# Verification of a HACCP-based Strategy for the Control of Histamine for the Fresh Tuna Industry.

J. John Kaneko MS, DVM
Jon W. Bell PhD
Donald R. Hawn BS

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PacMar, Inc.
3615 Harding Avenue, Suite 409
Honolulu, Hawaii 96816

## TABLE OF CONTENTS


| | | Page |
|---|---|---|
| I. | Report Title, Author, Organization, Grant Number and Date | 2 |
| II. | Abstract | 2 |
| III. | Executive Summary | 3 |
| IV. | Purpose: | 5 |
| | A. Detailed description of problem or impediment of fishing industry that was addressed. | 5 |
| | B. Objectives of the project. | 10 |
| V. | Approach: | 11 |
| | A. Detailed description of work that was performed. | 11 |
| | B. Project Management: List of individuals and/or organizations actually performing the work and how it was done. | 14 |
| VI. | Findings: | 15 |
| | A. Actual accomplishments and findings. | 15 |
| | Results Objective 1. | 15 |
| | Results Objective 2. | 33 |
| | Results Objective 3. | 35 |
| | Discussion | 37 |
| | B. If significant problems developed which resulted in less than satisfactory results, they should be addressed. | 43 |
| | C. Description of need, if any, for additional work. | 45 |
| V. | Evaluation: | 46 |
| | A. Describe: | 46 |
| | 1. Were the goals and objectives attained? | 46 |
| | 2. Were modifications made to the goals and objectives? | 46 |
| | B. Dissemination of Project results. | 46 |
| VI. | References: | 47 |

**Final Report**

## I. Report Title, Author, Organization, Grant Number and Date

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**Authors:** J. John Kaneko MS, DVM, Jon W. Bell PhD and Donald R. Hawn

**Organization:** PacMar, Inc., Honolulu, Hawaii

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## II. Abstract

A HACCP-based approach for controlling histamine formation in susceptible pelagic fish caught in the Hawaii tuna longline fishery was studied. Fish handling practices were evaluated by monitoring 68 longline sets, during 7 fishing trips on 5 different Hawaii tuna longline vessels. Two hundred thirty-one (231) fish were monitored using temperature data loggers to record fish temperature profiles after capture and handling on board the fishing vessels. After unloading, each fish underwent sensory examination for decomposition and was tested for histamine content. The chilling rates were analyzed and compared to critical time and temperature limits recommended for histamine control (≤ 50°F in ≤ 6 hours and ≤ 40°F in ≤ 24 hours after death) by the US Food and Drug Administration (2001). While some of the fish exceeded these time and temperature critical limits, none of the fish monitored exceeded the defect action limit of 50 ppm histamine. Only three (3) of these fish failed sensory examination, but all had muscle histamine levels below the defect action limit (1.5, 2.1 and 3.1 ppm). The mean histamine concentration of the 228 fish that passed sensory examination was 0.93 ppm (SD 0.96, max 9.0). The effectiveness of sensory examination as a histamine control measure during the inspection of fish landings was further evaluated. A total of 482 fish landed from 35 Hawaii longline vessels and 38 different fishing trips were examined for decomposition and tested for histamine. Three hundred sixty-six (366) of these fish passed sensory examination and had a mean histamine concentration of 1.06 ppm (SD 1.53, max 19.0). The 116 fish that failed sensory examination were accumulated from the landings and had a mean histamine concentration of 1.76 ppm (SD 6.94, max 71 ppm). Only one fish out of 482 exceeded the histamine defect action limit, with a concentration of 71 ppm, and this fish also failed sensory examination. Combined with the results of an earlier study (Kaneko, 2000), 100% of sampled fish that exceeded the histamine defect action limit (15 out of 1,065 fish) also failed sensory examination, and were rejected due to decomposition. These results show that the recommended critical limits for chilling rates for histamine-forming fish species may be extended by using standard onboard fish handling procedures in the Hawaii longline tuna fishery in the central North Pacific without significantly jeopardizing seafood safety when coupled with 100% sensory examination of the catch. A HACCP-based approach for the control of histamine on Hawaii fishing vessels that relies on vessel standard operating procedures and sensory examination is presented.

## III. Executive Summary

- Companies receiving fresh tuna and associated species directly from fishing vessels need a practical and effective approach to ensure that fish handling procedures are implemented at sea to control the accumulation of histamine, the causative agent of histamine poisoning (also known as scombroid fish poisoning).

- A study was conducted to verify the effectiveness of a HACCP-based approach (Hazard Analysis Critical Control Point) to control histamine onboard longline vessels in the fresh tuna fishery in Hawaii.

- This approach was previously studied (Kaneko, 2000) by documenting the fish handling practices, time and temperature controls and histamine control on Hawaii tuna longline vessels. The present study was conducted to verify important results of the earlier study.

- Fish species included in this study were: bigeye tuna, yellowfin tuna, albacore tuna, blue marlin, striped marlin, mahimahi, wahoo and escolar. Fish handling practices were evaluated by monitoring 68 longline sets, during 7 fishing trips on 5 Hawaii tuna longline vessels. Two hundred thirty-one (231) fish were monitored using temperature data loggers to record the fish temperature profiles during the post-harvest period on board the fishing vessels. After unloading, these fish underwent sensory examination for decomposition and were tested for histamine.

- Chilling rates were compared with the US Food and Drug Administration (FDA) recommended critical limits of time and temperature for histamine control on fishing vessels (FDA, 2001). The FDA recommended critical limits require fish to be chilled to ≤ 50°F in ≤ 6 hours and ≤ 40°F in ≤ 24 hours after death.

- The FDA recommended critical time limits for fish handling to control histamine begin at the time of death of the fish. The time of this event cannot be known for fish that die on longline gear. During this study, 43.3% of the fish monitored on longline fishing vessels were brought on board after dying on the line. There was no significant difference at the end of the trip between the mean histamine concentrations of fish that were alive (0.85 ppm, SD 0.73) and dead (1.07 ppm, SD 1.19) when brought on board.

- For bigeye tuna, a significant difference ($P < 0.05$) was found between the mean histamine concentrations of fish harvested alive (0.80 ppm, SD 0.64) and dead (1.21 ppm, SD 1.19). However, mean histamine levels for both groups were well below toxic levels (200 to 500 ppm) and the defect action limit (50 ppm) and do not present a public health hazard.

- The mean time to ≤ 50°F for all fish monitored was 6 hours and 4 minutes (SD 4 h 6 min). The maximum time recorded from the time of death to ≤ 50°F was 19 hours. This 146 lb bigeye tuna was retrieved alive and left whole, reached ≤ 40°F after 31

hours, passed sensory examination, was graded a No. 1 sashimi quality fish and contained 0.5 ppm histamine after unloading.

- The mean time to ≤ 40°F for all fish monitored was 12 hours and 40 minutes (SD 6 h 43 min). The maximum time recorded from the time of death to ≤ 40°F was 34 hours. This 128 lb bigeye tuna was retrieved alive and left whole, reached ≤ 50°F after 16 hours and 20 minutes, passed sensory examination, was graded No. 1 quality and contained 0.5 ppm histamine.

- While many of the fish monitored exceeded the critical limits (≤ 50°F in ≤ 6 hours and to ≤ 40°F in ≤ 24 hours), none of these fish exceeded the histamine defect action limit of 50 ppm.

- Only 3 of the 231 fish monitored for time and temperature at sea failed sensory examination after unloading. These fish had histamine levels of 1.5, 2.1 and 3.1 ppm, below the defect action limit. The mean histamine concentration of the 228 fish that passed sensory examination was 0.93 ppm (SD 0.96, max 9.0).

- These results strongly suggest that the critical time limits for chilling fish in the Hawaii longline tuna fishery may be extended. The extent to which the time to ≤ 50°F and ≤ 40°F can be safely extended is not fully known, but this study has documented safe fish handling of up to 19 hours to ≤ 50 °F and up to 34 hours to ≤ 40°F for bigeye tuna to be effective in controlling histamine production and safety risk.

- The effectiveness of sensory examination for decomposition as a histamine control measure during the inspection of fish landings was further evaluated. A total of 482 fish from 35 Hawaii longline vessels and 38 different fishing trips were examined for decomposition and tested for histamine. The mean histamine concentration of the 366 fish that passed sensory examination was 1.06 ppm (SD 1.53, max 19.0). The 116 fish that failed sensory examination had a mean histamine concentration of 1.76 ppm (SD 6.94, max 71 ppm). The difference in mean histamine concentration between the two groups was not significant (P = 0.05). The fish with the maximum histamine level of 71 ppm failed sensory examination and was the only fish that exceeded the defect action limit of 50 ppm histamine of the 482 fish sampled in this sensory examination study.

- For the bigeye tuna evaluated in this study, the difference in the mean histamine concentrations between fish that passed sensory examination (1.06 ppm, SD 1.34) and failed (2.88 ppm, SD 11.57) was significant (P <0.05).

- Combined with the results of the earlier study (Kaneko, 2000), a total of 1,065 histamine-forming fish were analyzed for sensory examination and tested for histamine. Sensory examination has been 100% effective in rejecting or culling fish (15 fish out of 1,065) that contained histamine concentrations above the defect action limit.

- This study shows that the detection of persistent and readily perceptible odors of decomposition is a reliable means of culling fish containing histamine concentrations above the defect action limit in this fishery. Sensory examination

is an effective and practical HACCP measure for controlling the histamine hazard as fish enter market channels from fishing vessels in the Hawaii tuna longline fishery.

- The effect of gilling and gutting fish on histamine formation was evaluated by comparing the chilling rates and histamine concentrations of 91 whole and 73 gilled and gutted longline-caught bigeye tuna. As expected, gilled and gutted fish were chilled at a faster rate than whole fish. No difference was detected between the mean histamine concentrations of whole bigeye (0.92 ppm, SD 1.16) and gilled and gutted fish (1.02 ppm, SD 0.70) during unloading.

- Epidemiological data on outbreaks of histamine poisoning reported to the State of Hawaii between September 1989 and October 2003 were evaluated. Tuna and mahimahi were the two most commonly implicated species. Between September 1989 and late December 1997, tuna and mahimahi were implicated in 69% of the histamine poisoning outbreaks and 82% of the number of illnesses. In the period between late December 1997 and October 2003, tuna and mahimahi were implicated in 78% of histamine poisoning outbreaks and 84% of the illnesses reported in Hawaii.

- The results of this work suggest that the critical limits for chilling rates for histamine-forming fish species in the Hawaii longline tuna fishery in the central North Pacific may be extended using standard fish handling practices without significantly jeopardizing seafood safety and histamine control when coupled with 100% sensory examination of the fish landed. Practical training for fishermen[1] to ensure that they understand how histamine forms and how it can be easily prevented through proper temperature control is key to controlling the histamine risk and safety in the Hawaii fishery with these verified procedures.

## IV. Purpose:

### A. Detailed description of problem or impediment of fishing industry that was addressed.

The goal of this project was to verify the efficacy of HACCP-based onboard fish handling procedures used by Hawaii longline vessels in controlling histamine formation and accumulation in susceptible pelagic fish species.

Context of the Project

The National Oceanographic and Atmospheric Administration Saltonstall-Kennedy Fisheries Research Program (NOAA/SK Program) funded the study “Development of a HACCP-based Strategy for the Control of Histamine for the Fresh Tuna Industry” that was completed in July, 2000 (Kaneko, 2000). The project resulted in several significant findings. That study evaluated the onboard fish handling methods of Hawaii’s tuna longline vessels and the effectiveness of sensory examination of the catch in preventing high histamine fish from entering the market. The study monitored fish handling practices in terms of time/temperature and histamine controls. The study demonstrated that the detection of sensory indicators of decomposition in fresh tuna and associated

[1] The term “fishermen” is used in this report without any disrespect for women involved in fishing.

pelagic fish at the time of unloading in the Hawaii fishery setting was effective in culling 100% of the fish in the study that exceeded the FDA histamine defect action limit of 50 ppm.

The project helped to support the application of scientifically derived information and industry knowledge to the assessment of histamine risk and the development of practical and effective controls. Since the end of the first project, the Food and Drug Administration (FDA) Office of Seafood has stepped up its efforts on histamine control in the U.S. seafood industry. Compliance with FDA recommendations for HACCP (Hazard Analysis Critical Control Point) controls for histamine on fishing vessels continues to be a serious issue especially for domestic seafood processors that receive histamine-forming fish species directly from vessels.

The current project was proposed to the NOAA/SK Program to assist the fresh tuna industry in Hawaii overcome continuing difficulty in complying with FDA HACCP regulations, and more specifically with FDA guidance for how best to control histamine formation from occurring at sea on fishing vessels. This project was designed to verify some of the findings of the previous study and expand this learning and knowledge to support the viability of the fresh tuna industry in Hawaii, produce safe seafood products and meet its obligations to comply with the principles of HACCP.

Description of the Problem or Impediment

The control of histamine poisoning continues to be the most important seafood-related public health problem facing the US fresh tuna industry. The application of HACCP principles in industry seafood safety control programs is mandated by federal regulation (21 CFR Part 123). The FDA seafood HACCP regulation is applicable to the seafood processing and wholesale sectors. Retail and fishing sectors are not directly impacted. Effective control of histamine formation requires rapid cooling during the initial post-harvest steps that occur on fishing vessels outside of the inspection jurisdiction of the FDA. As a result, the responsibility for ensuring histamine controls on fishing vessels falls on the companies that receive fish directly from fishing vessels. The crux of the problem is the establishment of practical process controls, monitoring procedures and records that can be relied upon to ensure that fish received from fishing vessels have been properly handled to control histamine.

The Hawaii seafood processing sector has encountered difficulties applying the FDA guidance for histamine control by the first receiver of histamine-forming fish from fishing vessels. Two approaches are recommended (FDA, 2001). The first approach relies on lot sampling and histamine testing of the selected fish (*Histamine Testing Approach*), while the second approach requires detailed monitoring and record-keeping by fishermen of post-harvest handling parameters (*Harvest Vessel Records Approach*). The Histamine Testing Approach applies representative sampling and will not be effective in detecting fish with high histamine levels when they occur at low frequency. The Vessel Harvest Records approach cannot be implemented because some of the recommended critical limits for time and temperature control are not always possible to achieve or to monitor.

An alternative approach tailored to the Hawaii fresh tuna industry is needed. A HACCP-based approach was developed and supported by research conducted in the earlier NOAA SK funded study (Kaneko, 2000). Industry compliance continues to be in

question by the FDA. Additional research is required to verify the effectiveness of the histamine control approach developed for the Hawaii fishery and to support the implementation of a practical HACCP-based program for this complex problem.

Background on histamine poisoning.

Histamine poisoning is also known as "*scombroid fish poisoning*" although non-scombroid fish species are often implicated. Mahimahi (also known as dorado), tuna and bluefish are the fish most commonly associated with reported cases of histamine poisoning in the US. It is caused by the consumption of fish containing toxic concentrations of histamine and other biogenic amines (Taylor et al., 1984). Histamine poisoning is a pseudo-allergic reaction and can be treated with antihistamines.

Histamine-forming fish species have naturally high levels of the free amino acid histidine in their muscle. Enzymes produced by spoilage bacteria can convert histidine to histamine. When fish are mishandled after capture (prolonged temperature abuse and poor sanitation), spoilage bacteria can proliferate. Certain species of bacteria are efficient histamine formers because they are capable of producing the enzyme histidine decarboxylase. Toxic levels of histamine may accumulate if histamine-forming bacteria species are present and the susceptible fish are subjected to inadequate temperature control at sea and through the processing and distribution channels.

Background on HACCP

HACCP is a process control philosophy that focuses on science-based food safety hazard analysis and the establishment of procedures demonstrated to control the identified safety hazard. The HACCP approach emphasizes the prevention of hazards instead of end product sampling and testing as a means of controlling seafood safety hazards. The FDA seafood HACCP regulation (21 CFR Part 123), implemented in December 1997, requires that all seafood processors conduct a hazard analysis of their products and processes, draft a HACCP plan for monitoring critical control points in the process, assign critical limits for process controls, establish a system for record-keeping, prepare plans for corrective actions when critical limits are exceeded and to establish procedures to periodically verify the efficacy of the HACCP plan.

