Development of High Levels of Histamine in Atlantic Herring (Clupea harengus harengus) and Gaspereau (Alosa pseudoharengus):

An examination of the effect of temperature and bacterial contamination on histamine accumulation in good quality fillets

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CONTENTS

ABSTRACT

M.W. Gilgan. 1987. DEVELOPMENT OF HIGH LEVELS OF HISTAMINE IN ATLANTIC HERRING (Clupea harengus harengus) AND GASPEREAU (Alosa pseudoharengus): An examination of the effect of temperature and bacterial contamination on histamine accumulation in good quality fillets

The accumulation of histamine was determined in matched sets of fillets cut from very good quality frozen herring. For the experiment the fillets were held at ice and room temperature (20 C), with and without inocculation with a known histamine forming bacterium, Proteus morganii. The progress of the histamine accumulation was followed for eight days. No histamine was produced in the fillets held at ice temperature. Fillets exposed to Proteus morganii and held at room temperature accumulated histamine to unacceptable levels (more than 10 mg/100g) in less than 24 hr with a maximum of 69 mg/100 g for the average of five replicates. A similar investigation was conducted with fillets from frozen, very good quality gaspereau (alewives). Again essentially no histamine was formed when the fillets were held at ice temperature and little histamine was formed at room temperature with natural bacterial contaminants. After Proteus morganii inocculation the histamine content of the fillets exceeded acceptance levels in less than 24 hours at room temperature. The maximum histamine levels reached were higher than with herring, reaching a five replicate average of 78 mg/100g in eight days at room temperature after Proteus morganii exposure.

Both herring and gaspereau fillets can accumulate histamine to levels well in excess of present acceptance levels (10 mg/100 g for the average of a standard Inspection sample or 30 mg/100 g for any single sample). It appears that any product samples which show high levels have been held at relatively high temperatures and were contaminated with histamine-forming bacteria likely due to unhygenic conditions.

Résumé

L'accumulation d'histamine a été mesurée dans des groupes de filets appariés provenant de harengs conngelés de très bonne qualité. Les filets ont été gardés en dessous du point de congélation ou à la température ambiante (20 C); certains d'entre eux ont été inoculés avec une bactérie productrice d'histamine Proteus morganni. L'évolution de la teneur en histamine a été suivie pendant huit jours. On n'a pas décelé la présence d'histamine dans les filets conservés en dessous du point de congélation. La teneur en histamine des filets conservés à la température ambiante et inoculés avec Proteus morganni a atteint des niveaux inacceptables (supérieurs a 10 mg/100 g) en moins de 24 h, la teneur maximale observée étant de 69 mg/100 g (moyenne de cinq essais). Une expérience semblable a été faite avec des filets de gaspareau (faux-hareng) congelé de très bonne qualité. Encore une fois la production d'histamine a été presque nulle dans les filets conserves a une température inférieure au point de congélation et très faible dans les filets conservés à la temperature ambiante lorsque seuls les contaminants bacteriens naturels étaient présents. Après inoculation avec Proteus morganni, la teneur en histamine des filets gardés a la temperature ambiante a dépassé les niveaux acceptables en moins de 24 h. Les teneurs en histamine maximales etaient supérieures à celles que l'on a mesurées chez le hareng, la moyenne de cinq essais étant de 78 mg/100 g pour une periode de huit jours.

Les filets de hareng et de gaspareau peuvent accumuler de l'histamine a des niveaux bien supérieurs aux actuellement acceptables (10 mg/100 g comme teneur moyenne pour un échantillon d'inspection standard ou 30 mg/100 g pour tout echantillon individuel). Il semble que tout echantillon présentant une teneur élevée ait été exposé à une température relativement élevée et contamine par des bacteries productrices d'histamine, vraisemblement à cause d'un manque d'hygiène.

INTRODUCTION

While toxicity is not generally associated with the herring group of fish, members of the Mackerel (scombroid) 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, 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.

Herring have been reported to have substantial accumulations of free histidine in their blood and tissues(5,6). When these fish or their products 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. The presence of histamine is also a sign of bacterial spoilage.

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 or 20 mg % (meaning 10 or 20 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 levels of histamine.

Several methods have been developed for the chemical determination of histamine in fish and fish products (7). 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., 8) are beginning to appear and may in time replace the older "wet chemistry" methods.

While the formation of histamine in herring has previously been studied, the gaspereau (alewives) have not. The purpose of this study was to determine the effect of icing and exposure to a known histamine forming bacterium, Proteus morganii, on a single lot of very good quality frozen herring or gaspereau which were frozen while very fresh. The study would demonstrate the likely maximum accumulation of histamine and the approximate maximum rate of accumulation.

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 inoculated with samples of the actively growing bacteria and incubated overnight at 27 C.

Aliquots of the broth (Ø.5 mL) were then transferred to an equal volume of methanol. Samples were then spotted as 2 mm zones on an activated (12Ø C for 1 hr) thin-layer chromatography plate (E. Merck, Kieselgel 6Ø F-254, 5x1Ø cm) and run in acetone/ conc. ammonia (2Ø:1)(9) for 5 cm (origin to front). The culture extracts were compared with standard zones of histidine and histamine after development with ninhydrin spray (3ØØ mg ninhydrin in 1ØØ mL 3 % glac. acetic acid in n-butanol).

