamine formation (ppm)							
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	1	1	2
3	0	0	0	1	1	2	5	12
4	0	0	1	2	4	10	27	82
5	0	1	2	4	12	39	134	480
6	0	1	3	11	38	147	588	2 006
7	1	2	7	27	114	512	1 953	4 561
8	1	4	15	67	330	1 486	4 137	6 151
9	2	7	31	163	866	3 200	5 785	6 661
10	3	12	64	380	1 925	4 948	6 494	6 788
11	4	21	130	829	3 422	6 033	6 727	6 817
12	6	37	258	1 622	4 859	6 525	6 797	6 824
13	10	65	497	2 756	5 840	6 717	6 817	6 825
14	15	113	908	4 009	6 372	6 787	6 823	6 825

(a): Applied assumptions: pH (6.5), water phase salt (1 %), initial concentration of *M. phsychrotolerans* (1 000 CFU/g), initial concentration of histidine (10 750 ppm), initial concentration of histamine (0 ppm), no lag phase.

Table 15: Predicted time (days) required for 100 ppm histamine formation in fishery products as affected by CO_2 in the packaging headspace at equilibrium and temperature during transport and storage

Temperature (° C)	CO_2 in the packaging headspace at equilibrium (%)			
	0	20	30	40
	Storage time (days) providing 100 ppm histamine formation			
0	14.7	16.4	17.4	18.6
1	10.9	12.2	12.9	13.7
2	8.4	9.4	10.0	10.6
3	6.6	7.5	7.9	8.4
4	5.4	6.0	6.4	6.9
5	4.5	5.0	5.3	5.7
6	3.8	4.2	4.5	4.8
7	3.2	3.6	3.8	4.1

5.3.2. Effect of storage temperature on the growth of *Listeria monocytogenes* in fishery products

The growth of *L. monocytogenes* in fishery products at temperature conditions from 0 to 7 °C and storage periods from one to 14 days was predicted using FSSP™. Figure 2 shows a representative comparison of *L. monocytogenes* growth at 2 and 5 °C. The effect of storage temperature and time on the growth of pathogens in fishery products with various levels of CO_2 in the package headspace is presented in Tables 16–19.

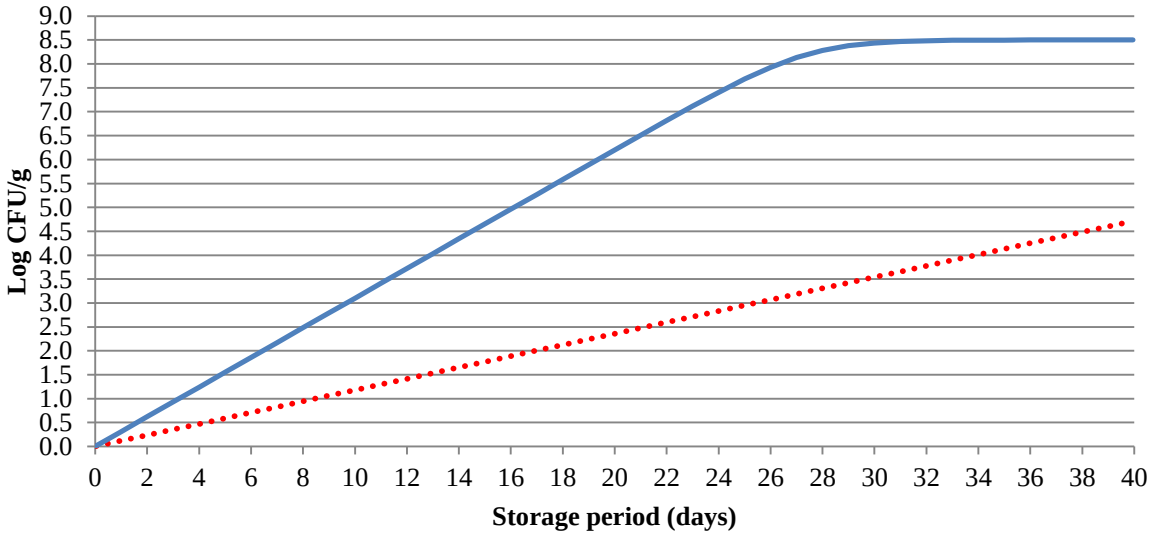

Figure 2: Predicted growth of *L. monocytogenes* in fishery products (pH 6.5, water phase salt = 1 %, CO_2 = 0 %) during transport and storage period of 40 days at 2 °C (···) and 5 °C (—), assuming no lag phase

Results in Table 16 indicate that the growth of *L. monocytogenes* is obviously affected by both the temperature and duration of storage. As an example, for a storage temperature of 3 °C, the growth in log CFU/g ranges from 0.17 after 1 day to 2.41 after 2 weeks of storage.

Table 16: Predicted growth of *Listeria monocytogenes* (in log CFU/g) in fishery products with 0 % CO_2 in the packaging headspace at equilibrium as affected by temperature during transport and storage at retail[(a)]

Time (days)	Storage temperature (° C)					
	2	3	4	5	6	7
	Growth of *Listeria monocytogenes* in log CFU/g					
1	0.12	0.17	0.23	0.30	0.38	0.47
2	0.24	0.34	0.47	0.61	0.78	0.96
3	0.35	0.51	0.70	0.92	1.17	1.45
4	0.47	0.69	0.94	1.23	1.57	1.94
5	0.59	0.86	1.18	1.54	1.96	2.43
6	0.71	1.03	1.41	1.85	2.36	2.92
7	0.83	1.20	1.65	2.16	2.75	3.41
8	0.94	1.37	1.88	2.47	3.14	3.90
9	1.06	1.55	2.12	2.78	3.54	4.38
10	1.18	1.72	2.36	3.09	3.93	4.87
11	1.30	1.89	2.59	3.40	4.33	5.36

Time (days)	Storage temperature (° C)					
	2	3	4	5	6	7
	Growth of *Listeria monocytogenes* in log CFU/g					
12	1.42	2.06	2.83	3.71	4.72	5.85
13	1.53	2.23	3.06	4.02	5.12	6.34
14	1.65	2.41	3.30	4.33	5.51	6.82

CFU: colony-forming unit.
(a): Applied assumptions: pH (6.5), water phase salt (1 %), initial concentration of *L. monocytogenes* (1 CFU/g), nitrite mg/kg (0), phenol, acetic acid, benzoic acid, citric acid, diacetate, lactic acid, sorbic acid (0 ppm), no lag phase.

Tables 17 to 19 show the effect of CO_2 in the packaging headspace on the growth of *L. monocytogenes* in addition to temperature and time of storage. As an example, for a storage temperature of 3 °C during a week, the growth in log CFU/g ranges from 0.97 with 20 % CO_2 to 0.74 with 40 % CO_2 compared to 1.20 when the concentration of CO_2 is 0 %.