HACCP relies on regular monitoring of critical control points in the processing sequence to prevent food safety hazards that are identified during the systematic hazard analysis and determined to be "*reasonably likely to occur*", based on the best available scientific and industry knowledge. HACCP is not a zero-risk system and the focus is on minimizing seafood safety hazards that are likely to occur.

FDA HACCP is also not a "*prescriptive program*" that dictates exactly how each company or industry sector will control recognized seafood safety hazards. Rather, processors are advised to follow FDA guidance contained within the Fish and Fishery Products Hazards and Controls Guide, now in its 3rd edition (FDA, 2001). When companies are unable to adapt this guidance to their individual operations or they reach different conclusions in their hazard analyses, they are encouraged to conduct research to strengthen the scientific understanding of the problem and demonstrate the effectiveness of controls used in their customized HACCP plans. In this way, science-based HACCP approaches can be developed to ensure effective food safety controls tailored to the myriad of seafood products, processes, facilities and industry settings.

Hawaii's fresh tuna industry has disagreed with FDA guidance on how to best control histamine risk on fishing vessels. The industry finds itself in the difficult position of trying to comply with the FDA HACCP regulation, while being unable to apply the FDA guidance that is not a prescriptive regulation, but agency guidance.

FDA Guidance for Histamine Control on Fishing Vessels

The FDA recommends two basic approaches for first receivers (or primary processors) for controlling histamine in fresh tuna and associated pelagic species received from the primary producers (fishing vessels) (FDA, 2001).

The Histamine Testing Approach (FDA, 2001).

First receivers using the Histamine Testing Approach shall at the time of delivery,

- Lot sample 1 fish (if wt >20 lb) per ton and test for histamine. If fish are smaller (wt <20 lb), sample 2 fish per ton.
- Conduct sensory examination for decomposition by sampling 118 fish in a lot (or each fish for lots <118 fish) and reject entire lot if rejects exceed 2.5% or 3 fish.
- Record the internal body temperature of the fish at the time of delivery to ensure that fish were handled in accordance with critical limits for receiving temperatures.

This approach is problematic because lot sampling and testing is not an effective method for detecting histamine in fish from longline fisheries due to the way fish are caught and handled in the post-harvest period. Longline fishing vessels deliver fish caught during multiple sets (deployments) of the gear made with hundreds of hooks, and each fish has a unique hooking and hauling time. Fish are harvested both alive and dead in the same set and may have very different times of death. This is in contrast to purse seining methods where schools of fish are caught in large nets, die at approximately the same time and are chilled, frozen and stored in large fish wells equipped with ammonia refrigeration providing relatively uniform handling conditions.

The recommended sampling rate may allow detection of histamine problems in fish lots when they occur at a high percentage of the catch caused by gross mishandling. However, this sampling rate is not sufficient to detect the few individual fish with high histamine or decomposition that may occur in longline vessel landings at extremely low frequency. Increasing the sampling rate would not be practical for longline fishery landings and does not emphasize HACCP-based prevention measures for histamine.

The Harvest Vessel Records Approach (FDA, 2001).

First receivers using the Harvest Vessel Records Approach shall at the time of delivery,

- Receive detailed fish handling records from the vessel operator demonstrating compliance with the critical limits of time and temperature control.
- Conduct sensory examination for decomposition by sampling 118 fish in a lot (or each fish for lots <118 fish) and reject entire lot if rejects exceed 2.5% or 3 fish.
- Record the internal body temperature of the fish at the time of delivery to ensure that fish were handled in accordance with critical limits for receiving temperatures.

Detailed vessel records monitoring onboard fish handling need to document the time of capture and death of each fish, and the temperature history details of the fish during the initial cooling period and subsequent storage phase. These guidelines have been summarized in Table 1.

TABLE 1. *FDA guidance* for Harvest Vessel Records for the control of histamine accumulation on fishing vessels.*

| **FISH** | **FISH HANDLING MONITORING RECORDS ON VESSEL** | | | |
|---|---|---|---|---|
| | **If Air / Water Temp ≥ 83 °F** | | **If Air / Water Temp < 83 °F** | |
| | whole fish | G&G fish | Whole fish | G&G fish |
| **Bigeye > 20 lb**<br>**Yellowfin > 20 lb**<br>**Albacore > 20 lb**<br>**Skipjack > 20 lb** | **Fish temp ≤ 50°F ≤ 6 h ≤ 40°F ≤ 24 h | Ice fish within ≤ 6 h | **Fish temp ≤ 50°F ≤ 6 h ≤ 40°F ≤ 24 h | Ice fish within ≤ 6 h |
| | | | | |
| **Bigeye ≤ 20 lb**<br>**Yellowfin ≤ 20 lb**<br>**Albacore ≤ 20 lb**<br>**Skipjack ≤ 20 lb** | Ice fish within ≤ 6 h | Ice fish within ≤ 6 h | Ice fish within ≤ 12 h | Ice fish within ≤ 12 h |
| | | | | |
| **Blue Marlin**<br>**Striped Marlin**<br>**Mahimahi**<br>**Wahoo**<br>**Escolar** | Ice fish within ≤ 6 h | Ice fish within ≤ 6 h | Ice fish within ≤ 12 h | Ice fish within ≤ 12 h |

*Based on HACCP guidance in FDA Fish and Fishery Products Hazards and Control Guide, 3rd ed, 7/01.
**Only categories that require fish temperature monitoring at sea. All time limits begin at time of death.

The problem with this guidance is that the critical limits for time and temperature control for whole fish are too restrictive and/or cannot be met. The guidance makes a distinction between fish smaller and greater than 20 lb whole weight. Fish size strongly affects fish chilling rates and time. In the Hawaii fishery, large tuna may be in excess of 200 lb and blue marlin in excess of 800 lb. While a 25 lb tuna can easily meet the critical limits, larger fish are difficult to impossible to chill to below 50°F within 6 hours of death using ice. Fish that die on the line before being retrieved cannot meet the critical limits that are based on a known time of death.

Once handled and properly iced in the fish hold, fish should remain there and fishermen should check to ensure the adequacy of the icing during the remainder of the fishing trip. When fish are left whole, the recommendation to document when each fish reaches 50°F and 40°F is an impractical and nearly impossible task for the vessel crew because it is difficult to access and monitor individual fish cooling rates when they are buried in ice. The best practice to ensure adequate chilling rates is to make sure the fish are properly iced.

An alternative approach is needed for longline fisheries. An appropriate program will account for the fishing method and environment and ensure histamine control while

promoting good fish handling practices and fish quality improvement. In the fresh tuna industry, price is directly related to individual fish quality and demand. Fishermen have clear economic incentives to improve fish handling practices, improve quality and simultaneously, improve histamine control and seafood safety. The real danger is to require fishermen to provide harvesting records that are not practical or possible to obtain, and may not actually be necessary to control histamine formation.

The practical question is how to design a HACCP-based system that emphasizes prevention, and establishes a set of standard operating procedures for fish handling on vessels using ice to chill and store fish that will reliably prevent histamine accumulation. The first receiver is responsible for verifying proper onboard handling methods by fishermen. How to document and verify that fish have been properly handled on board fishing vessels to control histamine production is the practical challenge.

### B. Objectives of the project.

The project goal was to verify the effectiveness of a practical alternative, HACCP-based approach for controlling histamine risk in the fresh tuna longline industry in Hawaii. The verification of the alternative HACCP-based approach relies on,

- Establishing a set of Vessel Standard Operating Procedures (VSOP).
- Verifying that the VSOP are able to meet FDA time and temperature guidelines.
- Determining that the VSOP are effective at preventing histamine accumulation.
- Requiring fishermen to sign a Letter of Assurance (LOA) for each trip attesting that they followed the VSOP.
- Verifying that sensory examination is an effective critical control measure for preventing high histamine fish from entering the market from fishing vessels.

Hazard analysis of histamine accumulation on board Hawaii longline fishing vessels should include the following.

- A review of epidemiological records on reported cases of histamine poisoning in Hawaii to search for information that might help to assess the risk associated with the type of fish, product form, source of the product, and the fishing method.
- Evaluation of time and temperature parameters during fish handling on board Hawaii longline fishing vessels related to fishing and post-harvest handling sequences and the ability to control histamine formation and accumulation.
- Evaluation of HACCP controls that first receivers can use to prevent fish containing high histamine levels from entering commerce from fishing vessels.

The Project Objectives.

Objective 1. Verify onboard fish handling procedures for controlling histamine accumulation.

Objective 2. Verify sensory examination as a reliable histamine control measure.

Objective 3. Conduct training workshops for vessel operators.

## V. Approach:

A. Detailed description of work that was performed.

### Methods Obj.1. Verify onboard fish handling procedures for controlling histamine accumulation.

Evaluation of epidemiological data on histamine poisoning in Hawaii.

Data on reported cases of histamine poisoning in Hawaii were reviewed to improve understanding of histamine risk in fish available to consumers in Hawaii. Data were obtained from the Epidemiology Branch of the State of Hawaii Department of Health for the period from 9/20/1989 through 10/5/03. The data set was then divided into two subsets, one leading up to the implementation date for the FDA seafood HACCP regulation (12/18/97) and the second for the period after implementation to evaluate potential effects and changes in the nature of the reported cases.

Evaluation of the onboard fish handling practices on Hawaii longline fishing vessels.

The study was designed to document the onboard temperature profiles of mixed pelagic fish caught by deep-set longline gear in Hawaii's fresh tuna fishery. Each individual fish was then evaluated for sensory indicators of decomposition, and muscle samples for histamine analysis were collected at the time the fish were delivered to the first receiver in Honolulu.

Cooperative Hawaii-based longline vessel owners and captains partnered with the project team to facilitate research on the vessels and to allow evaluation and testing of the fish at the end of the trip. These participating vessels were representative of deep-set tuna longline fishing gear, methods and fish handling practices. Research was conducted during commercial fishing trips to observe, monitor and verify fishing methods and fish handling procedures.

Details of fish harvesting methods were recorded. Monitoring of longline fishing sets included recording times for the start of the set, completion of the line setting, and the start and end of line hauling. Additional information collected included the longline set number, time the fish was hauled on board, the fish species, its condition (alive or dead), and initial core body temperature.

Observations of the fish handling methods were also recorded and evaluated. Methods used by the crew to handle the fish immediately after they were brought on board were monitored. These handling procedures included bleeding, gilling and gutting the fish, or leaving the fish uneviscerated (whole). The deck time from pulling the fish on board to placing it in ice in the fish hold on the vessel was recorded.

Continuous fish temperature profiles were recorded on board the vessel using water-resistant temperature loggers (Onset Computer Corporation, Stowaway® Tidbit XT). The stainless steel temperature probes (6-inch) were directed along the longitudinal axis of the vertebral column from within the gill cavity for placement into the deep muscle to record temperature. The temperature loggers and wires were secured to the gill arches using plastic cable tie wraps. Fish were identified with pectoral fin clips and heavy-duty plastic flagging ribbon around the caudal peduncle. Loggers were programmed to record temperature at 5-minute intervals the time of insertion into the fish until removed during vessel unloading.

For obtaining onboard time and temperature histories for blue marlin, cooperative commercial trolling vessels operating out of Oahu were selected. The captains received a set of temperature loggers along with data cards to complete for each blue marlin monitored. Data collected included, the date of the trolling trip, logger number, the time the fish was hooked, the time the fish was brought on board and the lapsed time on deck (deck time) before being iced.

At the end of each longline and trolling fishing trip, the vessels were unloaded and the fish were delivered in ice to the Honolulu Fish Auction. The auction staff coordinated with the project team to schedule meeting the vessels, collect data sheets, retrieve temperature loggers, record fish weights, conduct sensory examinations and collect muscle samples for histamine testing.

The fish monitored at sea were identified at the auction, and then confirmed for species identification and condition (whole or gilled and gutted) of the fish. These fish were weighed to the nearest pound using a platform scale certified for commercial use by the State of Hawaii. Each fish was graded for quality and evaluated for odors of decomposition. The fish were graded for quality using fresh tuna industry grading methods (Bartram et al., 1996). Quality grades were also applied to the other associated pelagic fish species. After this initial evaluation, a muscle sample was removed from the dorsal muscle mass, immediately posterior to the cleithrum for histamine analysis. This is the standard sampling location, in the area of muscle most likely to develop histamine (Baranowski et al., 1990). Samples were stored in plastic freezer bags, labeled and kept buried in ice for no longer than 6 hours before freezing. Sets of muscle frozen samples were delivered to the laboratory for histamine analysis using the standard AOAC fluorometric method (AOAC, 1995; Official Method 977.13 for histamine in seafood).

Time and temperature data were downloaded from data loggers and matched with the initial status of the fish (live or dead). Analysis of data from the temperature loggers resulted in the determination of the initial core body temperature, fish cooling rates to a deep core body temperature of below 50ºF and below 40ºF, and fish temperatures at 6 and 24 hours post-harvest. Fish temperatures for the duration of the trip were also determined to ensure that storage temperature was maintained below 40°F after the fish were initially chilled on the fishing vessel. Mean fish temperature profiles were calculated. The lower 5 and upper 95 percentiles of the temperature profiles were also calculated, and the maximum (or slowest) chilling rate was noted.

Data loggers recorded temperatures every 5 minutes. As a result, temperatures recorded at the critical times in the chilling period (1, 3, 6, 12, 24, and 30 h) did not

necessarily occur exactly on the hour. Temperatures recorded immediately before selected intervals were used for calculations.

Similarly, the key determination of the time required to chill fish to critical temperatures did not necessarily occur when the fish reached exactly 40°F or 50°F. The first time recorded after the fish dropped below the selected temperature was used.

Data were analyzed using analysis of variance methods (ANOVA) (SAS User's Guide, 1985) and Least Squares Means analysis to compare specific variable means.

The following variables were monitored and analyzed.

- Soak time (gear and hooks)
- Initial fish status (alive or dead at time brought on board)
- Initial fish temperature (core body)
- Initial fish handling (whole or gilled and gutted)
- Deck time (between being brought on board and placement in ice)
- Fish temperature 6 hours post-harvest
- Fish temperature 24 hours post-harvest
- Time to chill fish to 50°F
- Time to chill fish to 40°F
- Storage time (from time in ice to unloading)
- Sensory examination (all fish monitored on board and in market sampling)
- Histamine concentration (all fish monitored on board and in market sampling)

**Methods Obj. 2. Verification of sensory examination as a reliable histamine control measure.**

All fish monitored on board the vessels and retrieved at the auction underwent sensory examination after removal of the temperature loggers. The fish were then weighed and muscle samples were collected for histamine testing. Sensory examination resulted in a pass or fail decision, based on the presence or absence in the gills and muscle of persistent and readily perceptible odors of decomposition.

Additional fish were also sampled from normal commercial longline fish landings at the Honolulu Fish Auction for examination for decomposition and histamine testing. These fish formed the representative market sample used to evaluate the effectiveness of sensory examination as a practical histamine control measure. Special efforts were required to collect samples from decomposed (rejected) fish that only occur in Hawaii longline fishery landings at a low frequency.

Data collected for the market sample fish included fishing gear type, vessel name (kept confidential), fish species, weight, condition, quality grade and sensory examination (pass or fail). Muscle samples were collected, handled and analyzed for histamine concentration as previously described.

Data were analyzed using analysis of variance methods (ANOVA) (SAS User's Guide, 1985) and Least Squares Means analysis to compare specific variable means.

**Method Obj. 3. Conduct training workshops for vessel operators.**

Training workshops for vessel operators were conducted for the Hawaii Longline Association membership, which includes the owners, managers and captains of all Hawaii-based longline vessels. The purpose of this outreach effort was to inform vessel owners of the potential impacts of FDA HACCP regulations on vessel operations, to reinforce the inspection requirements of longline fishing vessels delivering histamine-forming fish to the market, to share information on the research project objectives and methods, and to seek participation from vessel operators in the onboard research.

