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DEVELOPMENT OF HIGH LEVELS OF HISTAMINE IN ATLANTIC MACKEREL (*Scomber scombrus* Linnaeus): An examination of some factors affecting histamine formation in the mackerel

by

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CONTENTS

Abstract v

Resume vi

Introduction 1

Materials and Methods 2

Bacterial Culture 2

Fish Samples 3

Histamine Analytical Procedure 3

Histamine Accumulation Trials 4

Determination of the Effects of Icing, Gutting and Contamination with Proteus morganii on Histamine Accumulation 4

Effect of Brine Treatment on the Accumulation of Histamine 5

Effect of Salt Curing (Pickling) on the Accumulation of Histamine in Fish Contaminated with Proteus morganii and Incubated for 24 hours 5

Results 5

Effect of Icing 5
Effect of Gutting 5
Effect of Proteus morganii contamination 6
Effect of Brine Treatment 6
Effect of Salt Pickling 6

Discussion 6

Conclusions 8

Acknowledgements 9

Bibliography 9

Table 1. Histamine accumulation in fresh, round mackerel at room vs. ice temperatures, and with natural bacteria vs. contaminated with Proteus morganii 11

Table 2. Histamine accumulation in fresh, gutted mackerel at room vs. ice temperatures, and with natural bacteria vs. contaminated with Proteus morganii 12

Table 3. Histamine formation in gutted small mackerel after brief exposure to brine 13

Table 4. Histamine accumulation in mackerel butterfly fillets exposed to Proteus morganii 24 hours at room-temperature (20 C) prior to salting 14

Figure 1. Comparison of the histamine accumulations achieved in round (a and b) and gutted (c and d) mackerel with natural bacterial contamination (a and c) and after contamination with Proteus morganii (b and d) held for the indicated times at ice or at room temperatures 15

M.W. Gilgan. 1987. DEVELOPMENT OF HIGH LEVELS OF HISTAMINE IN ATLANTIC MACKEREL (Scomber scombrus Linnaeus): An examination of some factors affecting histamine formation in the mackerel

ABSTRACT

An experiment was designed to determine the rate and degree of accumulation of histamine in very good quality mackerel under several conditions to which the mackerel might intentionally or otherwise be exposed to in the fishing or processing operation. The principal factors examined were icing, gutting and exposure to histamine-forming bacteria. Icing essentially prevented histamine formation even after contamination with histamine-forming bacteria. Gutting had essentially no effect but may have slightly improved keeping at 20 C. Exposure to histamine-forming bacteria accelerated the onset of the accumulation of histamine but the native bacteria of the mackerel were about as effective as the introduced, histamine-forming bacteria at causing the accumulation of histamine. The presence of a small amount of salt does not inhibit histamine formaion by either natural or the introduced histamine-former, Proteus morganii. On the other hand salt pickling apparently stops enzymic activity sufficiently quickly to prevent the accumulation of histamine in fish contaminated with histamine-forming bacteria.

Résumé

Une expérience a été conçue pour déterminer la vitesse et le dégré d'accumulation de 1'histamine dans du maquereau bleu d'excellente qualité dans diverses conditions auxquelles pourrait etre exposé, intentionnellement ou autrement, ce poisson au cours des opérations de pêche ou de transformation. Parmi les principaux facteurs étudies figurent la mise en glace, 1'éviscération et 1'exposition a des bactéries productrices d'histamine. La mise en glace prévenait essentiellement la formation de l'histamine, même aprbs contamination par des bactéries productrices d'histamine. L'éviscération n'avait pratiquement aucun effet, sauf qu'elle pouvait peur-être améliorer légérment la conservation du poisson à 2Ø C. L'exposition a des bactéries productrices d'histamine hâtait le début de 1'accumulation de >'histamine, mais les bactéries indigènes du maquereau bleu présentaient une efficacité presque égale a celle des bactéries productrices d'histamines introduites comme cause de l'accumulation de l'histamine. La présence d'une petite quantité de sel n'inhibait pas la formation de l'histamine par la bactérie Proteus morganii productrice d'histamine, qu'elle soit d'origine naturelle ou introduite. Par ailleurs, le saumurage arrêterait l'activitié enzymatique suffisamment tôt pour empêcher l'accumulation de l'histamine dans le poisson contaminé par des bacteries productrices d'histamine.