Table 17: Predicted growth of *Listeria monocytogenes* (in log CFU/g) in fishery products with 20 % CO_2 in the packaging headspace at equilibrium as affected by temperature during transport and storage at retail[(a)]

Time (days)	Storage temperature (° C)					
	2	3	4	5	6	7
	Growth of *Listeria monocytogenes* in log CFU/g					
1	0.09	0.14	0.19	0.25	0.32	0.39
2	0.19	0.28	0.38	0.50	0.64	0.80
3	0.28	0.42	0.57	0.76	0.97	1.21
4	0.38	0.55	0.77	1.01	1.30	1.62
5	0.47	0.69	0.96	1.27	1.62	2.02
6	0.57	0.83	1.15	1.52	1.95	2.43
7	0.66	0.97	1.34	1.78	2.28	2.84
8	0.76	1.11	1.54	2.03	2.60	3.25
9	0.85	1.25	1.73	2.29	2.93	3.66
10	0.95	1.39	1.92	2.54	3.26	4.06
11	1.04	1.53	2.11	2.80	3.58	4.47
12	1.14	1.67	2.31	3.05	3.91	4.88
13	1.23	1.81	2.50	3.31	4.24	5.29
14	1.33	1.95	2.69	3.56	4.56	5.69

CFU: colony-forming unit.
(a): Applied assumptions: pH (6.5), water phase salt (1 %), initial concentration of *L. monocytogenes* (1 CFU/g), nitrite mg/kg (0), phenol, acetic acid, benzoic acid, citric acid, diacetate, lactic acid, sorbic acid (0 ppm), no lag phase.

Table 18: Predicted growth of *Listeria monocytogenes* (in log CFU/g) in fishery products with 30 % CO_2 in the packaging headspace at equilibrium as affected by temperature during transport and storage at retail

Time (days)	Storage temperature (° C)					
	2	3	4	5	6	7
	Growth of *Listeria monocytogenes* in log CFU/g					
1	0.08	0.12	0.17	0.22	0.28	0.36
2	0.17	0.24	0.34	0.45	0.58	0.72
3	0.25	0.37	0.51	0.68	0.87	1.09

Time (days)	Storage temperature (° C)					
	2	3	4	5	6	7
	Growth of *Listeria monocytogenes* in log CFU/g					
4	0.33	0.49	0.68	0.90	1.16	1.46
5	0.42	0.61	0.85	1.13	1.45	1.82
6	0.50	0.74	1.02	1.36	1.75	2.19
7	0.58	0.86	1.19	1.59	2.04	2.56
8	0.66	0.98	1.36	1.81	2.33	2.92
9	0.75	1.10	1.53	2.04	2.63	3.29
10	0.83	1.23	1.70	2.27	2.92	3.66
11	0.91	1.35	1.88	2.50	3.21	4.02
12	1.00	1.47	2.05	2.72	3.50	4.39
13	1.08	1.59	2.22	2.95	3.80	4.76
14	1.16	1.72	2.39	3.18	4.09	5.12

CFU: colony-forming unit.
(a): Applied assumptions: pH (6.5), water phase salt (1 %), initial concentration of *L. monocytogenes* (1 CFU/g), nitrite mg/kg (0), phenol, acetic acid, benzoic acid, citric acid, diacetate, lactic acid, sorbic acid (0 ppm), no lag phase.

Table 19: Predicted growth of *Listeria monocytogenes* (in log CFU/g) in fishery products with 40 % CO_2 in the packaging headspace at equilibrium as affected by temperature during transport and storage at retail[(a)]

Time (days)	Storage temperature (° C)					
	2	3	4	5	6	7
	Growth of *L. monocytogenes* in log CFU/g					
1	0.07	0.10	0.15	0.19	0.25	0.32
2	0.14	0.21	0.29	0.39	0.51	0.64
3	0.21	0.32	0.44	0.59	0.77	0.97
4	0.29	0.42	0.59	0.79	1.03	1.29
5	0.36	0.53	0.74	0.99	1.29	1.62
6	0.43	0.64	0.89	1.19	1.55	1.95
7	0.50	0.74	1.04	1.39	1.80	2.27
8	0.57	0.85	1.19	1.59	2.06	2.60
9	0.64	0.96	1.34	1.79	2.32	2.93
10	0.71	1.06	1.49	1.99	2.58	3.25
11	0.79	1.17	1.64	2.19	2.84	3.58
12	0.86	1.28	1.79	2.39	3.10	3.90
13	0.93	1.38	1.94	2.59	3.36	4.23
14	1.00	1.49	2.08	2.79	3.62	4.56

CFU: colony-forming unit.
(a): Applied assumptions: pH (6.5), water phase salt (1 %), initial concentration of *L. monocytogenes* (1 CFU/g), nitrite mg/kg (0), phenol, acetic acid, benzoic acid, citric acid, diacetate, lactic acid, sorbic acid (0 ppm), no lag phase.

5.3.3. Effect of storage temperature on the growth of *Yersinia enterocolitica*, non-proteolytic *Clostridium botulinum* in fishery products

The growth of *Y. enterocolitica* at temperature conditions from 0 to 7 °C and non-proteolytic *C. botulinum* at temperature conditions from 4 to 7 °C in fishery products during storage periods from 1 to 14 days was predicted using the models from ComBase. The effect of storage temperature and time on the growth of these pathogens in fishery products is presented in Tables 20 and 21 for *Y. enterocolitica* and in Table 22 for non-proteolytic *C. botulinum*.

Table 20: Predicted growth of *Yersinia enterocolitica* (in log CFU/g) in fishery products as affected by storage temperature[(a)]

Time (days)	Storage temperature (°C)							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	Growth of *Y. enterocolitica* in log CFU/g							
1	0.45	0.53	0.63	0.74	0.86	1.00	1.16	1.33
2	0.90	1.07	1.25	1.47	1.72	2.00	2.31	2.66
3	1.35	1.60	1.88	2.21	2.58	2.99	3.47	3.99
4	1.80	2.13	2.51	2.94	3.43	3.99	4.61	5.31
5	2.25	2.66	3.13	3.67	4.29	4.98	5.74	6.55
6	2.70	3.19	3.76	4.41	5.13	5.94	6.79	7.56
7	3.15	3.72	4.38	5.13	5.96	6.83	7.62	8.09
8	3.60	4.25	5.00	5.84	6.74	7.56	8.07	8.26
9	4.05	4.78	5.61	6.52	7.39	8.00	8.24	8.29
10	4.49	5.30	6.20	7.13	7.86	8.20	8.29	8.30
11	4.94	5.82	6.76	7.62	8.12	8.27	8.30	8.30
12	5.38	6.31	7.25	7.95	8.23	8.29	8.30	8.30
13	5.81	6.78	7.65	8.14	8.28	8.30	8.30	8.30
14	6.23	7.20	7.93	8.23	8.29	8.30	8.30	8.30

CFU: colony-forming unit.
(a): Applied assumptions: pH (6.5), initial concentration of *Y. enterocolitica* (1 CFU/g), NaCl (1 %), CO_2 (0 %), no lag phase.