B. Project Management: List of individuals and/or organizations actually performing the work and how it was done.

| | |
|---|---|
| Principal Investigator: | John Kaneko MS, DVM, PacMar, Inc., Honolulu, Hawaii. Designed and managed the project. Coordinated the vessel-based research. Conducted sensory examinations, quality grading and collection of muscle samples for histamine analysis. Coordinated statistical analysis. Liaison with SK Program Manager and seafood industry members. Wrote the final report. |
| Financial Manager: | Thanh Lo Sananikone, PacMar, Inc., Honolulu, Hawaii. Managed the financial aspects of the project. |
| Research Associate: | Jon Bell PhD, PacMar, Inc., Honolulu, Hawaii. Assisted the PI in the research design, data analysis, design of figures and preparation of the final report. |
| Research Assistant: | Donald Hawn BS, Honolulu, Hawaii. Collected vessel-based research data, deployed temperature data loggers and coordinated with the PI to coordinate meeting the vessel at the end of the trip to collect data sheets, loggers and muscle samples. |
| Laboratory Services: | Wendy Minor, Food Quality Lab, Honolulu, Hawaii. Conducted the histamine analyses and maintained the laboratory QA/QC procedures. |
| Data management: | Tuan Ha, Honolulu, Hawaii Maintained database and assisted in statistical analysis. |

## VI. Findings:

A. Actual accomplishments and findings (corresponding to the Objectives).

**Results Obj. 1. Verify onboard fish handling procedures for controlling histamine accumulation.**

Evaluation of epidemiological data on histamine poisoning in Hawaii before and after the implementation of FDA HACCP regulation (effective December 18, 1997).

The Epidemiology Branch of the State of Hawaii Department of Health provided information on the reported outbreaks of histamine poisoning in Hawaii during the 14-year period between September 20, 1989 and October 5, 2003. Data were divided into two periods, pre-HACCP (September 20, 1989 to December 8, 1997) and post-HACCP (December 25, 1997 to October 5, 2003) and are presented in Table 2.

This table includes cases confirmed by histamine analysis of the implicated fish, probable cases based on history and clinical signs and suspected cases that have a lower degree of certainty.

The mean number of histamine outbreaks per year was 18.0 and the mean number of illnesses per year was 48.3 in the 1989 to 1997 period. Between late December 1997 and October of 2003 the mean annual number of reported outbreaks increased to 24.6 and the mean annual number of illnesses was 51.2. In the 1989 to 1997 period, the mean annual incident rate was 1.5 outbreaks and 4.0 illnesses per 100,000 people. In the more recent period between late 1997 and October 2003, the mean annual incident rate was 2.1 outbreaks and 4.3 illnesses per 100,000 people.

The two fish categories implicated most often were tuna (bigeye and yellowfin) and mahimahi. These two categories of fish were implicated in 69% of the outbreaks and 82% of the illnesses between 1989 and 1997, and 78% of the outbreaks and 84% of the illnesses in the period between late 1997 and October 2003.

The tuna category includes both bigeye and yellowfin tuna because it is often difficult for investigators to distinguish the species once the fish are processed and presented to the consumer. If greater detail concerning the tuna species, fishing and post-harvest handling methods and origin of the fish could be collected, improved insight might be provided on the relative risk of histamine poisoning. The percentage of reported histamine poisoning outbreaks associated with tuna (bigeye and/or yellowfin) remained relatively unchanged from 46% to 44% between the first and second period. At the same time, the percentage of histamine poisoning illnesses associated with tuna increased in the more recent period, from 25% to 41% of the illnesses.

Mahimahi caused 22% of the outbreaks and 56% of the illnesses in the first period. In the second period mahimahi was implicated in 34% of the outbreaks and 43% of the illnesses.

TABLE 2. *Fish species involved in cases of Histamine Poisoning in Hawaii between September 20, 1989 and October 5, 2003.*

| **FISH SPECIES*** | **FIRST PERIOD 9/20/89 to 12/08/97** | | **SECOND PERIOD 12/25/97 to 10/05/03** | |
|---|---|---|---|---|
| **Common, Hawaiian & Latin Names** | **No. of Outbreaks** | **No. of Illnesses** | **No. of Outbreaks** | **No. of Illnesses** |
| **Tuna** (yellowfin and bigeye) (ahi) (*Thunnus albacares or T. obesus*) | 68 (46.3%) | 97 (24.7%) | 63 (44.4%) | 121 (40.9%) |
| **Mahimahi** (mahimahi) (*Coryphaena hippurus*) | 33 (22.5%) | 225 (57.0%) | 48 (33.8%) | 128 (43.2%) |
| **Marlin** (Pac. Blue and striped) (kajiki and nairagi) (*Makaira nigricans or Tetrapterus audax*) | 12 (8.2%) | 16 (4.1%) | 5 (3.5%) | 10 (3.4%) |
| **Bigeye Scad** (akule) (*Trachiurops crumenophthalmus*) | 3 (2.0%) | 7 (1.8%) | 15 (10.6%) | 18 (6.1%) |
| **Wahoo** (ono) (*Acanthocybium solandri*) | 7 (4.8%) | 12 (3.0%) | 4 (2.8%) | 6 (2.0%) |
| **Albacore Tuna** (tombo ahi) (*Thunnus alalunga*) | 1 (0.7%) | 2 (0.5%) | 0 (0.0%) | 0 (0.0%) |
| **Skipjack** (aku) (*Katsuwonas pelamis*) | 2 (1.4%) | 3 (0.8%) | 0 (0.0%) | 0 (0.0%) |
| **Canned Tuna** (light meat) (*K. pelamis and/or T. albacares*) | 1 (0.7%) | 1 (0.3%) | 0 (0.0%) | 0 (0.0%) |
| **Spearfish** (hebi) (*Tetrapterus angustirostris*) | 0 (0.0%) | 0 (0.0%) | 1 (0.7%) | 2 (0.7%) |
| **Mackerel Scad** (opelu) (*Decapterus pinnulatus*) | 1 (0.7%) | 1 (0.3%) | 0 (0.0%) | 0 (0.0%) |
| **Jack** (ulua) (*Caranx* spp.) | 1 (0.7%) | 1 (0.3%) | 0 (0.0%) | 0 (0.0%) |
| **"Covina"** (Unknown spp.) | 1 (0.7%) | 1 (0.3%) | 0 (0.0%) | 0 (0.0%) |
| **Bonefish** (oio) (*Albula vulpes*) | 0 (0.0%) | 0 (0.0%) | 1 (0.7%) | 1 (0.3%) |
| **Escolar** (walu) (*Lepidocybium flavobrunneum*) | 0 (0.0%) | 0 (0.0%) | 2 (1.4%) | 4 (1.4%) |
| **Yellowtail** (*Seriola* spp.) | 0 (0.0%) | 0 (0.0%) | 2 (1.4%) | 2 (0.7%) |
| **Unknown fish** | 17 (11.6%) | 29 (7.3%) | 1 (0.7%) | 4 (1.4%) |
| **Total** | **147** | **395** | **142** | **296** |
| **Mean Histamine Outbreaks and Illnesses Per Year** | **18.0** | **48.3** | **24.6** | **51.2** |
| **Mean Annual Incident Rate (MAIR) per 100,000 people**** | **1.49** | **4.0** | **2.1** | **4.3** |

* Fish species confirmed, probable and suspected in cases of Histamine Poisoning reported to the Hawaii Department of Health.

**Assumes average Hawaii population of 1.2 million (1989 to 2003). Does not include the significant visitor population present in Hawaii throughout the year.

Changes in the quality of epidemiological investigation and reporting are most notable in the unknown fish category. The investigations of seafood illnesses have improved over

time with investigators identifying a greater percentage of the fish species implicated. Between 1989 and late 1997, 12% of the outbreaks and 7% of the illnesses were caused by fish that were not identified. In the second period, the percentage caused by fish that were not identified dropped to less than 1% of the outbreaks and approximately 1% of the illnesses.

Fish species of secondary importance in reported histamine poisoning cases include marlin (Pacific blue and/or striped), bigeye scad (akule) and escolar. Marlin were the third most commonly implicated species group in the first period and dropped to the fourth most common in the second period. Bigeye scad became the third most common species implicated in the second period. Fresh bigeye scad available in the Hawaii market are caught by surround net (modified purse seining method) and gill net and are not produced by pelagic longlining.

Fish species of minor importance in histamine cases in Hawaii include albacore and skipjack, which are both very popular and important food fish in Hawaii. They are both eaten raw and in cooked preparations. Skipjack is also a popular dried fish product. These two species caused only 3 histamine poisoning outbreaks and 5 illnesses in the first period. During the second period, no outbreaks or illnesses were reported associated with albacore or skipjack. The low number of reported cases of histamine poisoning associated with skipjack and albacore indicates a low risk in these fish in Hawaii. Fresh albacore is primarily caught by longline gear and a much smaller amount is caught by tuna handliners. Fresh skipjack in Hawaii is caught primarily by pole and line vessels (bait boats) that store fish in refrigerated seawater or directly in ice.

Escolar is a species of potential concern. This fish is not commonly eaten in Hawaii, but shipped to markets on the US mainland. No cases of histamine poisoning were reported in Hawaii associated with escolar in the first period. In the more recent period, 2 outbreaks and 4 reported illnesses caused by escolar were reported.

In the period between 1989 and late 1997, 14% of the outbreaks and 53% of the reported illnesses due to histamine poisoning in Hawaii were caused by imported seafood products. Imported mahimahi was responsible for 75% of the outbreaks and 96% of the illnesses caused by imported seafood products.

In the more recent period between late 1997 and October 2003, 18% of the histamine poisoning outbreaks and 29% of the illnesses were caused by imported seafood. Mahimahi was responsible for 81% of the outbreaks and 90% of the illnesses due to imported seafood.

The epidemiological data are valuable in focusing attention on the species most commonly implicated in histamine poisoning in Hawaii. This information can also be used to detect any new or emerging species or trends in reported histamine poisoning cases. Greater detail is needed to use the information to identify the source, the fishing gear, the quality of the product, and the product form to better guide efforts to improve seafood safety.

<u>Verify onboard fish handling procedures for controlling histamine accumulation.</u>

To verify the post-harvest fish handling procedures, standard operating procedures on fishing vessels during normal operations were monitored during research trips on

commercial fishing vessels. Research was conducted on 68 longline sets made during 7 commercial trips on 5 different longline vessels participating in the Hawaii tuna longline fishery.

The vessels that participated in this research were representative of Hawaii's deep-set longline fishing gear and methods used for targeting bigeye tuna in the central North Pacific Ocean. The typical Hawaii tuna longline fishing vessel operating during the study period in 2002 and 2003 used monofilament longline gear, set 1950 to 2000 hooks per longline set (NMFS, 2004), set during the morning, soaked the gear during the afternoon, and hauled the gear during the evening. Hawaii tuna longline vessels use hydraulic line shooters, machines that maintain slack in the mainline as it is deployed from the vessel in order to allow the gear to sink to depths were the bigeye tuna are found.

Under the current fishery management regime, in place since mid-2001, the deepest part of the mainline must be at least 100 m below the surface. Float lines suspending the mainline must be at least 20 m long, so that the shallowest part of the mainline is 20 m deep. Branch lines suspend hooks about 10 m below the mainline to ensure that the hooks settle in deep water to avoid interactions with sea turtles (WPRFMC, 2004). Greater than 15 branch lines must be set between floats to ensure deep-setting.

Deep-set tuna longline methods differ significantly from shallow-set longline fishing methods used for targeting broadbill swordfish. The typical Hawaii swordfish vessel used monofilament mainline, set about 800 hooks per set, set the gear in the evening, soaked the gear overnight and hauled the gear during the day (Ito et al., 1998). There are fewer than 10 hooks set between floats to ensure that the hooks remain in the upper water column where swordfish are more likely to be encountered. Swordfish fishing vessels do not use line shooters because they are trying to place the hooks in relatively shallow surface layers. Regulations in place since mid-2001 effectively eliminated the shallow-set swordfish longline fishery in Hawaii.

Bigeye tuna tend to occupy a greater depth in the water column and catch rates have been reported to be highest where water temperature (at depth) is 50.0 to 62.6°F (10 to 17°C) (Hanamoto, 1987). Boggs (1992) demonstrated using experimental longline fishing within the central North Pacific Ocean typically fished by Hawaii's longline fleet that the highest catch rate for bigeye tuna was at 360 to 400 m depths where water temperatures ranged from 46.4 to 50.0ºF (8 to 10°C).

Tagging studies to document the vertical movements of bigeye tuna near the main Hawaiian Islands revealed that fish body temperatures ranged from 59.0 to 80.6°F (15 to 27°C) (Musyl et al., 2003). The average body temperature was 69.8 to 71.6°F (21 to 22°C) during the day time when the fish were found in water layers with ambient temperatures of 41.0 to 78.8°F (5 to 26°C), with an average water temperature around 64.4°F (18°C). Body temperature rose to 77.0°F (25°C) during the night hours when fish made vertical migrations into shallower water layers where ambient water temperature was closer to 75.2°F (24°C).

Hooks on deep-set tuna longline gear are set deep in the water column where the temperature is significantly lower than at the sea surface. If fish die on the line after being hooked, the chilling phase begins immediately as heat is transferred from the fish body to the surrounding colder water. Histamine formation is known to be rapid at

temperatures above 70ºF and most rapid near 90ºF (FDA, 2001). The relatively cool temperatures at the hooking depth, even in tropical Pacific waters, may be one of the reasons histamine accumulation is not a more common problem with longline-caught tuna and other pelagic species in Hawaii.

Soak time.

The soak time for pelagic longline fishing gear is determined from the completion of gear-setting to the beginning of the gear-hauling phase. Hawaii longline vessels typically begin hauling from the end of the longline gear that was last to enter the water. Hauling in this sequence means that the last hook hauled, is also the hook that has been in the water for the longest period of time. The maximum hook soak time was calculated from the time the setting began and the last hook was hauled. Table 3 summarizes data from the 68 deep-set longline sets monitored during the study. Mean longline gear soak time was 6 h 13 min, with a minimum of 3 h 21 min and a maximum of 8 h 42 min. The mean maximum hook soak time was 20 h 55 min, with a minimum of 15 h 35 min and a maximum of 32 h 20 min. The maximum hook soak time occurred during a longline set with an unusually high catch rate.

TABLE 3. *Mean longline gear soak time and maximum hook soak time.*

| **No. of** | **Longline gear soak time (h:min)** | | | | **Maximum hook soak time (h:min)** | | | |
|---|---|---|---|---|---|---|---|---|
| **sets** | mean | SD | min | max | mean | SD | min | max |
| 68 | 6:13 | 1:10 | 3:21 | 8:42 | 20:55 | 2:14 | 15:35 | 32:20 |

In the first study (Kaneko, 2000), the mean longline gear soak time was 6 h 44 min with a maximum of 8 h 9 min. The mean maximum hook soak time was lower, at 18 h with a maximum of 20 h 29 min.

Initial fish status (live or dead) when brought on board.

The initial status of the fish (live or dead) was monitored at sea and recorded. The results are presented in Table 4. Of the 231 fish monitored on board Hawaii longline vessels, 57% (131 fish) were alive when they were hauled on board and 43% (100 fish) were already dead. The percentages of live and dead fish varied between species groups. The percentage of dead fish among the small numbers of albacore, blue marlin, striped marlin, wahoo, mahimahi and yellowfin observed ranged from 57 to 100%. Escolar were mostly alive when hauled on board (93% live out of 29 fish monitored). Bigeye tuna had the largest sample size of 164 fish and the majority of the fish were alive (60%) when hauled on board.

In the first study, the initial status of the fish when they were brought on board was also recorded. Of the 383 mixed pelagic fish observed, 40% were alive and 60% were dead when hauled on board (Kaneko, 2000). The difference is most likely due to the greater proportion of bigeye tuna in the current study.