INTRODUCTION

Members of the Mackerel family of fish have been known for some time to develop a peculiar type of toxicity. The sickness which develops from eating fish with this type of toxicity is referred to as scombroid poisoning. Scombroid poisoning has many of the symptoms associated with a histamine reaction and is now generally.attributed to histamine poisoning, although this is not universally accepted (1). The reported incidences of such poisonings and the general features of the problem have been well reviewed by several authors. One of the most complete and recent reviews is that of Arnold and Brown (1).

Histamine is essentially a mammalian hormone. It is a not well understood intermediary in many allergic reactions such as hay fever. The effects of histamine can be counteracted through the use of drugs referred to as antihistamines. Antihistamines have been reported to be effective at counteracting the effects of scombroid poisoning (2). This fact is the primary reason for considering the illness to be histamine poisoning.

Histamine is formed in living things by the action of an enzyme on the amino acid histidine. The enzyme is responsible for the removal of the acid function of the amino acid, a carboxylic acid group, and hence is called histidine decarboxylase. For the enzyme to be active it is necessary that the histidine be free and in solution, not bound in protein as it usually is. The enzyme is normally found at very low activity levels in animals. Some bacteria on the other hand, have been found to produce considerable histidine decarboxylase activity. Hence when these bacteria are exposed to free histidine they can rapidly convert it to histamine. One such bacterium which has been commonly associated with histamine formation in fish (3,4) and which is a very active histamine former, is Proteus morganii.

For reasons that are not yet understood, fish of the Mackerel family, the Scombroids, may have very high levels of free histidine in their blood and tissues (5). When these fish are exposed to bacteria capable of producing the histidine decarboxylase, histamine may be formed at a rate much greater than that of the general spoilage of the fish. Therefore the fish may accumulate very high levels of histamine without acquiring the obvious characteristics of spoiled fish. While as alluded to earlier the problem is not a simple one, the accumulated histamine can be toxic if the fish are eaten. This is scombroid poisoning.

There is no good agreement in the world as to what level of histamine accumulation represents the toxic level. As with most factors involved with human diet the problem is not a simple one

to establish due to the great variability of human sensitivity and diet and to the possible use of medications which can increase normal sensivity. The most generally accepted level for the accumulation of histamine in fish or fish products is 10 mg % (meaning 10 mg of free histamine per 100 g sample). Beyond that level the fish is considered to have spoiled even though it may not be obviously toxic. The level most generally accepted as toxic is 100 mg % (1). However, it is not unusual to hear of toxic reactions, which appear to be histamine poisonings, to much lower concentrations.

Several methods have been developed for the chemical determination of histamine in fish and fish products (6). These are essentially all adaptations of sensitive clinical methods. Most are based on the measurement of the fluorescent product of the quite specific reaction of histamine with o-phthalaldehyde. All are capable of meauring histamine with reasonable precision. Modern methods based on the liquid chromatograph (e.g., 7) are beginning to appear and may in time replace the older "wet chemistry" methods.

While the formation of histamine in mackerel has previously been studied, I wished to determine the effect of icing, gutting and exposure to a known histamine forming bacterium, Proteus morganii, on a single lot of v'ery fresh, good quality mackerel so that the effects of the treatments would be directly comparable. A small experiment was conducted to determine the effect of a uniform exposure of mackerel from the same lot to brine with and without prior exposure to the histamine forming bacterium. Similarly I wanted to determine the effect of salt curing on the formation of histamine in mackerel on which the histamine forming bacterium has been allowed to grow briefly prior to salting.

MATERIALS AND METHODS

BACTERIAL CULTURE

A culture of Proteus morganii was obtained from the National Collections of Industrial and Marine Bacteria, Torry Research Station, Aberdeen, Scotland, U.K. (ampule 235, prepared 11 jun. 1974). The culture was revived at this laboratory by a microbiologist and maintained on agar slants. One week prior to use for experimental contamination of the fish, cultures were transferred to tryptone broth at 27 C.

To ensure that the culture was able to decarboxylate histidine, sterile tryptone broth tubes containing 5, 0.5, 0.05 and 0.005 mg histidine/mL were innoculated with samples of the actively growing bacteria and incubated overnight at 27 C.