Table 21: Predicted time (days) required for equivalent growth of *Yersinia enterocolitica* as affected by temperature during transport and storage

Time (days)	Storage temperature (°C)							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	Growth (log CFU/g)	**Time (days) required for growth equal to that at 0 °C**						
3	**1.35**	2.5	2.2	1.8	1.6	1.4	1.2	1.0
4	**1.80**	3.4	2.9	2.5	2.1	1.8	1.6	1.4
5	**2.25**	4.2	3.6	3.1	2.6	2.3	2.0	1.7
6	**2.70**	5.1	4.3	3.7	3.2	2.7	2.3	2.0
7	**3.15**	5.9	5.0	4.3	3.7	3.2	2.7	2.4
8	**3.60**	6.8	5.8	4.9	4.2	3.6	3.1	2.7
9	**4.05**	7.6	6.5	5.5	4.7	4.1	3.5	3.0
10	**4.49**	8.5	7.2	6.1	5.2	4.5	3.9	3.4
11	**4.94**	9.3	7.9	6.7	5.8	5.0	4.3	3.7
12	**5.38**	10.2	8.6	7.4	6.3	5.4	4.7	4.1
13	**5.81**	11.0	9.3	8.0	6.8	5.9	5.1	4.4
14	**6.23**	11.8	10.1	8.6	7.3	6.3	5.5	4.7

CFU: colony-forming unit.

As with *L. monocytogenes*, the growth of *Y. enterocolitica* is influenced by both the temperature and the time of storage. Table 20 shows increasing growth as the temperature of storage rises and/or the duration of storage is prolonged. Table 21 shows instead different predicted durations of storage (in days), at temperatures ranging from 1 to 7 °C, to achieve equivalent growth to that predicted at 0 °C.

Table 22 shows the predicted growth of *C. botulinum* at temperatures ranging from 4 to 7 °C (the model cannot predict growth below 4 °C). Although this hazard is not expected to grow at

temperatures close to 0 °C, it should be considered if the temperature of storage is set at 4 °C or higher. For example, the growth after a week of storage ranges from 2.05 log CFU/g at 4 °C to 5.73 at 7 °C. It has to be noted that this predictions have been obtained using optimal growing conditions for *C. botulinum* and that there are other intrinsic factors that will influence growth and toxin production. This is therefore an estimate for a worst case scenario.

Table 22: Predicted growth of non-proteolytic *Clostridium botulinum* (in log CFU/g) in fishery products as affected by storage temperature[(a)]

Time (days)	Storage temperature (°C)			
	4	5	6	7
	Growth of *C. botulinum* in log CFU/g			
1	0.29	0.42	0.6	0.83
2	0.59	0.85	1.2	1.67
3	0.88	1.27	1.8	2.5
4	1.17	1.69	2.4	3.34
5	1.47	2.11	3	4.16
6	1.76	2.54	3.59	4.97
7	2.05	2.96	4.18	5.73
8	2.34	3.38	4.77	6.35
9	2.64	3.8	5.33	6.75
10	2.93	4.22	5.85	6.94
11	3.22	4.63	6.3	7.01
12	3.51	5.03	6.63	7.03
13	3.8	5.42	6.83	7.04
14	4.09	5.79	6.95	7.04

CFU: colony-forming unit.
(a): Applied assumptions: pH (6.5), initial concentration of *C. botulinum* (1 CFU/g), NaCl (1 %), no lag phase.

5.4. Uncertainty

In the EFSA context, the term 'uncertainty' is intended to cover '*all types of limitations in knowledge, at the time it is collected*' in the risk assessment process (EFSA, 2009). The need to address uncertainty is expressed in the Codex Working Principles for Risk Analysis. These state that '*constraints, uncertainties and assumptions having an impact on the risk assessment should be explicitly considered at each step in the risk assessment and documented in a transparent manner*' (CAC, 2007). The Scientific Committee of EFSA explicitly endorsed this principle in its guidance on transparency in risk assessment (EFSA, 2009). Therefore, it is recognised that, in the risk assessment process, it is important to characterise, document and explain all types of uncertainty arising in the process. Ideally, the analysis of the uncertainty in a risk assessment would require the following steps:

- identifying uncertainties;
- describing uncertainties;
- evaluating uncertainties arising from individual factors in their own right;
- evaluating the impact of individual uncertainty factors on the assessment outcome;
- evaluating the combined impact of multiple uncertainties on the assessment outcome, including evaluating how much the combined uncertainties downgrade the weight of the evidence.

The last three steps can be conducted at three levels: qualitative, deterministic and probabilistic.

In this report, the uncertainty analysis is performed by conducting a sensitivity analysis for histamine production, which has been identified as the main hazard for fishery products. In the approach used, the uncertainty sources for the predictions of histamine formation are mainly related to the assumptions made in applying the available predictive models. These assumptions refer to the use of representative values of pH, water phase salt, initial concentration of histidine, and initial concentration and lag phase of *Morganella psychrotolerans*. The values of pH, water phase salt, initial concentration of histidine and initial concentration of *Morganella psychrotolerans* can vary between fish species/batches while the lag phase depends on the physiological state of the cells, which is unknown. To analyse the uncertainty originating from the above sources, the time required for 100 ppm histamine formation at a reference temperature of 5 °C was also predicted for a range of each of the above factors based on data from the literature (Emborg and Dalgaard, 2008). The direction of the effect was quantitatively expressed as the difference, as a percentage, from the time predicted based on the initial assumption.

The predicted time required for 100 ppm histamine formation at 5 °C and 0 % CO_2 in the packaging headspace at equilibrium, based on the initial assumptions (shown in Table 7) is 4.5 days (see Table 15). The direction and the magnitude of the uncertainty of the time required for 100 ppm histamine formation compared to the above value, at a reference temperature of 5 °C is shown in Table 23 for the different uncertainty sources. For example the initial assumption for % NaCl was 1 %. Based on literature data the % NaCl of fresh fish varies from 0.2 to 2 %, which corresponds to a respective range of predicted time required for 100 ppm histamine formation from 4.02 to 5.21 days. Thus, the direction and magnitude of the uncertainty for the NaCl assumption is − 10.7 % to + 15.8 % compared to the time of 4.5 days predicted based on the initial NaCl assumption of 1 %.

Table 23: Sensitivity analysis for the predicted time (days) required for 100 ppm histamine formation in fishery products with 0 % CO_2 in the packaging headspace at equilibrium stored at 5 °C

Uncertainty source	Assumption	Source range	Prediction range (days)	Uncertainty	
				-	+
pH[(a)]	6.5	5.8-6.5	4.50-5.46	0 %	21.3 %
NaCl (%)	1	0.2-2	4.02-5.21	10.7 %	15.8 %
Initial concentration of histidine (ppm)	10750	5 000-10 750	4.50-4.50	0 %	0 %
Initial concentration of *Morganella psychrotolerans* (log CFU/g)	3	1-6	1.00-6.90	77.8 %	53.3 %
Lag phase (hours)	0	0-0.92[(b)] days	4.50-5.43	0 %	20.7 %

CFU: colony-forming unit.