TABLE 4: *Deck time and percentage of live and dead pelagic fish caught by Hawaii deep-set longline gear.*

| Common name | Live/Dead | N | % | Deck time (minutes) mean | min | max | SD |
|---|---|---|---|---|---|---|---|
| | Live | 98 | 60 | 22 | 5 | 70 | 14.1 |
| **Bigeye tuna** | Dead | 66 | 40 | 25 | 5 | 101 | 16.8 |
| | All | 164 | 100 | 23 | 5 | 101 | 15.2 |
| | Live | 2 | 17 | 10 | 8 | 11 | 1.5 |
| **Yellowfin tuna** | Dead | 10 | 83 | 21 | 7 | 69 | 19.2 |
| | All | 12 | 100 | 19 | 7 | 69 | 18.0 |
| | Live | 0 | 0 | | | | |
| **Albacore tuna** | Dead | 3 | 100 | 10 | 9 | 13 | 2.3 |
| | All | 3 | 100 | 10 | 9 | 13 | 2.3 |
| | Live | 1 | 25 | 11 | 11 | 11 | 0.0 |
| **Pacific Blue Marlin** | Dead | 3 | 75 | 9 | 5 | 11 | 3.5 |
| | All | 4 | 100 | 10 | 5 | 11 | 3.0 |
| | Live | 3 | 43 | 15 | 8 | 23 | 7.5 |
| **Striped Marlin** | Dead | 4 | 57 | 9 | 2 | 19 | 7.4 |
| | All | 7 | 100 | 11 | 2 | 23 | 7.7 |
| | Live | 0 | 0 | | | | |
| **Mahimahi** | Dead | 9 | 100 | 9 | 4 | 14 | 3.3 |
| | All | 9 | 100 | 9 | 4 | 14 | 3.3 |
| | Live | 0 | 0 | | | | |
| **Wahoo** | Dead | 3 | 100 | 57 | 57 | 58 | 0.6 |
| | All | 3 | 100 | 57 | 57 | 58 | 0.6 |
| | Live | 27 | 93 | 15 | 4 | 48 | 10.0 |
| **Escolar** | Dead | 2 | 7 | 8 | 7 | 8 | 0.5 |
| | All | 29 | 100 | 14 | 4 | 48 | 9.8 |
| | Live | 131 | 57 | 20 | 4 | 70 | 13.4 |
| **All Species** | Dead | 100 | 43 | 21 | 2 | 101 | 16.3 |
| | All | 231 | 100 | 20 | 2 | 101 | 14.7 |

The time and temperature limits for histamine control recommended by FDA (2001) begin at the time the fish dies. A significant number of fish die on the line before they are hauled on board, and the time of death is impossible to determine. Histamine testing results from this study indicate that the time of death is not a determining factor in producing elevated histamine level in these fish (Table 5) in the Hawaii fishery. Time of death does not appear to be a significant parameter for histamine controls for deep-set tuna longline methods. There was no difference between the mean histamine concentrations of live (0.85 ppm) and dead (1.07 ppm) fish. The difference in the mean histamine concentrations between bigeye tuna retrieved live (0.80 ppm) and dead (1.21 ppm) was significant ($P < 0.01$), but the actual histamine concentrations were all well below toxic levels (>200 to 500 ppm) and the defect action limit (50 ppm) for all species.

TABLE 5: *Comparison of histamine concentrations of pelagic fish retrieved alive and dead and caught on Hawaii deep-set longline gear and stored in ice.*

| Fish species | Live fish | | | Dead fish | | | P |
|---|---|---|---|---|---|---|---|
| | Histamine concentration (ppm) | | | Histamine concentration (ppm) | | | |
| | N | mean | SD | N | mean | SD | |
| **Bigeye tuna** | 98 | 0.80 | 0.64 | 66 | 1.21 | 1.31 | 0.009 |
| **Yellowfin tuna** | 2 | 0.75 | 0.35 | 10 | 1.47 | 1.47 | 0.52 |
| **Albacore** | | | | 3 | 0.67 | 0.29 | |
| **Pacific Blue Marlin** | 1 | 0.50 | 0.00 | 3 | 0.50 | 0.00 | 1.00 |
| **Striped Marlin** | 3 | 1.50 | 1.32 | 4 | 0.50 | 0.00 | 0.18 |
| **Mahimahi** | | | | 9 | 0.50 | 0.00 | |
| **Wahoo** | | | | 3 | 0.50 | 0.00 | |
| **Escolar** | 27 | 0.97 | 0.96 | 2 | 0.50 | 0.00 | 0.50 |
| **All** | 131 | 0.85 | 0.73 | 100 | 1.07 | 1.19 | 0.09 |

These data indicate that knowledge of the time of death is not necessary to ensure histamine control in the Hawaii deep-set longline fishery. The time the fish is brought on board is a more practical and usable “time zero”. Fishermen are generally aware of the importance of limiting the time that fish are left on deck, before being placed in the ice. For these reasons, it is recommended that time and temperature limits for onboard handling to control histamine formation begin at the time the fish are hauled on board.

Deck time.

Deck time is calculated from the time the fish is brought on board until it is placed into the ice. Deck time is affected by handling procedures, such as gilling and gutting and duration of bleeding, catch rate and weather conditions. Deck time can become extended when many fish are brought on board in rapid succession. The research procedures for implanting a temperature data logger and identifying the fish for recovery are rapid. These tasks were completed while the fish were being bled and did not add to the total deck time.

The mean deck time for all 231 fish was 20.3 minutes (Table 4). Mean deck times did not show much difference between fish that were hauled alive (20.0 min) and dead (20.8 min). The maximum deck time observed was 101 minutes for a 47 lb (G&G) bigeye tuna that was dead at the time it was retrieved during a set with a particularly high catch rate. This fish contained 1.9 ppm histamine at sampling after unloading, well below the defect action limit of 50 ppm histamine.

Initial fish body temperature.

Table 6 compares the initial core temperature of fish hauled alive with fish brought on board dead. The mean initial core temperature of mixed pelagic fish hauled alive was 75.6°F while fish hauled after dying averaged 65.6°F. The difference between the two means was not significant (P = 0.08).

TABLE 6: *Comparison of initial core temperature of pelagic fish retrieved alive and dead and caught by Hawaii deep-set longline gear.*

| Fish species | Live fish Initial core temp (°F) | | | Dead fish Initial core temp (°F) | | | P |
|---|---|---|---|---|---|---|---|
| | N | mean | SD | N | mean | SD | |
| **Bigeye tuna** | 98 | 77 | 4.7 | 66 | 64 | 5.3 | < 0.0001 |
| **Yellowfin tuna** | 2 | 75 | 2.3 | 10 | 66 | 8.4 | 0.03 |
| **Albacore** | 0 | | | 3 | 67 | 9.5 | |
| **Pacific Blue Marlin** | 1 | 79 | 0.0 | 3 | 74 | 7.8 | 0.46 |
| **Striped Marlin** | 3 | 76 | 2.5 | 4 | 71 | 7.1 | 0.30 |
| **Mahimahi** | 0 | | | 9 | 69 | 4.5 | |
| **Wahoo** | 0 | | | 3 | 69 | 5.5 | |
| **Escolar** | 27 | 71 | 6.1 | 2 | 66 | 0.1 | 0.27 |
| **All** | 131 | 76 | 5.4 | 100 | 66 | 6.1 | 0.08 |

The difference between the mean initial core temperatures of bigeye tuna hauled alive and dead was significant (P <0.0001). The mean initial core temperature for live bigeye tuna was 76.8°F compared with 64.2°F for dead bigeye. The lower core temperatures for bigeye tuna that were dead when hauled on board after dying on the line indicates that cooling of the fish begins in the relatively colder water temperatures encountered at the hook depths for Hawaii deep-set tuna longline gear and methods.

Fish Temperature Profiles.

**Chilling rate of mixed pelagic fish (whole and G&G) caught by deep-set longline gear in Hawaii's longline fishery.**

The mean fish chilling rate of 231 mixed pelagic fish monitored at sea using the temperature loggers is presented in Figure 1. The mean fish temperature profile is presented along with the temperature curves encompassing 5% and 95% of the data set. The maximum chilling rate is also included. It should be noted that the 231 fish include fish that were gilled and gutted (G&G) as well as those left whole, and fish that were alive and fish that were dead when hauled on board. The weight of these fish ranged from 9 to 300 lb, with a mean weight of 74 lb (SD 43.5).

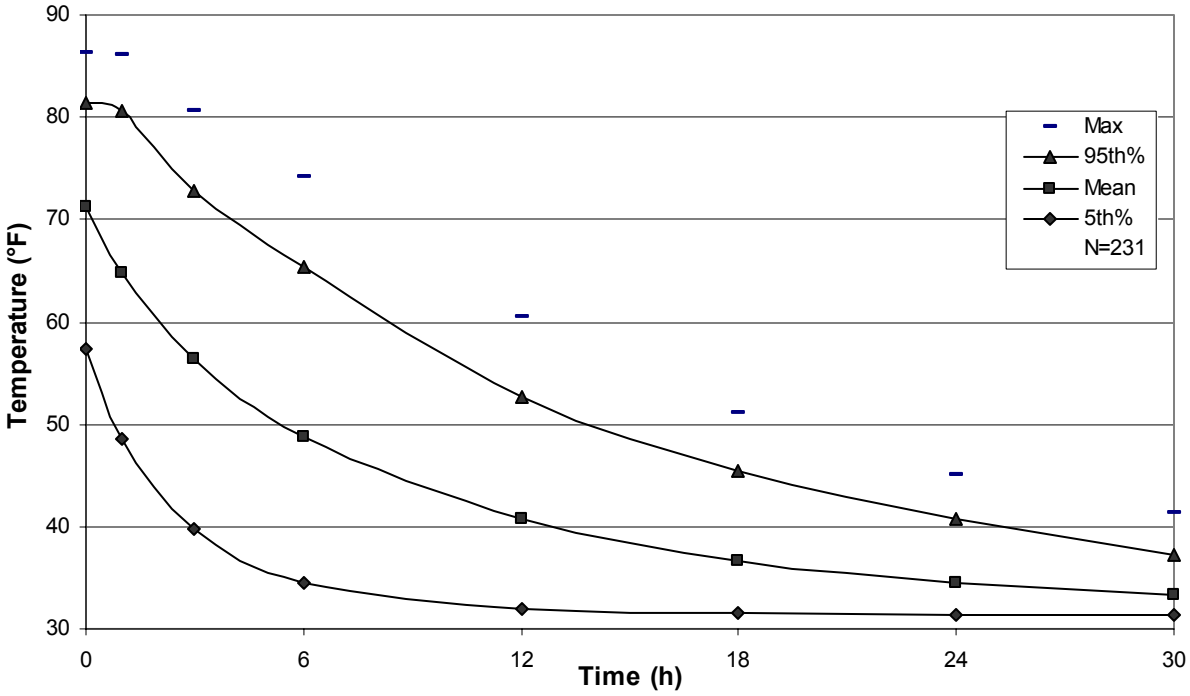


FIGURE 1. *Chilling rates of mixed pelagic histamine-forming fish (whole and gilled and gutted) caught in the Hawaii deep-set longline fishery and stored in ice.*

The mean fish temperature at 6 h was 48.8°F and 34.6°F after 24 h. The FDA recommended critical limits for chilling rates were met (≤ 50°F ≤ 6 h and ≤ 40°F ≤ 24 h) by the mean cooling curve, but not for all fish in the study. The maximum fish temperature after 6 h was 74.2°F and after 24 h was 45.0°F.

TABLE 7. *Time to chill mixed pelagic histamine-forming fish (whole, and gilled and gutted), caught in the Hawaii deep-set longline fishery to core temperature targets of 50°F and 40°F in ice.*

| **Fish temperature Target** | **Time (h:min) to reach temperature target** | | | | |
|---|---|---|---|---|---|
| | N | mean | SD | 95% | maximum |
| <50°F in <6 h | 231 | 6:04 | 4:06 | 14:35 | 19:00 |
| <40°F in <24 h | 231 | 12:40 | 6:43 | 25:20 | 34:00 |

The time required to chill the group of 231 mixed pelagic fish to the temperature targets of 50 and 40°F was also calculated (Table 7). The mean time to 50°F was 6 h 4 min. Ninety-five percent of the fish were below 50°F within 14 h 35 min. The maximum time required was 19 h. The mean time to 40°F was 12 h 40 min, 95% of the fish reached 40°F within 25 h 20 min and the maximum time to 40°F was 34 h. Although many of these fish exceeded the recommended critical limits, histamine levels for all fish were below the 50 ppm defect action limit.

The histamine testing results confirmed that all fish monitored at sea were safe for consumption and legal to sell. Of the 231 fish monitored on Hawaii longline fishing

vessels during this study, only 3 failed sensory examination. These fish were two yellowfin tuna with histamine concentrations of 2.1 and 1.5 ppm, and an escolar with a histamine level of 3.1 ppm, each well below the defect action limit. The 228 fish that passed sensory examination had a mean histamine concentration of 0.93 ppm (SD 0.96) and a maximum value of 9.00 ppm.

Any apparent discrepancy between the Tables presenting chilling time to target temperatures, and the predicted time at a given temperature derived from the temperature curves presented in the Figures, is related to how the data were collected in the temperature data loggers. Temperatures were recorded at 5 minute intervals, so that time data did not always occur exactly on the hour. Therefore the temperature recorded immediately before reaching the selected hour was used to determine the temperature curves. The time that fish reached the selected target temperatures was also similarly affected. The time the fish initially dropped below the target temperature was used to determine chilling time to target temperatures shown in the Tables.

**Chilling rate of <u>whole mixed pelagic histamine-forming fish</u> caught by deep-set longline gear in Hawaii's longline fishery.**

The mean chilling rate for 158 whole, mixed pelagic fish caught by deep-set longline gear is presented in Figure 2. The mean fish temperature after 6 h was 49.0°F and after 24 h was 34.8°F. The maximum fish temperature after 6 h was 74.2°F, and after 24 h was 45.0°F. The mean fish weight for this group was 76.8 lb (SD 47.3 lb), with a range of 9 to 300 lb.

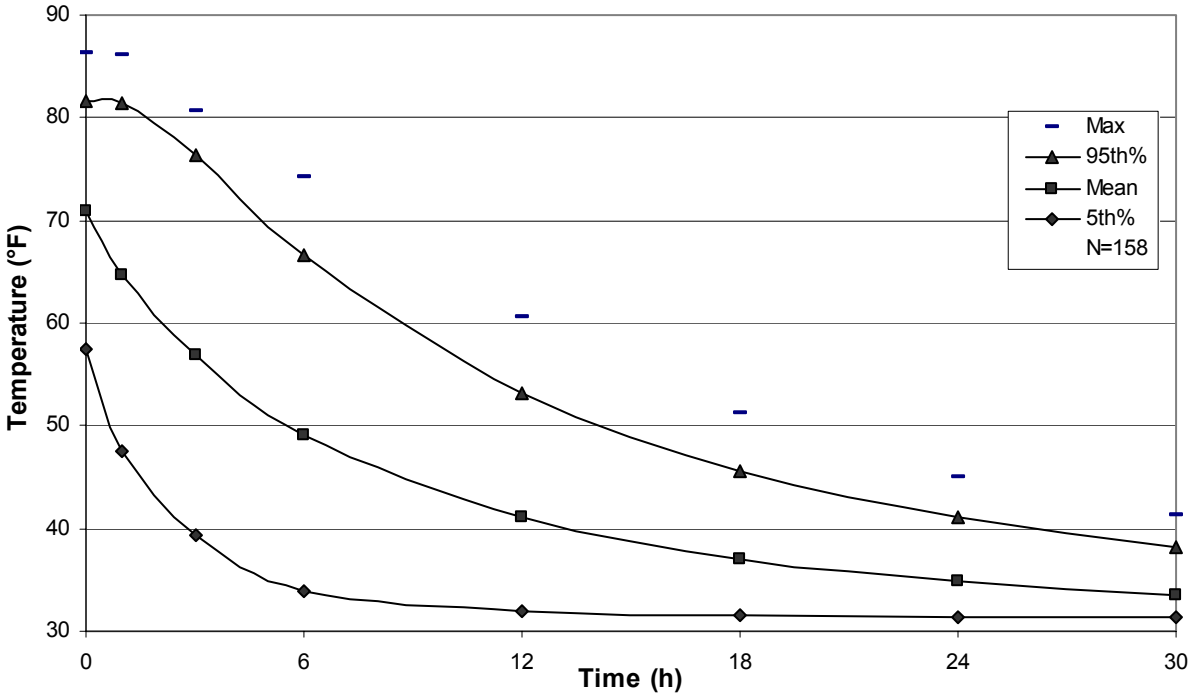


FIGURE 2. *Chilling rates of <u>mixed pelagic histamine-forming fish</u> (whole) caught in the Hawaii deep-set longline fishery and stored in ice.*

The time required to chill this group of 158 whole, mixed pelagic fish to the 50°F and 40°F temperature targets was calculated (Table 8). The mean time to 50°F was 6 h 21 min. Ninety-five percent of the fish were below 50°F within 14 h 50 min. The maximum time required to reach 50°F was 19 h. The mean time to 40°F was 12 h 57 min, 95% of the fish reached 40°F in 26 h 3 min and the maximum time to 40°F was 34 h. Although many of these fish exceeded the recommended critical limits, none of these fish exceeded the histamine defect action limit of 50 ppm.