Aliquots of the broth (0.5 mL) were then transferred to an equal volume of methanol. Samples were spotted as 2 mm zones on an activated (120 C for 1 hr) thin-layer chromatography plate (E. Merck, Kieselgel 60 F-254, 5x10 cm) and run in acetone/ conc. ammonia (20:1)(13) for 5 cm (origin to front). The culture extracts were compared with standard zones of histidine and histamine after development with ninhydrin spray (300 mg ninhydrin in 100 mL 3 % glac. acetic acid in n-butanol).

FISH SAMPLES

The mackerel were obtained from a trap to avoid net damage and to ensure that the fish were alive when transferred to the vessel. They were placed immediately on ice in an insulated container on board the fishing vessel. The container was transferred to a truck at the shore and transported to the laboratory. On arrival at the laboratory the fish were held in the same insulated container with an ample ice supply until the following morning (10 hr). The fish were then distributed to iced containers in numbers appropriate for the different sections of the experiment. All the fish were in excellent condition. The animals used for the main trial (tables 1 and 2) were of very uniform size, approximately 1 kg. The animals for the brine treatment and salt curing trials were of less uniform size, 0.5 to 1 kg.

HISTAMINE ANALYTICAL PROCEDURE

The detailed analytical procedure will be presented in a separate report. In brief the procedure used was an adaptation of that published by Taylor et al. 1978 (8), which is itself an adaptation of the orginial Shore (9) procedure.

The fish samples were filleted while partially frozen, skinned and the combined fillets homogenized in a food processor (General Electric, model PR10B) until it formed an even paste. A sample of the paste was then packed in a 50 mL plastic sample cup and stored in the refrigerator (ca. 6 C) if the sample was to be extracted that day or frozen for subsequent extraction. The sample was thoroughly remixed prior to weighing out 10.00 g. The weighed sample was further blended in ca. 50 mL methanol with a Polytron blender to an uniform suspension (1 min.). The suspension was transferred quantitatively to 100 mL volumetric flask diluted to near 100 mL and incubated at 60 C for 1 hr. When cooled to room temperature the suspension was volumed to 100.00 mL. A sample was transferred to a centrifuge tube, centrifuged and the clear supernatant fluid stored at -30 C in a glass scintillation vial with a tight sealing cap.

For analysis the methanol extract was rewarmed to room temperature, and remixed. All estimates were determined from the

analysis of duplicate aliquots. An appropriate replicate aliquot (0.200 mL) was transferred to a solution of 1 N sodium hydroxide saturated with sodium chloride and potassium sulfate. The alkali solution was extracted with an equal volume of water-saturated n-butanol and an aliquot of the n-butanol extract extracted with an equal volume dilute hydrochloric acid (0.1 N). An aliquot of the HCl extract was made alkaline with 1 N NaOH and reacted for precisely 4 min ,at room temperature with o-phthalaldehyde. The reaction was stopped by the timed addition of 3 N HCl. Appropriate standards (0.05 to 1.0 ug histamine) and blanks were prepared at the same time. After ca. 15 min at room temperature the fluorescent intensity of each sample was determined at an excitation wavelength of 360 nm and an emission wavelength of 450 nm with a Turner model 430 spectroflurometer. The histamine content of the samples was estimated from standard curves manually or with a programmable calculator by regression analysis. Spike recovery estimates were used to correct histamine estimates for recovery losses. For quality control purposes a check sample was included in each set of analyses. If the check sample value obtained was significantly at variance from the normal the whole analysis was repeated.

HISTAMINE ACCUMULATION TRIALS

DETERMINATION OF THE EFFECTS OF ICING, GUTTING AND CONTAMINATION WITH Proteus morganii ON HISTAMINE ACCUMULATION

The fresh, round mackerel were distributed to containers of ice as mentioned above in numbers appropriate to the experimental section. Each sample normally consisted of five fish. The group for each variable was sampled on each of day 1, 2, 4 and 8. Day 0 samples (controls) were reserved for each major grouping. After the groups were established, the fish for the gutted groups were then gutted, the heads and gills removed, the fish washed and returned to fresh ice in their assigned containers. When everything was ready all samples not to be treated with Proteus morganii were distributed to iced containers and transferred to a cold room (6 C), or waxed boxes and transferred to a fume cupboard (for room temperature). The samples to be treated with Proteus morganii were then dipped in a suspension of day old Proteus morganii cultures (4x5 mL) in tryptone broth in saline (0.85% sodium chloride in distilled water) and placed in appropriate containers for iced or room temperature holding and stored as above. The Proteus morganii contaminated samples were always handled last to minimize the chances of accidental contamination by the bacterium. The control samples (see tables 1 and 2) were now placed in a plate freezer, frozen and stored at -20 C until they could be processed for extraction. On the appropriate days samples of each category (see tables 1 and 2) were removed and immediately frozen.