(a): Since the pH can range from 5.8 to 6.5, the time required for 100 ppm histamine formation can be equal (0 %) or 21.3 % higher than that predicted assuming pH 6.5.

(b): Based on the relative lag equal to 2.55 (Emborg and Dalgaard, 2008).

The sensitivity analysis suggests that within the credible range of input parameters for the model, the initial concentration for *Morganella* is the dominant source of uncertainty. The plausible range of estimating the predicted time for 100 ppm histamine formation could be 34 % higher or 77 % lower than in the baseline model.

The effect of the model's accuracy, which is an additional uncertainty source, was evaluated qualitatively based on the bias and accuracy factors (Emborg and Dalgaard, 2008). The bias factor (1.15) indicates that the model systematically underestimates time. The accuracy factor (1.45) suggests that this underestimation has an average magnitude of 45 %.

The evaluation of combined effects was not carried out because we do not have information on the distribution of uncertainty sources.

5.5. Concluding remarks

The food safety hazards that would best be assessed when considering the effect of temperature vary quite substantially across the range of products in the TOR. Therefore the approach taken was to focus on those temperature-dependant hazards for which there exist legislative microbiological criteria (MC), and in particular those for which there exist epidemiological indicators of a burden of foodborne disease arising from fishery products in the EU. The growth of those pathogens at temperatures above 0 °C was specifically assessed. However, for many foods in the TOR there exist no such MC, so the assessment included an appraisal of those hazards for which there exist epidemiological indicators of foodborne risk in foods within the TOR. A further consideration in this work was that some of the food types covered by the TOR may not have a legal MC, but foods produced from these foods, e.g. processed ready to eat fishery products, may be subject to specific MC in EU legislation.

A maximal storage temperature obligation is one of the legislative approaches to ensure foodstuffs do not contain unacceptable concentration of microbial hazards at consumption. However, this requirement exists in parallel with various other obligations providing supporting (overlapping) legislative protections, such as the requirement for operators to establish product durability, the requirement to identify hazards and manage the resultant risks within food safety management system, the mandated microbiological criteria. Whilst a maximal storage temperature might provide some basic protection for products on the market as primary products where other protections are not likely to be protective, the continued utility of such a requirement for products subjected to a packaging step may need appraisal in context of the ancillary protections which come into effect for such packaged products.

Current EU legislation requires pre-packed fishery products to be stored at a temperature approaching that of melting ice. In addition, for products from certain species, food safety criteria are set for limits on histamine levels. For RTE products and molluscs, microbiological criteria are set for *Listeria monocytogenes* and *Salmonella*, respectively (see Section 2.6 for details). For products for which criteria are laid out in legislation, such criteria are used in this assessment as a guide to evaluate the impact of storage at a temperature different to that set out in legislation. The primary working assumption of the approach used in the latter case is that the temperature condition that could be applied to storage and transport of pre-packed fresh fishery products at retail level where icing is not possible is the condition that will still provide compliance to the safety criteria. Since *Salmonella* cannot grow during refrigeration storage the analysis was limited to histamine and *Listeria monocytogenes*.

For histamine, the results of the modelling approach showed that, for a fishery product with certain characteristics (pH 6.5, water phase salt = 1 %, initial histidine concentration = 10 750 ppm and initial concentration of *M. psychrotolerans* = 1 000 CFU/g), a shelf-life of 14.7 days and 0 % CO_2 in the packaging headspace at equilibrium which is stored in ice (0 °C), histamine formation would be 100 ppm (lower limit m of the safety criterion in Regulation (EC) No 2073/2005) at the end of its shelf-life, assuming an initial concentration of histamine of 0 ppm. Thus, an equivalent condition to the above baseline scenario is any combination of storage temperature, shelf-life and CO_2 concentration in the package that leads to histamine formation of 100 ppm at the end of shelf-life. The following are some examples of these combinations:

For a retail temperature of 3 °C:

- shelf-life of 6.7 days and 0 % CO_2 in the packaging headspace
- shelf-life of 7.5 days and 20 % CO_2 in the packaging headspace

- shelf-life of 8.5 days and 40 % CO_2 in the packaging headspace.

For a retail temperature of 5 °C:

- shelf-life of 4.5 days and 0 % CO_2 in the packaging headspace
- shelf-life of 5.0 days and 20 % CO_2 in the packaging headspace
- shelf-life of 5.7 days and 40 % CO_2 in the packaging headspace.

Based on the above, the available predictive models for histamine formation can be used by FBOs to adjust the fishery product durability (maximum shelf-life) and/or modify the packaging atmosphere of the product based on the retail temperature to ensure compliance with histamine safety criterion.

For *L. monocytogenes* EU Regulation (EC) No 2073/2005 set a limit of 100 CFU/g in RTE products. In this case the FBO can ensure compliance with such Regulation for products stored in retail at temperatures above 0 °C by adjusting the product durability (maximum shelf-life) and/or by modifying the packaging atmosphere of the product to ensure that the growth of the pathogen during distribution and storage will not lead to a concentration exceeding the limit of 100 CFU/g at the end of shelf-life. For this, the available predictive models were used assuming an initial concentration of 1 CFU/g. Following are some examples of these combinations for the latter case:

For a retail temperature of 3 °C:

- shelf-life of 11.6 days and 0 % CO_2 in the packaging headspace
- shelf-life of 14.4 days and 20 % CO_2 in the packaging headspace
- shelf-life of 18.8 days and 40 % CO_2 in the packaging headspace.

For a retail temperature of 5 °C:

- shelf-life of 6.5 days and 0 % CO_2 in the packaging headspace
- shelf-life of 7.8 days and 20 % CO_2 in the packaging headspace
- shelf-life of 10 days and 40 % CO_2 in the packaging headspace.

Apart from the non-compliance with the safety criteria, an increase of the storage temperature at retail above 0 °C may lead to growth of other pathogenic microorganisms able to grow at low temperatures such as *Y. enterocolitica*, *C. botulinum*. Growth of pathogens at storage temperatures above 0 °C may also occur in any packaged fishery products for which criteria are not laid out in legislation. In this case the temperature conditions that could be applied to storage and transport of packaged fresh fishery products at retail level where icing is not possible can be estimated as the condition that will provide equal or less growth of the pathogens than with icing leading to equal or less risk for the consumers. If we assume icing temperature to be 0 °C, this presents a challenge in the assessment, as for all relevant hazards, except for *Y. enterocolitica*, there are no available models predicting growth or pathogens do not grow at 0 °C. This means that *Y. enterocolitica* is the only hazard for which time-temperature scenarios during storage and transport at retail level equivalent to the currently mandated storage requirements can be estimated. For all other hazards, including *C. botulinum*, equivalent risk can be achieved by storing these products below the minimum growth temperature of those hazards (for example 3.3 °C for *C. botulinum*) that could be present in the product.