TABLE 8. *Time to chill mixed pelagic histamine-forming fish (whole) caught in the Hawaii deep-set longline fishery to core fish temperature targets of 50°F and 40°F in ice.*

| **Fish temperature Target** | **Time (h:min) to reach temperature target** | | | | |
|---|---|---|---|---|---|
| | N | mean | SD | 95% | maximum |
| <50°F in <6 h | 158 | 6:21 | 4:22 | 14:50 | 19:00 |
| <40°F in <24 h | 158 | 12:57 | 7:27 | 26:03 | 34:00 |

The histamine testing results confirmed that all of these fish were acceptable for sale and safe for consumption. Of the 158 whole, mixed pelagic fish monitored on Hawaii longline fishing vessels during this study, only 2 yellowfin and 1 escolar failed sensory examination. The mean histamine concentration for these 3 fish was 2.23 ppm. The 155 fish that passed sensory examination had a mean histamine concentration of 0.88 ppm (SD 1.05) and a maximum value of 9.00 ppm.

The individual fish that required 34 h to chill to 40°F was a 128 lb bigeye tuna. This fish was alive when brought on board with an initial core temperature of 80.12°F, was left whole and reached 50°F in 16 h 33 min. After unloading this fish passed sensory examination, received a grade of No. 1 and contained a very low histamine concentration of 0.5 ppm. This fish greatly exceeded the recommended critical limits, but was both high in quality and low in histamine concentration. This fish was handled properly and required 34 h to chill to 40°F, yet after continued ice storage it presented very little food safety risk at unloading.

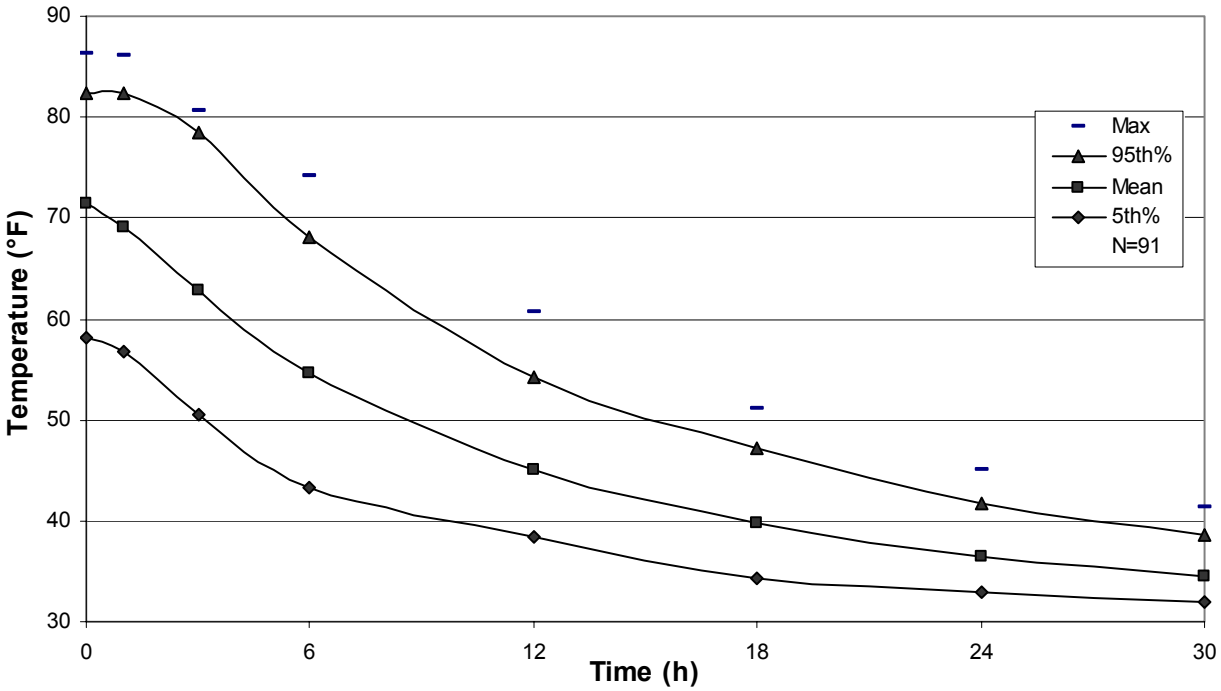


FIGURE 3. *Chilling rates of bigeye tuna (whole) caught in the Hawaii deep-set longline fishery and stored in ice.*

**Chilling rate of whole bigeye tuna caught by deep-set longline gear in Hawaii's longline fishery.**

During the study, a disproportionally large number of bigeye tuna were monitored at sea. The mean chilling rate for 91 whole bigeye tuna caught by deep-set longline gear is presented in Figure 3. The mean fish temperature after 6 h was 54.6°F with a maximum temperature of 74.2°F. The mean fish temperature after 24 h was 36.4°F and the maximum was 45°F. The mean fish weight was 99.12 lb (SD 34.35, min 25, max 207 lb).

The time required to chill the group of 91 whole, bigeye tuna to the 50°F and 40°F temperature targets was calculated (Table 9). The mean time to 50°F was 8 h 30 min. Ninety-five percent of the fish were below 50°F within 15 h 20 min. The maximum time required to reach 50°F was 19 h. The mean time to 40°F was 17 h 8 min, 95% of the fish reached 40°F within 27 h 20 min and the maximum time to 40°F was 34 h. Although many of these fish exceeded the recommended critical limits, none of these fish exceeded the histamine defect action limit of 50 ppm.

TABLE 9. *Time to chill bigeye tuna (whole) caught in the Hawaii deep-set longline fishery to core fish temperature targets of 50°F and 40°F in ice.*

| **Fish temperature target** | **Time (h:min) to reach temperature target** | | | | |
|---|---|---|---|---|---|
| | N | mean | SD | 95% | maximum |
| <50°F in <6 h | 91 | 8:30 | 3:47 | 15:20 | 19:00 |
| <40°F in <24 h | 91 | 17:08 | 5:48 | 27:20 | 34:00 |

The histamine testing results confirmed that all of these fish were safe for sale and consumption. All of the 91 fish in this group passed sensory examination. These fish had a mean histamine concentration of 0.92 ppm (SD 1.16) and a maximum value of 9.00 ppm.

**Chilling rate of gilled and gutted bigeye tuna caught by deep-set longline gear in Hawaii's longline fishery.**

Some fish were gilled and gutted after bleeding and before icing in the fish hold. Most Hawaii longline vessels do not gill and gut their catch. The crew gilled and gutted the fish during this particular trip, providing an opportunity to evaluate the difference in chilling rates and histamine control between whole and G&G bigeye tuna. Gilling and gutting removes approximately 10% of the round body weight, as well as the heat contained by the viscera and gills that are removed and discarded. Gilling and gutting also alters the body configuration, and allows ice to be packed into the peritoneal and gill cavities to increase heat removal or chilling rates. These fish ranged from 27 to 180 lb, with a mean weight of 70.7 lb (SD 33.5).

Figure 4 presents the mean chilling rate of G&G bigeye tuna. The mean fish temperature after 6 h is 48.2°F with a maximum of 71.4°F. The mean fish temperature after 24 h is 35.8°F and maximum of 43.3°F.

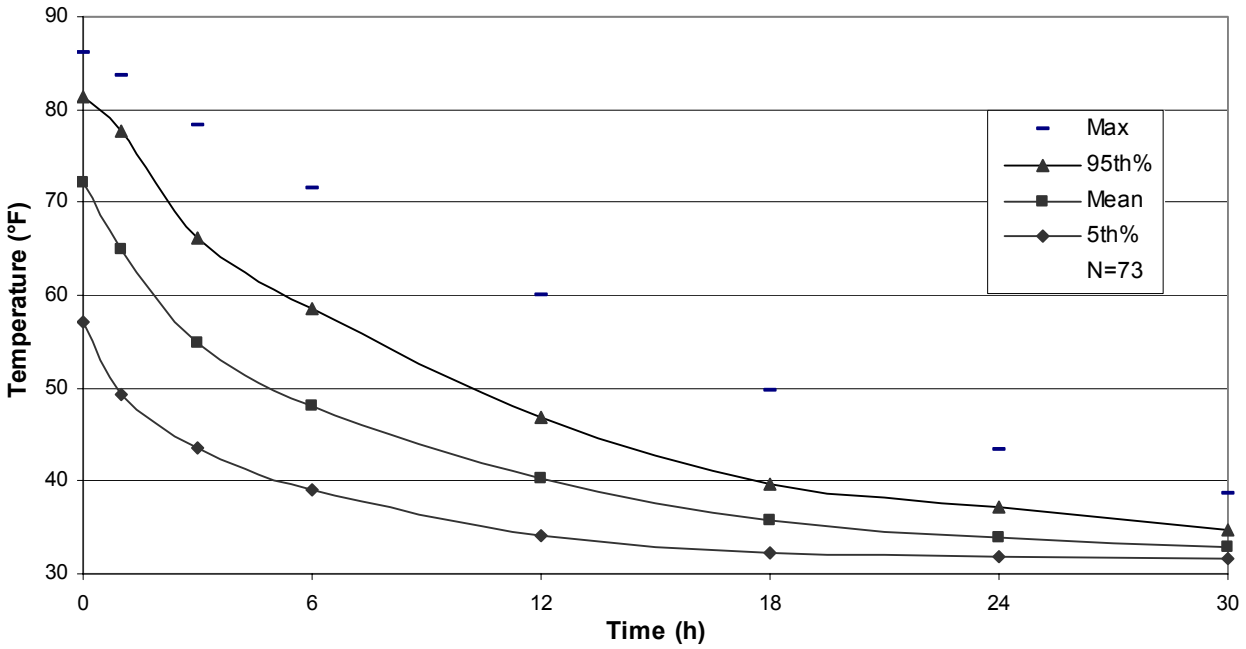


FIGURE 4. *Chilling rates of bigeye tuna (gilled and gutted) caught in the Hawaii deep-set longline fishery and stored in ice.*

The time required to chill the group of 73 G&G bigeye tuna to the temperature targets of 50 and 40°F was also calculated (Table 10). The mean time to 50°F was 5 h 23 min. Ninety-five percent of the fish were below 50°F within 10 h 12 min. The maximum time required to reach 50°F was 17 h 50 min. The mean time to 40°F was 11 h 57 min, 95% of the fish reached 40°F within 17 h 50 min and the maximum time to 40°F was 27 h 50

min. Although some of these fish exceeded the recommended critical limits, none of these fish exceeded the histamine defect action limit of 50 ppm.

TABLE 10. *Time to chill bigeye tuna (gilled and gutted) caught in the Hawaii deep-set longline fishery to core fish temperature targets of 50°F and 40°F in ice.*

| **Fish temperature target** | **Time (h:min) to reach temperature target** | | | | |
|---|---|---|---|---|---|
| | N | mean | SD | 95% | maximum |
| <50°F in <6 h | 73 | 5:23 | 3:23 | 10:12 | 17:50 |
| <40°F in <24 h | 73 | 11:57 | 4:47 | 17:50 | 27:50 |

The histamine testing results confirmed that all of these fish were safe for sale and consumption. Each of the 73 G&G bigeye tuna monitored on Hawaii longline fishing vessels during this study passed sensory examination. The mean histamine concentration was 1.02 ppm (SD 0.70) and the maximum value was 3.00 ppm.

The individual fish that required 17 h 50 min to chill to 50°F was a 158 lb G&G bigeye tuna. This fish was alive when brought on board, had an initial core temperature of 81.0°F, and reached 40°F in 27 h 20 min. After unloading, this fish passed sensory examination, received a grade of No. 1, and had a very low histamine content of 0.5 ppm.

The individual fish that required 27 h 50 min to chilled to 40°F was a 180 lb G&G bigeye tuna. This fish was alive when brought on board, had an initial core temperature of 80.5°F, and reached 50°F in 17 h 30 min. After unloading, this fish passed sensory examination, received a grade of No.1, and contained a 0.5 ppm histamine.

Both of these fish exceeded the recommended critical limits, but were both high in quality and low in histamine concentration and food safety risk.

**Comparison of chilling rates of whole and G&G bigeye tuna, caught by deep-set longline gear in Hawaii's longline fishery.**

Figure 5 compares the mean chilling rates of whole bigeye tuna with those that were gilled and gutted. Initial fish temperatures were similar for both groups. The mean chilling rate of the G&G fish was much greater than that of the whole fish. Lower body temperatures for the G&G fish were maintained through the initial 24 hours of cooling in ice.

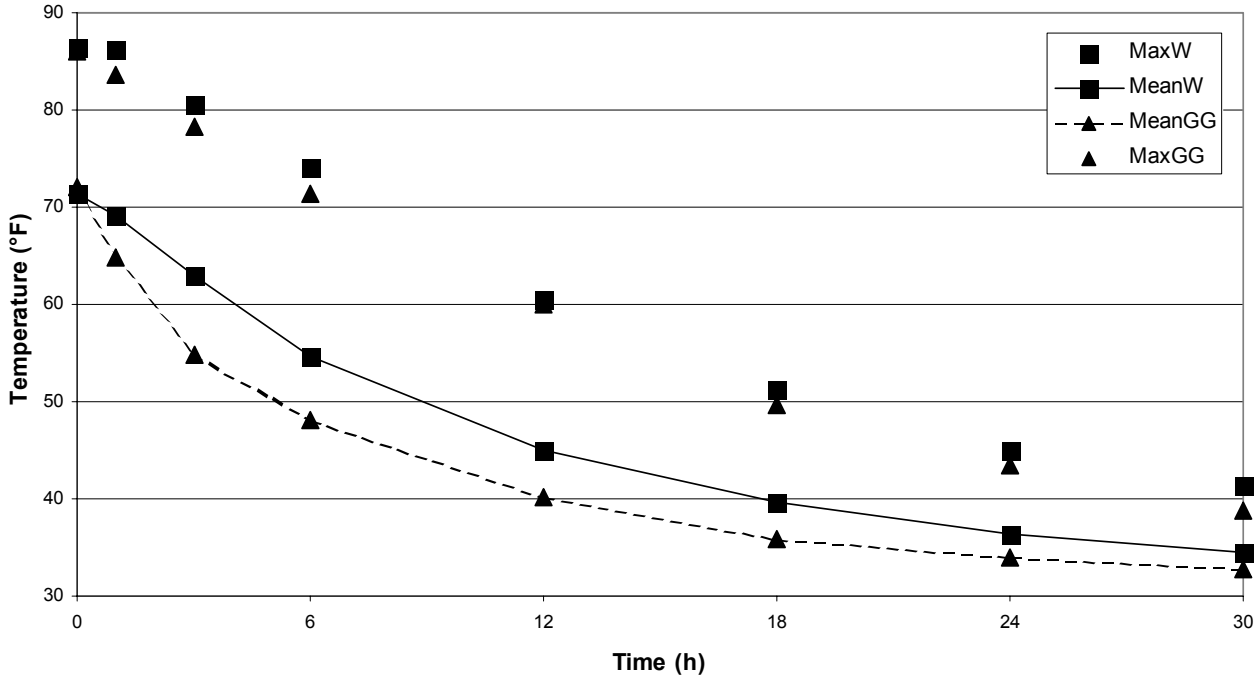


FIGURE 5. *Chilling rates of bigeye tuna (whole and gilled and gutted) caught in the Hawaii deep-set longline fishery and stored in ice.*

Table 11 compares some of the harvest and post-harvest factors affecting histamine risk for whole and G&G bigeye tuna caught by Hawaii deep-set longline gear.