EFFECT OF BRINE TREATMENT ON THE ACCUMULATION OF HISTAMINE

Gutted, headless mackerel (fifteen) were dipped in a brine (10% w/v NaCl in water) and stored in waxed boxes in a fume cupboard. Unselected animals (ten) from the same group of mackerel were dipped in the Proteus morganii suspension and then in the brine and stored as with the contaminated fish. Since the animals were not different from those of the main experiment except in size, no time zero samples was taken as the control for the main experiment would suffice for these animals also. When the samples were taken for the indicated categories on the specified days (see table 3) they were immediately frozen.

EFFECT OF SALT CURING (PICKLING) ON THE ACCUMULATION OF HISTAMINE IN FISH CONTAMINATED WITH Proteus morganii AND INCUBATED FOR 24 HOURS

Round mackerel (ten) were dipped in the Proteus morganii suspension and held in a waxed box at room temperature as described above for 24 hours. The fish were then split along the spine, gutted and placed in dry salt in a manner usual for pickling. They were held covered, so as to exclude air from the fish, at 6 C in a plastic bucket for approximately one month then sampled and extracted as with the usual samples.

RESULTS

EFFECT OF ICING

In all cases icing completely prevented the formation of histamine (tables 1 and 2). The values were so low that none of the iced samples were really different from zero. Due to the very low values and instrumental error some readings were actually slightly below zero, but since this has no physical meaning these have been reported as zero.

EFFECT OF GUTTING

Gutting had only minimal effect on histamine accumulation. Gutted mackerel held at room temperature with only natural bacterial contamination had not exceeded 10 mg % by day 2. All other groups held at room temperture for two days exceeded the generally accepted tolerance for histamine. Gutted fish showed lower variability in histamine accumulation.

EFFECT OF PROTEUS MORGANII CONTAMINATION

Individual round fish contaminated with Proteus morganii showed very high accumulations of histamine by day 2 (table 1), much higher than the equivalent uncontaminated group. However

some members of the equivalent uncontaminated group also showed quite high values as well. It would appear that the natural flora of these mackerel were quite capable of causing pronounced histamine accumulations. The Proteus morganii contamination resulted in greater and more rapid accumulation, of histamine (tables 1 and 2).

Gutted fish exposed to Proteus morganii accumulated histamine as quickly as ungutted at room temperature (figure 1). By day 4 the histamine accumulation in this group was much greater than was seen in any other samples in this trial.

EFFECT OF BRINE TREATMENT

The brine dip may have inhibited the formation of histamine in that the day 4 sample values were all less than those shown for equivalent groups above (table 3 vs table 2). However it would not appear that this would be useful as the fish were already well above the average acceptable histamine level two days after brine dipping, even without Proteus morganii contamination.

EFFECT OF SALT PICKLING

It appears that under the favorable conditions of low temperture and good contact with dry salt, the mackerel fillets already contaminated with growing Proteus morganii did not further accumulate significant amounts of histamine during the pickling process (table 3).

DISCUSSION

It is abundantly clear from the results of these trials that the answer to controlling histamine accumulation in mackerel is to keep the fish at ice temperature. The problem could be eliminated if fishermen, distributers and processors would hold these fish at or near ice temperture until processed into a stable form (canned or frozen). While the results in these trials were the most clear cut similar results have been reported elsewhere (10, 11). Since it appears that most of the bacteria which cause the histamine formation are of enteric origin, and presumalby reach the fish through sewage contamination or unhygenic conditions at the plant, during transportation or on-board the fishing vessel, it is not suprising that their activity is thoroughly inhibited by ice temperature. Proteus morganii has been reported to produce little of the histidine decarboxylase enzyme below 15 C (12). While the fish in the above trials would eventually have spoiled if kept on ice due to the action of their natural cold tolerant bacteria it would not

appear that the spoiled fish would have had significant levels of histamine. There was no sign of histamine formation with the natural bacteria or after contamination of the fish with an active histamine-former, like Proteus morganii, even after eight days storage in ice.