For *Y. enterocolitica*, the results of the modelling showed that, for a fishery product with certain characteristics (pH 6.5, water phase salt = 1 %,) and a shelf-life of 14 days, which is stored in ice (0 °C), the conditions that lead to equivalent growth to that at 0 °C are the following combinations:

- shelf-life of 10.1 days at 2 °C

- shelf-life of 8.6 days at 3 °C
- shelf-life of 7.3 days at 4 °C
- shelf-life of 6.3 days at 5 °C
- shelf-life of 5.5 days at 6 °C

In conclusion, packaged fresh fishery products for which icing is not possible during transport and retail can be stored at refrigeration temperatures (e.g. 3–5 °C), with the prerequisite that FBOs adjust the fishery product durability (maximum shelf-life) and/or modify the packaging atmosphere of the product based on the retail temperature to ensure compliance with current EU and international rules. To ensure compliance, the FBOs can perform challenge tests or use the available predictive models for the growth of pathogens and histamine formation, taking into account variability and uncertainty of all factors that affect microbial behaviour.

CONCLUSIONS AND RECOMMENDATION

CONCLUSIONS

Answer to the terms of reference

To assess, in the light of the current EU and international rules, which temperature conditions, including a possible tolerance, could be applied for storage and transport of pre-packed fresh fishery products, gutted or entire, including some parts of them, at retail level where icing is not possible.

- In the context of this report, which focuses on fresh or thawed unprocessed fishery products and cooked and chilled products from crustaceans and molluscs stored at temperatures approaching that of melting ice, the main temperature-dependent hazards are histamine formation, *Listeria monocytogenes*, *Clostridium botulinum*, and *Yersinia enterocolitica*.

- The hazard most frequently implicated in outbreaks associated with fish and fishery products is histamine. To assess bacterial growth and histamine production and evaluate different storage scenarios, the growth potential of relevant bacteria on fishery products during storage and transport at retail level was estimated using published predictive microbiology growth models assuming favourable growth conditions.

- The requirement to maintain products at a temperature approaching that of melting ice is an empirical requirement. It is generally interpreted to mean the contact of the fishery product with ice. Compliance is therefore difficult to demonstrate or verify in the case of packaged fishery products. Compliance with the requirement can be approached only by translating the requirement into an objective measure, which, in this instance, is assumed to be 0 °C. While there is a temperature requirement for storage and transport of fishery products, this requirement does not exist for other refrigerated products of animal origin.

- Any assessment of temperature and its effect on bacterial growth can be meaningful only in the context of a time period. As the current legal requirement extends for long, potentially interrupted, and variable durations, the potential outcomes of different temperature scenarios are best considered on the basis of potential shelf-life durations. It is worth noting that the group of products in the TOR includes foods with likely short shelf-life such as packaged fresh fishery products, as well as products which have been processed to have a relatively long shelf-life, e.g. cooked and chilled products from crustaceans.

- For products for which microbiological criteria are laid out in legislation (Regulation (EC) No 2073/2005), such criteria are used to evaluate the impact of storage at a temperature different to that set out in legislation. Growth of pathogens at storage temperatures above 0 °C may also occur in any pre-packed fishery products for which criteria are not laid out in legislation. In

this case the temperature that could be applied to storage and transport can be estimated as the temperature that will provide equal or less growth of the pathogens.

- The primary working assumption for those products for which there exist food safety criteria was that all storage temperatures used should continue to provide compliance with the relevant food safety criteria.

- The results from the modelling showed that pre-packed fresh fishery products can be stored at refrigeration temperatures above 0 °C (e.g. 3–5 °C) and be compliant with the current EU and international rules. Examples of combinations of product durability (maximum shelf-life) and packaging atmosphere that should enable compliance with the safety criteria for various storage temperatures at retail are provided.

- Apart from the non-compliance with the safety criteria, an increase of the storage temperature at retail above 0 °C may lead to growth of pathogenic microorganisms for which food safety criteria do not apply and are capable of growth at low temperatures. In this case the temperature that could be applied to storage and transport of pre-packed fresh fishery products at retail level can be estimated as the temperature that will provide equal or less growth of those pathogens.

- For histamine, the modelling results showed that, for a fishery product with certain characteristics subject to the current temperature requirement, histamine formation would be 100 ppm (lower limit m of the safety criterion in Regulation (EC) No 2073/2005) at the end of its shelf-life. Thus, an equivalent condition to this baseline scenario is any combination of storage temperature, shelf-life and CO_2 concentration in the package that leads to histamine formation of 100 ppm at the end of shelf-life. For example, for a retail temperature of 3 °C, 100 ppm would be reached under the following conditions:

  - shelf-life of 6 days and 0 % CO_2 in the packaging headspace
  - shelf-life of 7 days and 20 % CO_2 in the packaging headspace
  - shelf-life of 8 days and 40 % CO_2 in the packaging headspace.

- For *L. monocytogenes* EU Regulation (EC) No 2073/2005 sets a limit of 100 CFU/g in RTE products at the end of shelf-life. In this case the FBO can ensure compliance with such limit for products stored in retail at temperatures above 0 °C by adjusting the product durability (maximum shelf-life) and/or by modifying the packaging atmosphere of the product to ensure that the growth of the pathogen during distribution and storage will not exceed such limit. For this, the available predictive models were used assuming an initial concentration of 1 CFU/g. and show that, for a retail temperature of 3 °C for example, this limit would be respected with:

  - shelf-life of 11 days and 0 % CO_2 in the packaging headspace
  - shelf-life of 14 days and 20 % CO_2 in the packaging headspace
  - shelf-life of 18 days and 40 % CO_2 in the packaging headspace.

- Apart from the non-compliance with the safety criteria, an increase of the storage temperature at retail above 0 °C may lead to growth of other pathogenic microorganisms able to grow at low temperatures such as *Y. enterocolitica* and *C. botulinum*. *Y. enterocolitica* is the only hazard for which models predicting growth at 0 °C are available and time–temperature scenarios during storage and transport at retail level equivalent to the currently mandated storage requirements can be estimated. For all other hazards, including *C. botulinum*, equivalent growth can be achieved by storing these products below the minimum growth temperature of those hazards (for example 3.3 °C for *C. botulinum*) that could be present in the product. For *Y. enterocolitica*, the results of the modelling showed that, for a fishery

product with certain characteristics (pH 6.5, water phase salt = 1 %) and a shelf-life of 14 days, which is stored in ice (0 °C), the conditions that lead to equivalent growth to that at 0 °C are, for example, the following combinations:

- shelf-life of 10 days at 2 °C
- shelf-life of 7 days at 4 °C
- shelf-life of 5 days at 6 °C.