TABLE 11. *Comparison of harvest and post-harvest factors and histamine concentration of whole and gilled and gutted bigeye tuna caught by Hawaii deep-set longline gear.*

| **Bigeye Tuna** | **Whole** N=91 mean (±) SD | **Gilled and Gutted** N=73 mean (±) SD | **Probability** |
|---|---|---|---|
| Fish Wt (lb) | 99.1 (±) 34.4 | 70.7 (±) 33.5 | 0.00000035 |
| Initial core temp (°F) | 71.5 (±) 7.9 | 72.1 (±) 7.8 | 0.592 |
| Hook soak time (h:min) | 16:47 (±) 3:49 | 15:15 (±) 2:54 | 0.005 |
| Time to 50°F (h:min) | 8:30 (±) 3:47 | 5:23 (±) 3:22 | 0.00000017 |
| Time to 40°F (h:min) | 17:08 (±) 5:48 | 11:57 (±) 4:47 | 0.000000006 |
| Total stored time (d) | 9.17 (±) 3.98 | 11.97 (±) 3.33 | 0.0000071 |
| Quality score ( 1 - 5 ) | 2.0 (±) 0.9 | 2.7 (±) 0.8 | 0.0000006 |
| Hx (ppm) | 0.92 (±) 1.16 | 1.02 (±) 0.70 | 0.521 |

Fish weight. The mean fish weight of the G&G bigeye tuna was significantly lower than whole bigeye tuna monitored in this study. Some of this difference results from the weight loss associated with the removal of the viscera and gills. However, the difference in mean fish weight between the two groups (99 lb for whole vs. 78 for G&G fish) is greater than the estimated 10% weight loss due to gilling and gutting. Both lower fish weight and packing of ice into the empty visceral and gill cavities for G&G fish contributed to the increased chilling rates and lower body temperatures.

Initial core temperature. There was no difference in mean initial core fish temperatures between whole (71.5°F) and G&G bigeye (72.1°F). As expected, the initial core temperature was not influenced by gilling and gutting the fish after being brought on board.

Hook soak time. Hook soak time was calculated as the time between the setting of the first hook and hauling of each fish on board during a longline set. Hook soak time is not influenced by whether or not fish are gilled and gutted or left whole, after the fish is brought on board. The mean hook soak time for whole bigeye tuna (16 h 47 min) was significantly greater ($P < 0.005$) than for the G&G fish (15 h 15 min) in this study, but is not considered to impact the other parameters for comparison of G&G and whole bigeye tuna. The difference in hook soak times is a function of the position of the hooks that caught fish during the observed longline sets.

Chilling time to 50°F. As expected, gilled and gutted fish chilled more rapidly than fish left whole. The mean time to reach 50°F was significantly greater ($P < 0.001$) for whole bigeye tuna (8 h 30 min) and G&G fish (5 h 23 min).

Chilling time to 40°F. The mean time to reach 40°F was also significantly greater ($P < 0.001$) for whole bigeye (17 h 8 min) than for G&G fish (11 h 57 min).

Total fish storage time. Total fish storage time would be expected to impact the quality and safety of fresh fish delivered by fishing vessels. Fish quality should to decline as iced storage time increases. Gilling and gutting should extend iced shelf life or the usable storage period for acceptable quality fish, by chilling fish more rapidly and by removing major sources of bacteria in the discarded viscera and gills. The total storage time was calculated from the time the fish was hauled on board until it was sampled during unloading. The mean storage time was significantly greater ($P < 0.001$) for the G&G fish (approx. 12 days), than for the fish left whole (approx. 9 days) in this study. This is a result of differences in when the fish were caught within longline sets and when the sets were made in relationship to the fishing trip and unloading date.

Quality scores. Fresh tuna quality grades range from No. 1 (highest quality) to No. 4 (lowest acceptable quality) to No. 5 (rejected for decomposition). The mean quality score of the 91 whole bigeye tuna monitored in this study was 2.0. The mean quality score of the 73 G&G bigeye tuna graded was significantly higher 2.7 ($P < 0.001$) reflecting the lower quality of these fish. While gilling and gutting significantly reduced the time required to chill these fish to 50°F and 40°F, the expected quality improvement was not observed after unloading. One possible explanation is the significantly longer storage time of the G&G fish on the vessel prior to unloading and associated negative impact on decomposition and quality of the fish determined by sensory examination and quality grading.

Histamine concentration. While gilling and gutting fish resulted in reduced chilling times for bigeye tuna, mean histamine concentrations for whole bigeye tuna (0.92 ppm), and G&G fish (1.02 ppm) were not significantly different. Mean histamine concentrations for both groups were well below the defect action limit, and none of the individual fish exceeded the defect action limit. These results showed that there was minimal overall histamine risk, and no difference between whole and G&G bigeye tuna when the fish were properly handled and iced using standard vessel handling practices in the Hawaii deep-set longline fishery.

**Chilling rate of whole yellowfin tuna caught by deep-set longline gear in Hawaii's longline fishery.**

Twelve (12) yellowfin tuna were monitored at sea during the study. The mean chilling rate for the yellowfin tuna is presented in Figure 6. The mean temperature was 52.1°F after 6 h with a maximum of 64.2°F. The mean temperature was 35.0°F after 24 h with a maximum of 37.7°F. Most of the yellowfin did not achieve the guideline of chilling to below 50°F within 6 h, but all fish were below 40°F after 24 h. The group of yellowfin tuna ranged from 40 to 119 lb with a mean weight of 85.6 lb (SD 23.4).

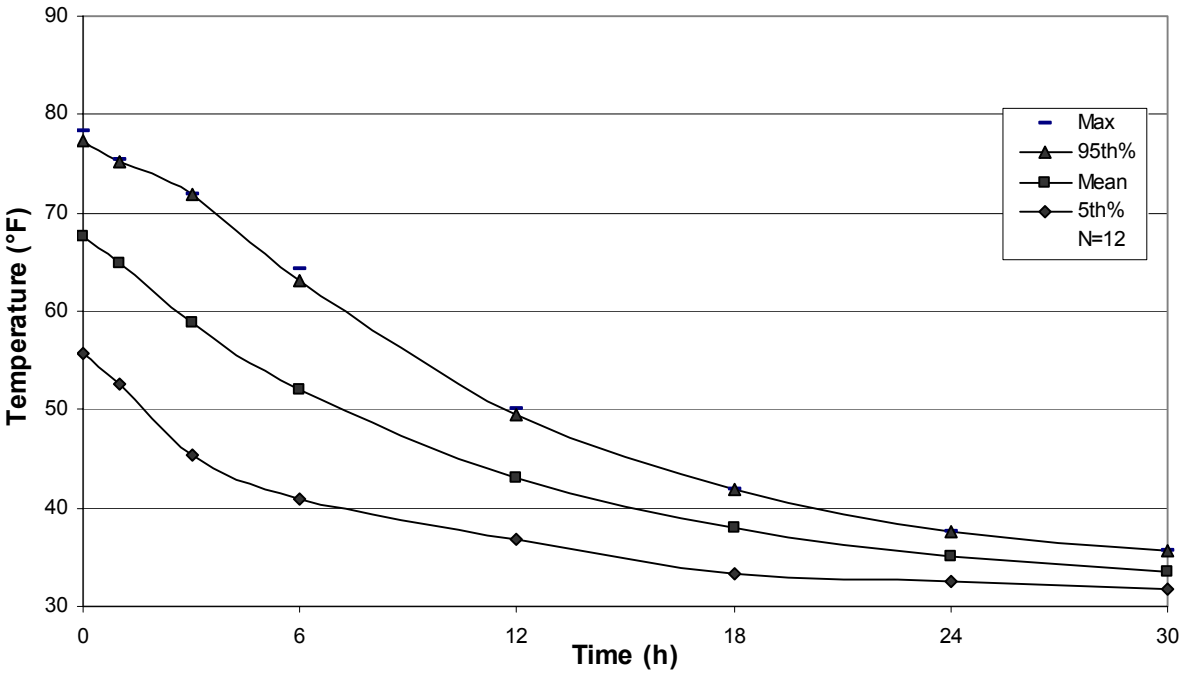


FIGURE 6. *Chilling rate of yellowfin tuna (whole) caught in the Hawaii deep-set longline fishery and stored in ice.*

The time required to chill the group of 12 yellowfin tuna to the FDA recommended critical limits for chilling fish to 50 and 40°F was also determined (Table 12). The mean time to 50°F was 7 h 2 min. Ninety-five percent of the fish were below 50°F within 11 h 48 min. The maximum time required to reach 50°F was 12 h 10 min. The mean time to 40°F was 14 h 23 min, 95% of the fish reached 40°F within 20 h 24 min and the maximum time recorded to reach 40°F was 20 h 30 min. Although many of these fish exceeded the recommended critical limit to 50°F, all of these fish were subsequently chilled to below 40°F within 24 h. None of these fish exceeded the histamine defect action limit.

TABLE 12. *Time to chill yellowfin tuna (whole) caught in the Hawaii deep-set longline fishery to core fish temperature targets of 50°F and 40°F in ice.*

| Fish temperature target | Time (h:min) to reach temperature target | | | | |
|---|---|---|---|---|---|
| | N | mean | SD | 95% | maximum |
| <50°F in <6 h | 12 | 7:02 | 4:21 | 11:48 | 12:10 |
| <40°F in <24 h | 12 | 14:23 | 5:26 | 20:24 | 20:30 |

The histamine testing results confirmed that all of these fish were safe for sale and consumption. Two of these 12 yellowfin tuna monitored on Hawaii longline fishing vessels during this study failed sensory examination. These fish had histamine concentrations of 2.1 and 1.5 ppm, each well below the defect action limit. The 10 yellowfin that passed sensory examination had a mean histamine concentration of 1.26 ppm (SD 1.48) and a maximum value of 4.1 ppm.

The individual fish that required 12 h 10 min to be chilled to 50°F was a 97 lb yellowfin tuna. This fish was dead when brought on board, had an initial core temperature of 80.8°F, and reached 40°F in 20 h 30 min. After unloading, this fish passed sensory examination, received a grade of No. 3, and had a low histamine concentration of 4.0 ppm.

**Discussion Obj. 1. Verify onboard fish handling procedures for controlling histamine accumulation.**

Is it necessary to chill fish to below 50°F within 6 hours of death to control histamine formation in Hawaii's longline fishery?

No. This study shows that many fish were hauled alive, with known times of death, and exceeded 6 hours to reach 50°F. However, none of these fish accumulated unsafe levels of histamine nor exceeded the defect action limit. This study also shows that not all fish are alive when they are retrieved from longline gear. For fish that die on the line, it is not possible to determine the time of death, nor start the calculation of chilling time at this event. This study verifies previous results (Kaneko, 2000) that determined there was no difference in mean histamine concentration between fish hauled alive and dead on the line in the Hawaii deep-set longline fishery. The time that a fish (dead or alive) is hauled on board the vessel is strongly recommended for practical use as the initial time to determine chilling temperature and time parameters.

Is it necessary to chill fish to below 40°F within 24 hours of death to control histamine formation in Hawaii's longline fishery?

No. Many of the fish monitored in this study exceeded this recommended time and temperature critical limit and did not accumulate unsafe levels of histamine nor exceed the defect action limit.

Is there evidence that gilled and gutted bigeye tuna should have different fish handling requirements from those left whole in the Hawaii longline fishery to control histamine?

No. The study did not find a difference in mean histamine concentration between G&G and whole bigeye tuna. Although chilling rates were shown to be more rapid for G&G fish, there was no significant difference in accumulation of histamine. Gilling and gutting at sea can be recommended for more rapid chilling for fish quality improvement as well as histamine control, but the time and temperature and histamine results do not support the substantial difference in the fish handling recommendations of the FDA described in Table 1 (FDA, 2001) between whole and G&G fish when applied to the Hawaii fishery.

**Conclusion Obj. 1 Verify onboard fish handling procedures for controlling histamine accumulation.**

The vessel research results show that the standard onboard fish handling practices used in the Hawaii deep-set longline fishery are not always capable of meeting the FDA fish handling guidance for critical limits for time and temperature to control histamine formation. Many fish in the study exceeded the critical limits of ≤ 50°F in ≤ 6 hours and ≤ 40°F in ≤ 24 hours.

However, histamine testing results showed that every fish that exceeded the critical limits to 50 and 40°F, accumulated histamine to levels well below the histamine defect action limit and was safe for consumption.

These results demonstrate that the onboard handling practices for chilling and storing pelagic histamine-forming fish caught in the Hawaii deep-set longline fishery are effective in controlling histamine formation.

Investigating exactly how much additional time can be considered safe for chilling fish to reach 50 and 40°F was not a goal of the this study and was not determined. However, this study included individual large bigeye tuna caught in the Hawaii deep-set longline fishery that required up to 19 h to reach 50°F and 34 h to reach 40°F, and still resulted in very low histamine levels and effective control of histamine formation.

**Results Obj. 2. Verification of sensory examination as a reliable histamine control measure in combination with effective onboard fish handling practices.**

A total of 482 mixed pelagic fish caught by Hawaii deep-set longline gear were evaluated after unloading for sensory indicators of decomposition and tested for histamine content (Table 13). These fish were sampled from the landings of 35 longline vessels and 38 different fishing trips. During the study period there were between 91 and 100 active longline vessels in Hawaii's fishery. From this sample, 366 fish passed sensory examination and 116 fish failed (reject due to odors of decomposition). It is important to note that this level of decomposed rejects does not reflect the actual occurrence in Hawaii longline fish landings. The occurrence of rejects was actually very low (<1%) within the fishing vessel landings, making repeated special and directed efforts necessary to obtain the 116 reject samples for histamine testing.

Not one fish that passed sensory examination exceeded the 50 ppm defect action limit. The mean histamine concentration of these acceptable fish was 1.06 ppm, with a maximum of 19.0 ppm. The mean histamine concentration of fish that failed sensory examination was 1.76 ppm. The single fish that exceeded the defect action limit contained 71.0 ppm histamine and failed sensory examination.