While it would be nice to think that these results would always be the case a little caution should be used. While the major histamine formers have implied enteric origins (Eschericia coli, Proteus morganii, Klebsiella and Shigella), and hence would be relatively high temperture organisms, there may be exceptions which will allow histamine formation at low temperatures.

It was expected that gutting would accelerate the formation of histamine, particularly after exposure to Proteus morganii. The cut surfaces were expected to allow easier access to the tissues by the microorganisms and ,their secreted enzymes. In fact, in this case at least, the round fish accumulated histamine more quickly than the gutted ("damaged") fish. This may have been due to the fact that the guts would essentially liquify under these high temperature conditions thereby allowing the rapid growth of normal gut bacteria. It would appear that at least one of these bacteria, or less likely one of those found on the surface of the fish, was able to synthesize histamine from histidine since it is generally accepted that sterile fish tissues do not produce significant amounts of histamine (1).

Contamination of the fish with Proteus morganii was used to ensure that histamine formation would be demonstrated. The Proteus morganii contaminated fish were intended to be a worst case demonstration. It was not expected that the natural bacteria would be so effective at histamine formation. Clearly if the present trial was typical, histamine accumulation is to be expected if the fish are kept under poor conditions, even without special bacterial contamination due to unhygenic conditions. It should be noted that while holding the fish at room temperture is in some ways an extreme example, it is not more extreme than the condition of fish piled in an open boat in the sunshine in July.

To evaluate the effect of low levels of salt on histamine formation rates, trial fish were dipped in 10% brine. Brine was used rather than solid salt to ensure uniform exposure. It appears that the brine treatment did not retard the rate of histamine formation due to the natural bacterial contaminants but, in fact, may have accelerated it in the early stages of formation (table 3 vs. table 2). Retardation of histamine formation after Proteus morganii contamination probably did result from the brine treatment and may warrant further study.

On the other hand salt pickling must have fairly quickly stopped histamine accumulation due to Proteus morganii that was

already in progress (table 4). It was not certain before the trial that the salt penetration would be rapid enough to prevent further deterioration due to secreted enzymes, even though the surface bacteria would be quickly inhibited. One could speculate that the free enzyme might have been able to diffuse into the fish ahead of the salt for a time and continue to create histamine even after salting had started. At least under these more or less optimum conditions for salting of low temperature and good salt contact little or no further histamine formation occurred (table 4 vs. table 1).

CONCLUSIONS

1. Thorough icing completely inhibited the accumulation of histamine in round or gutted mackerel. It seems probable that this would be generally true.

2. Gutting did not result in accelerated accumulation of histamine despite the formation of cut surfaces. Therefore gutting to improve the other quality characteristics of mackerel should not affect the histamine accumulation significantly even if the fish were held under bad conditions.

3. Mackerel caught and transported without significant damage can rapidly accumulate sufficient histamine to fail the usual acceptance level standard (10 mg %) when held under poor conditions (less than 48 hours).

4. Light salting had no significant effect on the accumulation of histamine in mackerel when held under bad conditions (room temperature).

5. Salt pickling quickly stops the formation of histamine when conducted with good salt contact at low (6 C) temperature.

6. The presence of appreciable amounts of histamine in mackerel products strongly indicates that at some point in the storage or transport of the dead fish they were held for an extended period at a relatively high temperature.

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Table 1

Histamine accumulation in fresh, round mackerel at room *vs.* ice temperatures, and with natural bacteria *vs.* contaminated with *Proteus morganii*