RECOMMENDATION

- If a temperature limit is deemed necessary then future legislation should include a clear temperature value instead of generic statements such as 'temperature approaching that of melting ice'.

REFERENCES

Allen DG, Green DP, Bolton GE, Jaykus LA and Cope WG, 2005. Detection and identification of histamine-producing bacteria associated with harvesting and processing mahimahi and yellowfin tuna. Journal of Food Protection, 68, 1676–1682.

Arumugaswamy RK, Ali GRR and Hamid S, 1994. Prevalence of *Listeria monocytogenes* in foods in Malaysia. International Journal of Food Microbiology, 23, 117–121.

Balasubramanian S, Rajan MR and Raj SP, 1992. Microbiology of fish grown in a sewage-fed pond. Bioresource Technology, 40, 63–66.

Baranyi J and Roberts TA, 1994. A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology, 23, 277–294.

Bermejo A, Mondaca MA, Roeckel M and Marti MC, 2003. Growth and characterization of the histamine-forming bacteria of jack mackerel (*Trachurus symmetricus*). Journal of Food Processing and Preservation, 26, 401–414.

Boxman ILA, Tilburg J, te Loeke N, Vennema H, Jonker K, de Boer E and Koopmans M, 2006. Detection of noroviruses in shellfish in the Netherlands. International Journal of Food Microbiology, 108, 391–396.

CAC (Codex Alimentarious Commission), 1999. Principles and guidelines for the conduct of Microbiological Risk Assessment. Food Hygiene Series. CAC/GL 30.

CAC (Codex Alimentarius Commission), 2007. Procedural Manual - 17th Edition. Available at: ftp://ftp.fao.org/docrep/fao/010/a1472e/a1472e.pdf

Chang SC, Kung HF, Chen HC, Lin CS and Tsai YH, 2007. Determination of histamine and bacterial isolation in swordfish fillets (*Xiphias gladius*) implicated in a food borne poisoning. Food Control, 19, 16–21.

Chen HC, Lee YC, Hwang DF, Chiou TK and Tsai YH, 2011. Determination of histamine in mahi-mahi fillets (*Coryphaena hippurus*) implicated in a foodbrone poisoning. Journal of Food Safety, 31, 320–325.

Colavita G, Miotti Scapin R, Giaccone V, Lucci A, Manuppella A, Laurelli T, Antonelli V and Ciallella ML, 2000. A large outbreak of food poisoning due to enterotoxin D-producing *Staphylococcus aureus* from shrimp salad. Igiene Moderna, 114, 205–215.

Dalgaard P, Emborg J, Kjolby A, Sorensen ND and Ballin NZ, 2008. Histamine and biogenic amines: formation and importance in seafood. Improving Seafood Products for the Consumer, 292–324.

Dalgaard P, Madsen HL, Samieian N and Emborg J, 2006. Biogenic amine formation and microbial spoilage in chilled garfish (*Belone belone belone*) - effect of modified atmosphere packaging and previous frozen storage. Journal of Applied Microbiology, 101, 80–95.

David ST, McIntyre L, MacDougal L, Kelly D, Liem S, Schallie K, McNabb A, Houde A, Mueller P, Ward P, Trottier YL and Brassard J, 2007. An outbreak of norovirus caused by consumption of oysters from geographically dispersed harvest sites, British Columbia, Canada, 2004. Foodborne Pathogens and Disease, 4, 349–358.

Davies AR, Capell C, Jehanno D, Nychas GJE and Kirby RM, 2001. Incidence of foodborne pathogens on European fish. Food Control, 12, 67–71.

Davies AR and Slade A, 1995. Fate of Aeromonas and Yersinia on modified-atmosphere-packaged (MAP) cod and trout. Letters in Applied Microbiology, 21, 354–358.

Di Pinto A, Terio V, Pinto P and Tantillo G, 2012. Detection of potentially pathogenic *Aeromonas* isolates from ready-to-eat seafood products by PCR analysis. International Journal of Food Science and Technology, 47, 269–273.

Domenech-Sanchez A, Laso E, Perez MJ and Berrocal CI, 2011. Emetic disease caused by *Bacillus cereus* after consumption of tuna fish in a beach club. Foodborne Pathogens and Disease, 8, 835–837.

Dorsa WJ, Marshall DL, Moody MW and Hackney CR, 1993. Low-temperature growth and thermal inactivation of *Listeria monocytogenes* in precooked crawfish tail meat. Journal of Food Protection, 56, 106–109.

Duflos G, Dervin C, Malle P and Bouquelet S, 1999. Relevance of matrix effect in determination of biogenic amines in plaice (*Pleuronectes platessa*) and whiting (*Merlangus merlangus*). Journal of AOAC International, 82, 1097–1101.

EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessment carried out by EFSA. Part 2: general principles. The EFSA Journal 2009, 1051, 1–22.

EFSA (European Food Safety Authority), 2013. Analysis of the baseline survey on the prevalence of *Listeria monocytogenes* in certain ready-to-eat (RTE) foods in the EU, 2010-2011 Part A: *Listeria monocytogenes* prevalence estimates. EFSA Journal 2013;11(6):3241, 75 pp. doi:10.2903/j.efsa.2013.3241

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA Journal 2015;13(1):3991, 162 pp. doi:10.2903/j.efsa.2015.3991

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2010. Scientific Opinion on risk assessment of parasites in fishery products. EFSA Journal 2010;8(4):1543, 91 pp. doi:10.2903/j.efsa.2010.1543

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2011a. Scientific Opinion on risk based control of biogenic amine formation in fermented foods. EFSA Journal 2011;9(10):2393, 93 pp. doi:10.2903/j.efsa.2011.2393

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2011b. Scientific Opinion on an update on the present knowledge on the occurrence and control of foodborne viruses. EFSA Journal 2011;9(7):2190, 96 pp. doi:10.2903/j.efsa.2011.2190

Emborg J and Dalgaard P, 2008. Modelling the effect of temperature, carbon dioxide, water activity and pH on growth and histamine formation by *Morganella psychrotolerans*. International Journal of Food Microbiology, 128, 226–233.

Emborg J, Dalgaard P and Ahrens P, 2006. *Morganella psychrotolerans* sp nov., a histamine-producing bacterium isolated from various seafoods. International Journal of Systematic and Evolutionary Microbiology, 56, 2473–2479.

Emborg J, Laursen BG and Dalgaard P, 2005. Significant histamine formation in tuna (*Thunnus albacares*) at 2 degrees C - effect of vacuum- and modified atmosphere-packaging on psychrotolerant bacteria. International Journal of Food Microbiology, 101, 263–279.

Emborg J, Laursen BG, Rathjen T and Dalgaard P, 2002. Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere-packed salmon (*Salmo salar*) at 2 degrees C. Journal of Applied Microbiology, 92, 790–799.