TABLE 13. *Sensory examination for decomposition and histamine concentration (ppm) of mixed pelagic fish caught by Hawaii deep-set gear and stored in ice.*

| Fish | Passed sensory examination | | | | | | Failed sensory examination | | | | | | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SPP* | | Weight (lb) | | Hx (ppm) | | | | Weight (lb) | | Hx (ppm) | | | Hx |
| | N | mean | SD | mean | SD | max | N | mean | SD | mean | SD | max | Diff |
| **BE** | 186 | 84.2 | 35.9 | 1.06 | 1.34 | 11.50 | 37 | 65.0 | 32.3 | 2.88 | 11.57 | 71.0 | 0.038 |
| **YF** | 55 | 87.7 | 32.4 | 1.19 | 1.20 | 6.31 | 21 | 59.3 | 26.8 | 1.11 | 2.17 | 10.40 | 0.827 |
| **AL** | 3 | 41.7 | 4.5 | 0.67 | 0.29 | 1 | 4 | 43.8 | 7.4 | 0.50 | 0.00 | 0.50 | 0.286 |
| **BM** | 27 | 280.2 | 156.3 | 0.70 | 0.42 | 2 | | | | | | | |
| **SM** | 8 | 64.4 | 16.4 | 0.88 | 0.88 | 3 | 1 | 70.0 | 0.0 | 0.50 | 0.00 | 0.50 | 0.699 |
| **MM** | 30 | 17.1 | 4.5 | 0.63 | 0.64 | 4.00 | 29 | 14.3 | 3.9 | 1.96 | 4.32 | 21.80 | 0.102 |
| **ON** | 3 | 57.3 | 0.6 | 0.50 | 0.00 | 0.50 | 8 | 39.8 | 12.8 | 0.50 | 0.00 | 0.50 | 1.000 |
| **WA** | 54 | 21.8 | 13.7 | 1.42 | 2.76 | 19 | 16 | 21.3 | 13.9 | 0.68 | 0.65 | 3.10 | 0.294 |
| **ALL** | 366 | 83.5 | 80.0 | 1.06 | 1.53 | 19 | 116 | 42.8 | 31.3 | 1.76 | 6.94 | 71.0 | 0.074 |

*AL = albacore; BE = bigeye; BM = pacific blue marlin; MM = mahimahi; ON = wahoo; SM = striped marlin; WA = escolar; YF = yellowfin

No significant difference in mean histamine concentrations was found between fish that passed and fish that failed sensory examination for decomposition at the 95% confidence level ($P<0.05$) for all mixed pelagic fish combined, and for individual species groups, mahimahi, escolar and yellowfin tuna. Few albacore, blue marlin, wahoo, and striped marlin were sampled. The mean histamine concentrations for each of the species sampled that passed or failed sensory examination were well below the defect action limit (50 ppm) and toxic levels.

There was a significant difference ($P <0.05$) in mean histamine concentration between bigeye tuna that passed and failed sensory examination. The 186 bigeye tuna that passed sensory examination had a mean histamine concentration of 1.06 ppm and a maximum of 11.5 ppm. The 37 bigeye tuna that failed sensory examination had a mean histamine concentration of 2.88 ppm and a maximum of 71.0 ppm for the one fish that exceeded the defect action limit.

While the mean histamine concentrations of fish that passed and fish that failed sensory examination were not significantly different, it is important to note that the only fish that exceeded the defect action limit, also failed sensory examination. Sensory examination methods identify and reject unacceptable quality fish (based on decomposition) and at the same time are effective in culling fish containing histamine levels above the defect action limit from the market in this fishery.

The 12 highest histamine concentrations.

Information on the fish with the twelve highest histamine concentrations from both the vessel monitoring and market sampling is presented in Table 14. The data from these fish are presented to determine potential causes for the higher histamine levels found in this study. The mean histamine concentration of these fish was 15.5 ppm (SD 18.2) with a range of 4.1 to 71.0 ppm. Only 1 fish exceeded the histamine defect action limit, but was below the concentration associated with toxicity. Five of these fish failed sensory examination, including the only fish that exceeded the defect action limit.

TABLE 14. *Top twelve (12) highest histamine concentration found in fish caught by Hawaii deep-set longline gear in this study.*

| Fish* | Weight (lb) | Type** | Quality Grade | Sensory Exam | Histamine (ppm) |
|---|---|---|---|---|---|
| Bigeye tuna | 79 | M | 5 | fail | 71.0 |
| Mahimahi | 17 | M | 5 | fail | 21.8 |
| Escolar | 41 | M | 3 | pass | 19.0 |
| Bigeye tuna | 89 | M | 4 | pass | 11.5 |
| Mahimahi | 14 | M | 5 | fail | 11.0 |
| Yellowfin tuna | 64 | M | 5 | fail | 10.4 |
| Bigeye tuna | 207 | V | 4 | pass | 9.0 |
| Bigeye tuna | 68 | M | 3 | pass | 8.5 |
| Escolar | 10 | M | 2 | pass | 8.0 |
| Yellowfin tuna | 85 | M | 3 | pass | 6.3 |
| Bigeye tuna | 38 | M | 5 | fail | 5.9 |
| Yellowfin tuna | 96 | V | 2 | pass | 4.1 |
| ALL | | | | | mean 15.5 (SD 18.2) |

*all fish were left whole, not gilled and gutted

**M = market sample from auction; V = vessel sample monitored at sea.

These 12 fish included 5 bigeye tuna, 3 yellowfin tuna, 2 mahimahi and 2 escolar. Ten of these 12 fish were collected from the market landings and onboard handling data were not collected (available). Only two of these fish were caught during the at sea vessel monitoring study when onboard handling data were collected.

The 207 lb bigeye tuna passed sensory examination, received a low quality grade of No. 4 and had a histamine concentration of 9.0 ppm after unloading. This fish was dead when hauled on board, had an initial core temperature of 57.4°F, and reached 50°F in 7 h 20 min and 40°F in 21 h.

The 96 lb yellowfin tuna passed sensory examination, received a quality grade of No. 2 and had a histamine concentration of 4.1 ppm after unloading. This fish was dead when hauled on board, had an initial core temperature of 75.9°F, and reached 50°F in 11 h 30 min and 40°F in 19 h.

**Discussion Obj. 2. Verification of sensory examination as a reliable histamine control measure in the Hawaii longline fishery.**

The previous study (Kaneko, 2000) investigating histamine controls in this fishery showed similar results. In that study all fish that exceeded the 50 ppm histamine defect action limit were from market sample fish rejected due to odors of decomposition (failing sensory examination). The mean histamine level of all 14 fish that exceeded the histamine defect action limit was 410 ppm (SD 632.5) with a maximum of 1,960 ppm and minimum of 53.3 ppm. Of the 14 high histamine fish found in that study, 10 were from swordfish fishing trips and 4 were from tuna trips.

In the current study, only 1 fish exceeded the 50 ppm defect action limit. The highest histamine concentration was 71.0 ppm, still well below the toxic concentration of greater than 200 to 500 ppm (FDA, 2001). The number of fish with high histamine levels, and the highest individual histamine concentrations tested were both lower in the current

study than for the previous study. These decreased histamine levels and occurrences are likely affected by a major change in the Hawaii longline fishery that occurred between the completion of the first study and the beginning of the current study. Also importantly, the vessel that produced the fish with the 3 highest histamine concentrations in the first study (453, 1,790 and 1,960 ppm) was no longer active in the fishery following the change in fishery management regulations in 2001.

Prior to 2001, Hawaii's longline fishery consisted of three distinct types of fishing strategies: 1) targeting bigeye tuna, 2) swordfish or 3) mixed tuna/swordfish. The strategies to target different fish corresponded to gear configurations and fishing methods that place hooks at deep, shallow or intermediate target water depth. New fishery management rules eliminated the shallow-set, swordfish-style and mixed tuna/swordfish-style longlining in Hawaii in mid-2001. Currently only deep-set tuna fishing methods and gear are permitted in Hawaii's longline fishery although a model swordfish fishery may be allowed in the future.

Swordfish longlining is characterized as a shallow-set, night-soak method with relatively lengthy fishing trips exceeding 30 to 45 days (Ito, et al., 1998). When the first histamine control study was performed, most of the decomposed fish rejected during the unloading were from the extended swordfish trips. Hawaii's tuna longline fishing method is a deep-set, day-soak method involving shorter trip lengths of 14 to 21 days. Longline vessels targeting tuna landed decomposed and rejected fish at a much lower level than vessels making swordfish trips during the first study.

**Conclusion Obj. 2. Verification of sensory examination as a reliable histamine control measure.**

The results from the histamine testing and sensory examinations performed in the current study and comparison with those obtained in the first study strongly suggest that sensory examination of the mixed pelagic fish caught by Hawaii deep-set longline gear is an effective seafood safety procedure to control the risk of fish containing high histamine concentration entering the market from fishing vessels.

**Results Obj. 3. Conduct training workshops for vessel operators.**

An opportunity to cooperate with NOAA Fisheries (Pacific Islands Regional Office, PIRO) became available to facilitate the training of the Hawaii-based longline fleet. PIRO conducts mandatory protected species workshops as part of the annual requirement for longline permit holders in Hawaii's pelagic longline fishery. The PI for this study was asked by the NOAA training coordinator to participate in these workshops and provide training for fishermen regarding onboard fish handling, quality control, seafood safety, histamine controls and fishermen's responsibilities under HACCP regulations.

Four workshops were conducted (August 27, September 3, 10, and 17, 2002) in Honolulu. One hundred and forty (140) Hawaii longline vessel operators and owners attended the 4 workshops sessions. The training sessions allowed for discussion of the cause and prevention of bacterial decomposition and histamine formation, current FDA guidance for histamine controls on fishing vessels, current efforts to develop and verify HACCP-based approaches for histamine control at sea for the Hawaii longline fishery and to solicit participation from fishermen with the research project.

A second training outreach effort to the Hawaii longline fleet was made. The PI provided an update on the status of HACCP compliance in Hawaii and the histamine research activities of this project during a second series of 4 workshops (September 26, October 3, 10 and 17, 2003).

**Conclusion Obj. 3. Conduct training workshops for vessel operators.**

Controlling histamine requires proper fish handling by fishermen at sea. Training for fishing vessel crews is essential to improve the control of histamine poisoning. Training must be practical and convey the tangible benefits of rapid chilling and proper cold storage of histamine-forming fish species in ways important to fishermen: improved fish quality, increased economic returns and the continued ability to sell fish. This is especially true in fresh tuna and swordfish fisheries where fish quality grades are important determinants of price (Bartram, et al., 1996). Fish handling training that improves understanding of histamine formation and promotes simple and effective control measures is needed as a continuing effort to help experienced and new fishermen recognize their critical role in the production of safe seafood and the control of histamine poisoning in the consuming public.

**Discussion: The Vessel Standard Operating Procedure (VSOP) Approach to controlling histamine formation on vessels in the Hawaii fresh tuna fishery.**

Histamine is a good example of a seafood safety problem that can be prevented through HACCP-based process controls. The critical limits of time and temperature control enable processors to maintain the safety of histamine-forming fish after they reach the first receiver and enter commercial processing, marketing and distribution channels. The greatest challenge is to establish an effective method for companies that receive and process susceptible fish to extend onboard HACCP controls to the fishing vessels where histamine formation must be controlled initially through proper fish handling and rapid chilling. These onboard procedures are beyond the receiving company's direct control, and are not inspected by the FDA.

The Histamine Testing Approach (FDA, 2001) is not practical and will not work for the Hawaii fresh tuna industry. Representative sampling and histamine testing has potential to detect histamine problems in fish landings only when the prevalence rate of histamine is high. Testing is not likely to detect individual fish with high histamine concentrations that occur at a very low frequency, especially in landings from hook and line fisheries such as longline and troll fisheries.

The Vessel Harvest Records Approach (FDA, 2001) is not likely to be entirely effective. There will always be a level of uncertainty regarding the accuracy of records produced by fishermen (and seafood processors). Fishermen are highly unlikely to incriminate themselves by providing evidence that critical limits were exceeded while at sea. This is especially true if the records required are perceived of as unnecessary, overly restrictive, and impractical to generate and merely add to the existing record-keeping burden imposed by government regulation.

Harvesting records will not make fish safe, but proper fish handling will. Histamine formation and accumulation on fishing vessels will be effectively controlled when fishermen understand that it is in their best interest to do so. Once this is recognized,

fishermen will adjust their fish handling practices and can adopt reasonable record-keeping requirements. The danger of requiring fishermen to produce additional records that are impractical or impossible to obtain, is to demonstrate a lack of understanding and respect for the realities and practicalities of fishing operations. This approach risks making the HACCP principles and regulations appear to be an illegitimate and unimportant paper exercise.

However, the control of histamine is a critical issue in the fresh tuna industry, and fishermen need to understand their responsibility in the control process. While the incidence of high histamine fish in the landings in the Hawaii longline fishery is low, fish mishandling, decomposed fish and histamine production can occur. Fishermen must be convinced that proper post-harvest fish handling practices are mutually beneficial to them and the companies that buy their fish. Proper fish handling on fishing vessels will not only control histamine formation, but will also improve fish quality and dockside value of the catch. This incentive will encourage fishermen to adjust and improve fish handling practices and simultaneously improve the control of histamine.

This adoption of improved and successful handling practices can be best accomplished through training and working directly with the fishermen. An effective training program will not alienate good operators and will focus on fishermen that do not fully understand the relationship between onboard fish handling, histamine accumulation, spoilage, the resulting fish quality and the value of the catch.

One of the corrective actions recommended by the FDA (2001) for companies receiving fish directly from fishing vessels when critical limits for histamine control are exceeded at receiving is to stop accepting fish from vessels that do not comply with the company's HACCP plan. This approach does not promote better understanding and working relationships between the producer and the buyer. This approach does not actively promote improved handling or histamine control. This action merely shifts the potential seafood safety hazards to other companies or buyers, some of which do not fall under FDA inspection. Direct sales from fishing vessels to the retail sector are occurring in Hawaii as a result of the FDA HACCP regulations. The fish from some fishermen, being unable to meet the critical time and temperature limits, especially for troll-caught fish, are entering the market through retailers that are not required to have HACCP Plans. This recommended corrective action has the unintended potential of increasing the risk of histamine poisoning to the consuming public.

An alternative approach to successfully improve histamine control and fish quality produced by the fleet is to require fish handling and seafood safety training for fishermen as a mandatory corrective action to be taken when vessels deliver excessive amounts of decomposed fish.

A unique opportunity for reliable histamine controls is presented by the practices of the Hawaii fresh tuna fishery, the nature of fishing operations, marketing system and the process control steps that exists after unloading. The overwhelming majority of fresh pelagic fish are marketed through an auction system, which requires 100% sensory examination of fish as they are inspected at the auction before marketing and distribution. The results of this study verify the effectiveness of the histamine control program of combining standard vessel handling practices with sensory examination for decomposition after unloading. This study supports a scientific and customized HACCP-

based approach to controlling histamine on vessels, tailored to the Hawaii fresh tuna industry.

The message to fishermen is simple. Chill fish quickly and properly and keep them cold. The best practice for fishermen to improve quality and control histamine is to ice the fish as soon possible, chill the fish rapidly and keep the fish properly iced while they are on the vessel. Removing whole fish from iced storage to routinely monitor temperatures and meet generic compliance recommendations is not only impractical, but also detrimental to reliable HACCP-based histamine control. The results of this study verify standard onboard handling practices on Hawaii longline vessels for controlling histamine production and associated food safety risk.

The message to companies buying these fish is also simple. Confirm fish core temperatures and perform sensory examination of the fish upon receipt and if a fish is decomposed, reject it. If the fish is acceptable, keep it cold. An effective HACCP and food safety control program for a company buying fish is to have a good working relationship with the fishermen and understand their operations at sea. Carefully evaluate fish landings for temperature, evidence of decomposition and mishandling, and fish quality. Then strengthen the program by providing meaningful feedback to the fishermen on the quality of the catch and training on proper fish handling practices.

An alternative HACCP-based approach is recommended for the Hawaii fresh tuna industry. The Vessel Standard Operating Procedure (VSOP) was introduced in the first study (Kaneko, 2000). The model VSOP (Figure 7) has been modified as a result of the current study findings and describes standard fish handling practices that have been shown to control histamine accumulation in the Hawaii fresh tuna fishery. The owner and captain of each vessel delivering fish must sign a VSOP document annually to be kept on file by the receiving company.

***COMPANY X, Inc.***
**VSOP**
**(Vessel Standard Operating Procedures)**
**Onboard Fish Handling for Histamine Control in the Hawaii Tuna Fishery.**

**Fishing Vessel:** _____

**Owner:** (print)_____ (sign)_____ (date _____)

**Captain:** (print)_____ (sign)_____ (date_____)

*This is to certify that the following standard operating procedures for onboard fish handling are practiced on this vessel. Any significant deviation from these practices will be noted and the receiver notified prior to unloading. This VSOP is submitted in cooperation with the receiver's HACCP Plan designed for the prevention of histamine accumulation in susceptible fish species.*

**Fishing Method:** longline / handline / troll

**Refrigeration Method:** ice slurry / ice alone / ice with refrigerated fish hold / RSW

**Sanitation Procedures:**

The fish holds are cleaned and sanitized after each trip using a dilute chlorine bleach solution (specifically, sodium hypochlorite solution of 100 ppm). Clean, new ice made from potable water is loaded into the fish hold at the start of each fishing trip. Fish holds are never used to store fuel. Fish holds are kept free of chemicals, lubricants and other potential contaminants used on board.