	HISTAMINE (mg %)				
DAY	Ø	1	2	4	8
TREATMENT					
		NATURAL BACTERIA			
ICED	0.1	0.6	0.5	0	0.4
	0	0.6	0.5	0.3	0.3
	0.1	0.4	0.5	0.7	0.6
	0.3	0.5	0.5	0	0.6
	0	0.6	0.3	0.3	0.5
(MEAN +- SD)	0.1	0.5	0.5	0.3	0.5
	+-0.1	+-0.1	+-0.1	+-Ø.3	+-0.1
ROOM TEMP (2Ø C)		2.3	86.9	34.9	65.4
		2.5	10.2	137.7	278.7
		2.2	58.8	13.7	395.0
		2.7	5.0	454.3	229.8
		2.7	3.9	47.9	55.5
(MEAN +- SD)		2.5	33.0	137.7	204.9
		+-0.2	+-37.8	+-183.2	+-144.5
		PROTEUS CONTAMINATED			
ICED	0.4	0	0.6	Ø.1	Ø.2
	Ø.3	Ø.1	Ø.5	Ø.1	Ø.2
	Ø.5	Ø.1	Ø.5	Ø.3	Ø.3
	Ø.2	Ø.1	Ø.5	Ø.3	Ø.1
	Ø.2	Ø.1	Ø.3	Ø.3	Ø.1
(MEAN +- SD)	0.3	0.1	0.5	0.2	0.2
	+-0.1	+-0	+-0.1	+-0.1	+-0.1
ROOM TEMP (2Ø C)		0.7	386.9	89.5	195.9
		0.4	14.9	122.1	161.1
		0.4	9.2	40.6	-
		0.3	295.4	81.4	214.3
		0.4	3.5	34.9	-
(MEAN +- SD)		0.4	142.0	73.7	190.4
		+-0.2	+-184.7	+-36.2	+-27.

Table 2

Histamine accumulation in fresh, gutted mackerel at room vs. ice temperatures, and with natural bacteria vs. contaminated with Proteus morganii

	HISTAMINE (mg %)			
DAY	0	1	2	4	8
TREATMENT					
		NATURAL BACTERIA			
ICED	0.2	0.3	0.1	0.1	0.1
	0.2	0.3	0.1	0.3	0.1
	0.2	0.2	0.2	0.1	0.3
	0.2	0.2	0	0.1	0.1
	0.2	0.2	0.1	0.2	0
(MEAN +- SD)	0.2 +-0	0.2 +-0.1	Ø.1 +-0.1	0.2 +-0.1	Ø.1 +-0.1
ROOM TEMP (20 C)		4.1	7.2	150.4	221.3
		2.2	5.0	81.8	186.5
		2.2	2.1	138.0	-
		1.7	4.9	162.9	-
		2.2	2.3	161.8	-
(MEAN +- SD)		2.5 +-0.9	4.3 +-2.1	139.0 +-33.5	203.9 +-24.6
		PROTEUS CONTAMINATED			
ICED		0.1	0	0	0.5
		Ø.1	Ø	Ø	Ø.2
		Ø.1	Ø	Ø	Ø.1
		Ø	Ø.3	Ø	Ø.2
		Ø	Ø.3	Ø	Ø.2
(MEAN +- SD)		Ø.1 +-Ø.1	Ø.1 +-Ø.2	Ø	Ø.6 +-Ø.8
ROOM TEMP (20 C)		0	24.7	460.2	249.7
		0	22.7	386.0	207.6
		0.1	13.8	433.0	198.4
		0.1	6.1	416.0	190.4
		0.1	10.8	-	204.1
(MEAN +- SD)		0.1 +-0.1	15.6 +-7.9	423.8 +-31.1	210.0 +-23.1

Table 3

Histamine formation in gutted small mackerel after brief exposure to brine

	HISTAMINE (mg %)		
DAY	I	2	4
TREATMENT			
	NATURAL BACTERIA		
BRINE DIPPED	0	24.7	178.1
ROOM TEMP	Ø	23.1	180.7
(2Ø C)	0	14.0	183.2
	0	6.8	188.8
	0	11.7	348.5
(MEAN +- SD)	Ø	16.1 +-7.6	215.9 +-74.3
	PROTEUS CONTAMINATED		
BRINE DIPPED	Ø.6		245.4
ROOM TEMP	Ø.8		250.5
(2Ø C)	1.1		250.5
	Ø.7		280.8
	1.6		168.Ø
(MEAN +- SD)	1.0 +-0.4		239.0 +-42.1

Table 4

Histamine accumulation in mackerel butterfly fillets exposed to *Proteus morganii* 24 hours at room temperature (20 C) prior to salting

	HISTAMINE (mg %)
	2.5
	1.3
	1.3
	3.8
	6.3
	1.6
	1.3
	1.5
	1.2
	2.2

(MEAN +- SD)	2.3+-1.6

Figure 1

Comparison of the histamine accumulations achieved in round (a and b) and gutted (c and d) mackerel with natural bacterial contamination (a and c) and after contamination with Proteus morganii (b and d) held for the indicated times at ice (—⊙—) or at room (—⊡—) temperatures.