EU (European Union), 2014a. Facts and figures on the Common Fisheries Policy– Basic statistical data – 2014 Edition. Luxembourg: Publications Office of the European Union 2014, 44 p. ISBN 978-92-79-34192-2 doi: 10.2771/35745

EU (European Union), 2014b. The EU Fish Market. 2014 Edition. European Market Observatory for Fishery and Aquaculture Products (EUMOFA). Available at: https://www.eumofa.eu/documents/10157/bf18cf2c-1b33-440d-8870-e05b2644b58b

FAO (Food and Agriculture Organisation of the United Nations), 2014. The State of World Fisheries and Aquaculture. Opportunities and challenges. Available at: http://www.fao.org/3/a-i3720e/index.html

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 2013 Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products. Meeting report. Rome: FAO Headquarters 1–111. Available at: http://apps.who.int/iris/bitstream/10665/89216/1/9789240691919_eng.pdf?ua=1

Fletcher GC, 2010. Research of relevance to histamine poisoning in New Zealand. A review. Ministry of Agriculture and Forestry, MAF Technical Paper No: 2011/70. Available at: http://www.foodsafety.govt.nz/elibrary/industry/2011-70-histamine-poisoning.pdf

Fletcher GC, Summers G, Winchester RV and Wong RJ, 1995. Histamine and histidine in New Zealand marine fish and shellfish species, particularly kahawai (*Arripis trutta*). Journal of Aquatic Food Product Technology, 4, 53–74.

Friesema I, de Jong A, Hofhuis A, Heck M, van den Kerkhof H, de Jonge R, Hameryck D, Nagel K, van Vilsteren G, van Beek P, Notermans D and van Pelt W, 2014. Large outbreak of *Salmonella* Thompson related to smoked salmon in the Netherlands, August to December 2012. Eurosurveillance, 19, 13–20.

Gawade L, Barbuddhe SB and Bhosle S, 2010. Isolation and confirmation of *Listeria* species from seafood off Goa region by Polymerase Chain Reaction. Indian Journal of Microbiology, 50, 385–389.

Gui M, Binzhao Song J, Zhang Z, Hui P and Li P, 2014. Biogenic amines formation, nucleotide degradation and TVB-N accumulation of vacuum-packed minced sturgeon (*Acipenser schrencki*) stored at 4 °C and their relation to microbiological attributes. Journal of the Science of Food and Agriculture, 94, 2057–2063.

Guyader FSl, Bon F, DeMedici D, Parnaudeau S, Bertone A, Crudeli S, Doyle A, Zidane M, Suffredini E, Kohli E, Maddalo F, Monini M, Gallay A, Pommepuy M, Pothier P and Ruggeri FM, 2006. Detection of multiple noroviruses associated with an international gastroenteritis outbreak linked to oyster consumption. Journal of Clinical Microbiology, 44, 3878–3882.

Hewitt JH, Begg N, Hewish J, Rawaf S, Stringer M and Theodoregandi B, 1986. Large outbreaks of *Clostridium perfringens* food poisoning associated with the consumption of boiled salmon. Journal of Hygiene, 97, 71–80.

Huss HH, 1993. Assurance of seafood quality. FAO Fisheries Technical Paper. No. 334. Food and Agriculture Organization of the United Nations Rome, 1994, 169 pp. Available at: http://www.fao.org/docrep/003/t1768e/t1768e1700.htm.

Huss HH, Reilly A and Ben Embarek PK, 2000. Prevention and control of hazards in seafood. Food Control, 11, 149–156.

Hwang D-F, Chen T-Y, Chang S-H, Chou S-S, Deng J-F and Chai T-J, 1999. Biogenic amines and bacterial isolates of marlin implicated in food poisoning. Food Science and Agricultural Chemistry, 1, 223–228.

ICMSF (International Commission on Microbial Specifications for Food), 1996. Microorganisms in foods 5: Characteristics of microbial pathogens. Blackie Academic & Professional. Springer, London; UK, 514 pp.

ICMSF (International Commission on Microbial Specifications for Food), 2005. Microorganisms in Foods 6: Microbial Ecology of Food Commodities. 2nd edition. Chapter 3. Fish and fish products. Kluwer Academic & Plenum Publishers, New York, 174–249.

Iwamoto M, Ayers T, Mahon BE and Swerdlow DL, 2010. Epidemiology of seafood - Associated infections in the United States. Clinical Microbiology Reviews, 23, 399–411.

James JS and James C, 2014. Chapter 20 – Refrigeration and Freezing. In: Food Safety Management. A Practical Guide for the Food Industry. Eds Motarjemi Y and Lilieveld H, Academic Press, 481–510.

Janda JM and Abbott SL, 2010. The Genus Aeromonas: Taxonomy, Pathogenicity, and Infection. Clinical Microbiology Reviews, 23, 35–73.

Kanki M, Yoda T, Ishibashi M and Tsukamoto T, 2004. *Photobacterium phosphoreum* caused a histamine fish poisoning incident. International Journal of Food Microbiology, 92, 79–87.

Klausen NK and Huss HH, 1987. Growth and histamine production by *Morganella morgani* under various temperature conditions. International Journal of Food Microbiology, 5, 147–156.

Lampila LE and McMillin KW, 2012. Major microbial hazards associated with packaged seafood. In: Advances in Meat, Poultry and Seafood Packaging. Ed Kerry JP, Woodhead Publishing in Food Science Technology and Nutrition, 59–85.

Lee YC, Lin CM, Huang CY, Huang YL, Chen HC, Huang TC and Tsai YH, 2013. Determination and Frying Loss of Histamine in Striped Marlin Fillets Implicated in a Foodborne Poisoning. Journal of Food Protection, 76, 860–866.

Malle P, Valle M and Bouquelet S, 1996. Assay of biogenic amines involved in fish decomposition. Journal of AOAC International, 79, 43–49.

McLaughlin JB, DePaola A, Bopp CA, Martinek KA, Napolilli NP, Allison CG, Murray SL, Thompson EC, Bird MM and Middaugh JP, 2005. Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. New England Journal of Medicine, 353, 1463–1470.

Mejlholm O, Bøknæs N and Dalgaard P, 2014. Development and evaluation of a stochastic model for potential growth of *Listeria monocytogenes* in naturally contaminated lightly preserved seafood. Food Microbiology, 45, 276–289.

Mejlholm O and Dalgaard P, 2007a. Modeling and predicting the growth boundary of *Listeria monocytogenes* in lightly preserved seafood. Journal of Food Protection, 70, 70–84.

Mejlholm O and Dalgaard P, 2007b. Modeling and predicting the growth of lactic acid bacteria in lightly preserved seafood and their inhibiting effect on *Listeria monocytogenes*. Journal of Food Protection, 70, 2485–2497.

Mejlholm O and Dalgaard P, 2009. Development and validation of an extensive growth and growth boundary model for *Listeria monocytogenes* in lightly preserved and ready-to-eat shrimp. Journal of Food Protection, 72, 2132–2143.