**Fish Handling Procedures:**

Fish are handled carefully to prevent bruising, kept clean and chilled rapidly in order to prevent the formation of histamine in susceptible fish species.

Fish are landed individually, gaffed and immediately stunned and then bled using gill and/or tail cuts. Fish are rinsed with clean seawater and placed immediately into ice, ice slurry or RSW. Fish may be kept whole, gilled and gutted or headed and gutted. This process takes no more than 60 minutes from the time of boarding.

The adequacy of icing (ice slurry or RSW) is checked and recorded at least twice per day during the remainder of the chilling and storage time. Fish are kept properly stored to maintain fish temperature <40ºF after the initial chilling period and until fish are unloaded. The objective is to chill fish to an internal temperature of ≤ 50°F within 6 hours and to ≤ 40º F within 24 hours after boarding.

These procedures have been shown to effectively control histamine formation to less than 50 ppm for susceptible fish caught in the Hawaii tuna fishery.

Figure 7. *Vessel Standard Operating Procedure (VSOP) document.*

The VSOP is supported by a Letter of Assurance (LOA) that is signed and submitted to the receiver each time the vessel delivers fish (Figure 8). The LOA certifies to the receiver that the VSOP was followed during the fishing trip and provides the basic information companies need about the fishing trip when they inspect the delivered catch. In addition, a record of twice daily ice checks should be received to document the adequacy of icing during the trip.

***COMPANY X, INC.***
**VESSEL HACCP RECORD**

**Letter of Assurance (LOA)**

*This certifies that the fish delivered to COMPANY X from the described fishing trip were handled in accordance with the current signed VSOP (vessel standard operating procedures) document on file at COMPANY X. This information is provided as a component of the COMPANY X VSOP program for compliance with US FDA HACCP regulations (21 CFR Part 123).*

**Vessel Name:** _____

**Fishing Method: (circle one)** (longline / handline / troll)

**Captain:** (print)_____(sign)_____(date)_____

**Trip details:**

Date trip started: _____ Time departed: _____

Date first fish caught: _____ Time fish caught: _____

Date last fish caught: _____ Time fish caught: _____

Date of unloading: _____ Time started: _____

Last fish caught: ( ) dead for more than 34 hours at delivery
( ) dead for more than 24 hours at delivery
(check one) ( ) dead for 12 to 24 hours at delivery
( ) dead for less than 12 hours at delivery

Were all fish iced within 60 minutes of hauling on board? (yes / no)

**Cooling Methods:**

Tons or lbs. of ice at start of trip: _____

Icemaker capacity: _____/day

Refrigerated hold? (circle) (yes / no)

Tons or pounds of fish: _____ total estimate

*To be completed by COMPANY X:

Icing adequate at time of unloading? (yes / no)

VSOP on file at COMPANY X? (yes / no)

Icing Records indicate proper icing? (yes / no)

Signature of COMPANY X staff: _____

Figure 8. *Letter of Assurance (LOA) of compliance with VSOP.*

The HACCP Plan of the receiving company would require the following,

1. Confirmation of a current VSOP on file, signed by the captain.
2. Receiving an LOA associated with the fishing trip that produced the catch.
3. Receiving a record of Daily Ice Checks made during the trip.
4. Fish temperature checks on 2 fish per ton in the lot.
5. Conduct sensory examination on 100% of the histamine-forming fish in the lot.

Critical Limits (CL) would include,

1. Presence of a current VSOP on file, signed by the owner and captain.
2. Presence of a completed LOA indicating proper handling signed by the captain.
3. Presence of a record of Daily Ice Checks (at least twice per day) indicating that fish were adequately iced during the trip.
4. All fish temperatures must be below 40°F for fish that are received more than 34 hours after being brought on board.
5. No more than 5% rejected by sensory examination for decomposition in the lot of histamine-forming fish by weight.

Corrective Actions (CA) would include,

1. Contact the captain to update the VSOP, or test the lot for histamine, or reject the lot.
2. Contact the captain to complete the LOA, or test the lot for histamine, or reject the lot.
3. Contact the captain to obtain the record of Daily Ice Checks, or if records are incomplete or do not indicate proper ice storage, test for histamine, or reject the lot.
4. Check the temperature of all fish in the lot. Reject all fish that exceed 40°F if more than 34 hours after being brought on board, and require a seafood safety training session before the beginning of the next fishing trip.
5. Cull and dispose of all rejects, and require a seafood safety training session before the beginning of the next fishing trip.

**Conclusion: Verification of a HACCP-based strategy for the control of histamine in the Hawaii Fresh Tuna Fishery.**

The FDA's recommended critical control limits (FDA, 2001) for time to ≤ 50°F and ≤ 40°F provide good guidance for companies to develop HACCP Plans for the control of histamine formation on fishing vessels. When possible, companies should implement these recommended critical limits to control histamine accumulation in the fish they receive. For those companies that are unable to achieve these critical limits, studies should be conducted to determine critical limits that provide an equivalent level of histamine control that are appropriate for their fisheries, fishing methods and marketing systems.

In order to establish critical limits, study components should include the fishing environmental factors, and the fishing gear and operations. The species and size composition of the catch should also be evaluated to demonstrate the effectiveness of

the histamine control processes and alternative critical limits. Such a study should utilize a HACCP approach towards controlling histamine formation specific to, and within the context of, the fishing and processing setting of the assessed fishery. Research activities need to verify the effectiveness of the alternative critical limits or HACCP approach. This thorough approach is consistent with the principles of HACCP and the FDA seafood HACCP regulation.

It is unlikely that determining critical limits for chilling rates (times and temperatures) for only two size classes of fish, such as tuna less than 20 lb and those greater (described in Table 1) will be adequate. This is especially true for pelagic fish that can weigh over 800 lb.

Establishing critical limits of time and temperature to control histamine must be practical and usable. They will need to consider the effects of fish species, size classes, status of fish landed on board (alive or dead), onboard handling procedures (gilled and gutted or not), fishing method, fishery environment and other variables on the risk of histamine formation. Determining safe, reliable and universally effective fish handling limits for all fisheries may be complex and extremely difficult. The complexity and difficulty may require an individual fishery to demonstrate safe handling in their fishery setting.

The potential effects of these variables have been recognized in the Hawaii longline tuna fishery. Multiple research studies have been conducted that demonstrate safe fish handling on board vessels and effective histamine control, but exact critical limits have not been determined.

Results from these studies show that high temperature abuse (above 83°F) of fish caught on Hawaii deep-set longline vessels using standard vessel operating procedures is unlikely to occur. These fish handling practices focus on immediate and rapid chilling, and result in reliable control of histamine formation and food safety risk.

These results show that sensory examination of the mixed pelagic fish caught by Hawaii deep-set longline gear on vessels using standard vessel operating procedures to handle and chill the catch is an effective seafood safety program to control the entry of fish containing high histamine concentration from fishing vessels to the market.

B. If significant problems developed which resulted in less than satisfactory results, they should be addressed.

Reliance on the captains and crew of commercial trolling boats to record fishing data and properly implant the temperature data loggers proved to be a less than satisfactory project design. This impacted the investigation of troll-caught blue marlin.

<u>Troll-caught Pacific Blue Marlin.</u>

Commercial trollers in Hawaii produce histamine-forming fish including mahimahi, wahoo, blue marlin, striped marlin, yellowfin tuna, and skipjack. Large blue marlin present the greatest challenge for vessel crews to chill these fish to below 40°F within 24 hours. Fishing trips on Hawaii trolling boats are single day trips. The fish are often delivered to the market in less than 24 hours post-capture. Many of these fish, especially the larger individuals, are still in their initial chilling phase when unloaded and delivered.

Captains of commercial trolling boats on Oahu that participated in the study were trained on how to record the required data and how to implant the temperature loggers. Captains were issued sets of temperature loggers and data sheets to carry with them on fishing trips and to use them when a blue marlin was caught. Difficulties arose during the study. The catch rate of blue marlin on Oahu was poor for the trolling vessels that participated in the study. Captains carried temperature loggers for months without catching a blue marlin. When the few blue marlin were caught, problems with temperature logger placement were encountered and confirmed at the time the loggers were retrieved from the fish. The main problem was that the stainless steel temperature probes were not placed in the deep muscle layers near the spine, but ended up in the shallow muscle layers nearer to the skin. This problem resulted in unrepresentative cooling rates expected for deep core muscle.

Data from the longline vessel studies provided some information on chilling rates for a limited number of blue marlin. The temperature profiles of 4 blue marlin were monitored on longline vessels during this study and are presented in Table 15. The catch rate of blue marlin in Hawaii's deep-set longline fishery is relatively low because the depth of the gear is outside of the common vertical distribution of the blue marlin. Two of these fish (300 and 166 lb) exceeded the recommended critical limit of 6 h to 50°F, but were chilled to below 40°F within the 24 h and both contained very low histamine levels (0.5 ppm) at the time the fish were sampled after unloading.

TABLE 15: *Chilling rate and histamine concentration of Pacific Blue Marlin caught in the Hawaii deep-set longline fishery and stored in ice.*

| Gear type* | Weight (lb) | Live or Dead | Initial Core Temp (°F) | Temp after 6 hours (°F) | Temp after 24 hours (°F) | Time to 50°F (h:min) | Time to 40°F (h:min) | Hist (ppm) |
|---|---|---|---|---|---|---|---|---|
| LL | 300 | Dead | 70.8 | 62.8 | 38.3 | 12:50 | 21:30 | 0.5 |
| LL | 166 | Live | 78.7 | 59.9 | 35.9 | 10:30 | 18:30 | 0.5 |
| LL | 107 | Dead | 83.0 | 50.1 | 32.8 | 6:10 | 10:30 | 0.5 |
| LL | 47 | Dead | 68.8 | 41.4 | 31.9 | 3:20 | 6:40 | 0.5 |

*TL = trolling gear, LL = deep-set longline gear

An alternative approach was selected to collect time and temperature data from 9 troll-caught blue marlin. These fish were unloaded and received from commercial trollers before the fish were chilled to below 40°F. Information on the fishing trip was collected from the fishing boat captain, the fish were weighed, an initial core temperature was taken and the fish were iced. A second temperature was taken some time later and recorded. The data set includes the time the fish was brought on board (provided by the fisherman), an initial receiving temperature (and lapsed time) and a second temperature (and lapsed time) after the fish were further chilled. A decision was then made whether to sell, reject or test the fish for histamine depending on whether or not the critical limit of 24 h to below 40°F was met.

The time and temperature data collected were not continuous, and the exact time when fish were chilled to 50 and 40°F was not known. Instead, an estimation of these parameters was calculated. Using the time the fish was brought on board as time zero (troll caught fish are alive when retrieved to the boat), the lapsed times for the first and second core temperature measurements were recorded and individual graphs of the

cooling rates were generated. When the first and second temperatures straddled 50°F and 40°F (>50°F and <40°F), the cooling rate was calculated as a straight-line, linear function. Cooling rates are not linear and using a linear approach produced an over-estimation (conservative towards meeting the recommended critical limits) of the actual time to 50°F and time to 40°F. These data are presented in Table 16.

TABLE 16: *Estimated chilling time and histamine concentration of Pacific Blue Marlin caught by trolling gear in Hawaii and stored in ice.*

| | Weight (lb) | 1st Temp (°F) | Lapsed* time (h:min) | 2nd Temp (°F) | Lapsed* time (h:min) | Est time to 50° F (h:min) | Est time to 40° F (h:min) | Histamine (ppm) |
|---|---|---|---|---|---|---|---|---|
| | 811 | 53.6 | 19:30 | 36.0 | 63:26 | 28:00 | 54:00 | 0.50 |
| | 453 | 55.2 | 14:11 | 37.8 | 39:24 | 21:00 | 35:00 | 0.50 |
| | 292 | 44.1 | 26:50 | 38.6 | 40:26 | N/A | 37:00 | 1.02 |
| | 255 | 47.5 | 20:53 | 38.2 | 30:15 | N/A | 28:15 | 1.80 |
| | 240 | 46.3 | 19:30 | 38.1 | 32:20 | N/A | 30:30 | 0.50 |
| | 144 | 42.6 | 21:56 | 34.0 | 45:05 | N/A | 28:50 | 0.50 |
| | 140 | 51.3 | 19:17 | 38.0 | 40:30 | 21:00 | 37:00 | 0.50 |
| | 138 | 43.8 | 24:45 | 35.8 | 39:01 | N/A | 31:30 | 0.50 |
| | 132 | 49.7 | 14:17 | 39.6 | 25:46 | 14:00 | 25:00 | 12.6 |
| mean | 289.4 | | | | | 21:00 | 34:07 | 2.05 |
| SD | 221.8 | | | | | 5:42 | 8:30 | 3.98 |
| N | 9 | | | | | 4 | 9 | 9 |

*Lapsed time = time passed since fish was brought on board.

The 9 troll-caught blue marlin were relatively large and ranged from 132 to 811 lb (whole weight) with a mean weight of 289.4 lb (SD 221.8). They were estimated to have greatly exceeded the 6 h to below 50°F critical limit. When possible, estimated times to reach 50°F ranged from 14 to 28 h with a mean of 21 h (SD 5 h 42 min). The time to reach 40°F exceeded 24 h and ranged from 25 to 54 h with a mean of 34 h 7 min (SD 8 h 30 min).

Even after exceeding the recommended critical limits, all of these fish passed sensory examination. They were all tested for histamine and had levels below the defect action limit, and were determined to be safe for consumption. The range of histamine levels was 0.5 ppm to 12.6 ppm with a mean histamine concentration of 2.05 ppm (SD 3.98).

C. Description of need, if any, for additional work.

Determining the upper time and temperature limits of safe fish handling and histamine controls for troll-caught histamine-forming fish requires additional investigation. The troll fishery in Hawaii produces the highest quality mahimahi and wahoo available in the Hawaii market. These boats are capable of rapid chilling and proper temperature control of the catch during day trips. However, when large fish such as Pacific blue marlin or yellowfin tuna are caught, these boats face limitations on the size of the fish hold and difficulty meeting FDA guidelines for chilling rates to control histamine. Limited testing indicates that, as in the longline fishery, the critical limits for time to ≤ 50°F in ≤ 6 hours and ≤ 40°F in ≤ 24 hours after death can be extended for troll-caught fish. Additional examination of safe fish handling limits for troll-caught fish is needed.

## VII. Evaluation:

A. Describe:

1. Were the goals and objectives attained? How? If not why?

Objective 1. Verify onboard fish handling procedures for controlling histamine accumulation.

Yes. Onboard fish handling practices, fish chilling rates and the resulting histamine concentrations were evaluated for 231 histamine-forming fish caught by deep-set longline gear in Hawaii's tuna fishery.

Objective 2. Verify the relationship between odors of decomposition and histamine accumulation.

Yes. A total of 482 histamine-forming fish were subjected to sensory examination for decomposition and tested for histamine. Sensory examination was successful in culling 100% of the high histamine fish in this study and the previous study of this fishery (Kaneko, 2000). The 15 fish in the two studies that exceeded 50-ppm histamine (the defect action limit) were first rejected because of odors of decomposition.

Objective 3. Conduct training workshops for vessel operators.

Yes. Eight workshops were conducted as an outreach effort to the Hawaii longline fishery. A total of 140 longline vessel owners and captains attended the workshops. Most of the Hawaii longline fishery limited entry permit holders were represented in the workshops.

3. Were modifications made to the goals and objectives?

No.

B. Dissemination of Project Results.

Copies of the final report for this project will be shared with the NOAA Pacific Island Regional Office, NOAA Pacific Islands Science Center, the United Fishing Agency Ltd., Hawaii Longline Association, the Western Pacific Regional Fisheries Management Council, and the National Sea Grant Project team (Training and Education in Support of Effective Controls of Scombroid Poisoning).

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