Mejlholm O and Dalgaard P, 2015. Modelling and predicting the simultaneous growth of *Listeria monocytogenes* and psychrotolerant lactic acid bacteria in processed seafood and mayonnaise-based seafood salads. Food Microbiology, 46, 1–14.

Mejlholm O, Gunvig A, Borggaard C, Blom-Hanssen J, Mellefont L, Ross T, Leroi F, Else T, Visser D and Dalgaard P, 2010. Predicting growth rates and growth boundary of *Listeria monocytogenes* - An international validation study with focus on processed and ready-to-eat meat and seafood. International Journal of Food Microbiology, 141, 137–150.

Morii H and Kasama K, 2004. Activity of two histidine decarboxylases from *Photobacterium phosphoreum* at different temperatures, pHs, and NaCl concentrations. Journal of Food Protection, 67, 1736–1742.

Ozogul F and Ozogul Y, 2006. Biogenic amine content and biogenic amine quality indices of sardines (*Sardina pilchardus*) stored in modified atmosphere packaging and vacuum packaging. Food Chemistry, 99, 574–578.

Papadopoulou C, Economou E, Zakas G, Salamoura C, Dontorou C and Apostolou J, 2007. Microbiological and pathogenic contaminants of seafood in Greece. Journal of Food Quality, 30, 28–42.

Peck MW, Goodburn KE, Betts, R. P. and Stringer SC, 2006. *Clostridium botulinum* in vacuum packed (VP) and modified atmosphere packed (MAP) chilled foods. Institute of Food Research, Norwich, UK; Food Safety and Technology Management Consultant; Campden & Chorleywood Food Research Association, Chipping Campden, UK, 225 pp. Available at: http://www.ifr.ac.uk/safety/Final_project_report0707.pdf

Pinto AL, Teixeira P, Castilho F, Felicio MT, Pombal F and Gibbs PA, 2006. Prevalence and serotyping of *Listeria monocytogenes* in Portuguese live bivalve molluscs sampled in various steps along the sanitary control process. Aquaculture Research, 37, 1112–1116.

Potasman I, Paz A and Odeh M, 2002. Infectious outbreaks associated with bivalve shellfish consumption: A worldwide perspective. Clinical Infectious Diseases, 35, 921–928.

Prato R, Lopalco PL, Chironna M, Barbuti G, Germinario C and Quarto M, 2004. Norovirus gastroenteritis general outbreak associated with raw shellfish consumption in South Italy. BMC Infectious Diseases, 4, DOI: 10.1186/1471-2334-1184-1137.

Reilly PJA and Twiddy DR, 1992. *Salmonella* and *Vibrio cholerae* in brackishwater cultured tropical prawns. International Journal of Food Microbiology, 16, 293–301.

Rodriguez-Jerez JJ, Moraventura MT, Lopezsabater EI and Hernandezherrero M, 1994. Histidine, lysine and ornithine decarboxylase bacteria in Spanish salted semipreserved anchovies. Journal of Food Protection, 57, 784–787.

Ruiz-Capillas C and Moral A, 2004. Free amino acids and biogenic amines in red and white muscle of tuna stored in controlled atmospheres. Amino Acids, 26, 125–132.

Saheki K, Kobayashi S and Kawanishi T, 1989. *Salmonella* contamination of eel culture ponds. Nippon Suisan Gakkaishi, 55, 675–679.

Sciarrone R, Maini P, Simoncello I, Stevanin A, Gottardello L and Renzulli G, 1997. An outbreak of Clostridium perfringens associated with consumption of "baccala alla vicentina" a stock-fish Italian speciality. Igiene Moderna, 107, 1–9.

Senderovich Y, Izahi I, Halpern M, 2010. Fish as reservoirs and vectors of Vibrio cholerae. PloS ONE, 5, 1–5.

Simmons G, Garbutt C, Hewitt J and Greening G, 2007. A New Zealand outbreak of norovirus gastroenteritis linked to the consumption of imported raw Korean oysters. The New Zealand Medical Journal, 120, U2773.

Solano R, Lafuente S, Sabate S, Tortajada C, de Olalla PG, Hernando AV and Cayla J, 2013. Enterotoxin production by *Staphylococcus aureus*: An outbreak at a Barcelona sports club in July 2011. Food Control, 33, 114–118.

Tao ZH, Sato M, Yamaguchi T and Nakano T, 2009. Formation and diffusion mechanism of histamine in the muscle of tuna fish. Food Control, 20, 923–926.

Tham W, Ericsson H, Loncarevic S, Unnerstad H and Danielsson-Tham ML, 2000. Lessons from an outbreak of listeriosis related to vacuum-packed gravad and cold-smoked fish. International Journal of Food Microbiology, 62, 173–175.

Thayumanavan T, Vivekanandhan G, Savithamani K, Subashkumar R and Lakshmanaperumalsamy P, 2003. Incidence of haemolysin-positive and drug-resistant *Aeromonas hydrophila* in freshly caught finfish and prawn collected from major commercial fishes of coastal South India. FEMS Immunology and Medical Microbiology, 36, 41–45.

Tsai YH, Chang SC, Kung HF, Wei CI and Hwang DF, 2005. Histamine production by *Enterobacter aerogenes* in sailfish and milkfish at various storage temperatures. Journal of Food Protection, 68, 1690–1695.

van Spreekens K, 1986. Histamine production by the psychrophilic flora. In: Seafood quality determination. Eds Kramer DE and Liston J, Elsevier Science Publishers, Amsterdam, the Netherlands, 309–318.

Vazquez-Sanchez D, Lopez-Cabo M, Saa-Ibusquiza P and Rodriguez-Herrera JJ, 2012. Incidence and characterization of *Staphylococcus aureus* in fishery products marketed in Galicia (Northwest Spain). International Journal of Food Microbiology, 157, 286–296.

Wall R, Dymond N, Bell A, Thornley C, Buik H, Cumming D and Petersen N, 2011. Two New Zealand outbreaks of norovirus gastroenteritis linked to commercially farmed oysters. The New Zealand Medical Journal, 124, 63–71.

Wyatt LE, Nickelson R and Vanderzant C, 1979. Occurrence and control of *Salmonella* in freshwater catfish. Journal of Food Science, 44, 1067–1073.

ABBREVIATIONS

a_w	Water activity
BIOHAZ Panel	EFSA Panel on Biological Hazards
CFU	Colony-forming units
EFSA	European Food Safety Authority
EU	European Union
EUMOFA	European Market Observatory for Fisheries and Aquaculture
FBO	Food Business Operator
GHP	Good Hygiene Practice
HACCP	Hazard Analysis and Critical Control Point
m	Lower limit of the safety criterion for fish species
MAP	Modified Atmospheric Packaging
MC	Microbiological Criteria
MPN	Most Probable Number
ppm	Parts per million
RTE	Ready-to-eat
TOR	Term of Reference