Since histidine decarboxylase might be an inducible enzyme in P. morganii, subcultures were "activated" by growing them for two passes, 48 hours, in the presence of 5 mg free histidine/mL. For exposure, two 5 mL samples of the desired P. morganii culture ca. 24 hours old were diluted to 1 L with distilled water (1Ø^7 cells/mL) and immediately used as a dip for the selected fillets.

FISH SAMPLES

The herring were obtained from a trap to avoid net damage and to ensure that the fish were alive when transferred to the vessel. The fish were caught in the vicinity of Eastern Passage, Nova Scotia, on May 28, 1982, brought to the laboratory live, frozen whole and stored at -3Ø.

The gaspereau were obtained from spawning run (May 19, 1982) in the LaHavre River, Lunenburg County, N.S.. The fish were thoroughly iced and promptly brought to the laboratory in insulated containers. At the laboratory the whole fish were placed in polyethylene bags and frozen, then stored at -3Ø C.

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 (1Ø), which is itself an adaptation of the original Shore (11) procedure.

Each fillet was homogenized individually in a food processor (General Electric, model PR1ØØB) until it formed an even paste. A sample of the paste was then packed in a 5Ø 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 1Ø.ØØ g. The weighed sample was further blended in ca. 5Ø mL methanol with a Polytron blender to an uniform suspension (1 min.). The suspension was transferred quantitatively to 1ØØ mL volumetric flask diluted to near 1ØØ mL and incubated at 6Ø C for 1 hr. When cooled to room temperature the suspension was volumed to 1ØØ.ØØ mL. A sample was transferred to a centrifuge tube, centrifuged and the clear supernatant fluid stored at -3Ø 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 (Ø.2ØØ 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 (Ø.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 (Ø.Ø5 to 1.Ø ug histamine as free base) 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 36Ø nm and an emission wavelength of 45Ø nm with a Turner model 43Ø 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

1. DETERMINATION OF THE EFFECTS OF ICING AND P. MORGANII CONTAMINATION ON HISTAMINE ACCUMULATION IN HERRING FILLETS

At the time of the experiments the frozen herring were partially thawed and fillets were cut from either side. Groups of five matched, skinned fillets were then placed in labelled polythene bags; one set for holding at ice temperature and the other at room temperature (ca. 2Ø C) to be sampled at days zero to eight as shown in table 1. Similar matched sets of skinned fillets were prepared to compare histamine formation at room temperature with the natural microbial contamination against that occurring after contamination with P. morganii. At the time the samples were taken they were either blended and extracted with methanol immediately or were stored at -3Ø C until they could be extracted. The histamine accumulations found are shown in table 1.

2. DETERMINATION OF THE EFFECTS OF ICING AND P. MORGANII CONTAMINATION ON HISTAMINE ACCUMULATION ON GASPEREAU FILLETS

Like the herring, the gaspereau were partially thawed and filleted. Groups of five matched, skinned fillets were then placed in labeled plastic bags to allow direct comparison of the histamine accumulation which would occur when the samples are held at ice or room temperature with normal bacterial

contamination. At the same time similar sets of fillets were prepared to compare histamine accumulation which occurred when contaminated with P. morganii which was exposed to free histidine in the culture broth (activited) prior to use as the inocculum for the fillets and that which occurred with the usual P. morganii cultures (unactivated). The results of these trials are shown in table 2 and in figure 2.

RESULTS AND DISCUSSION

Very little histamine formation occurred in the herring fillets at room temperature with the natural bacterial contamination (table 1). This was true with both sets of samples held at room temperature. While the samples did spoil, the spoilage did not result in histamine formation. Therefore in this case the icing cannot be said to have prevented histamine formation even though it did delay normal spoilage. It has already been demonstrated in this laboratory that icing completely prevented histamine formation in mackerel with either natural microflora or after P. morganii contamination(12). Herring and gaspereau were not expected to be different and apparently they were not. Psychrophilic histamine-forming bacteria have been reported to exist (13) but their importance in the usual cases of histamine accumulation remains to be established. The rapid (figure 1) and extreme accumulation of histamine in the herring fillets exposed to P. morganii clearly shows that histamine accumulation could be a serious problem in fillets of these fish. In fact the accumulation of histamine was more extreme than would be expected for herring. The highest level observed in defective commercial herring products in this laboratory has been approximately 45 mg %. The unusually high accumulation found in this study may reflect the relatively pure culture P. morganii contamination with minimal competing bacteria present. The work of Hughes (6) indicates that the histidine levels are seasonal and highest in the spring for herring landed in Britain. Since the herring for this experiment were caught in May they may have had very high histidine levels as well.

Since it is generally accepted that the level of histamine accumulation is not related to the observed quality (7), no attempt was made to relate the deteriorating quality of the fillets to the histamine content.

The results in figure 1 clearly show how rapid the histamine accumulation can be in these fish under the extreme conditions of bacterial contamination and high holding temperature. While the bacterial contamination would be much more severe than would

occur in normal conditions of the fishery, the highest temperatures expected in the industry could be greater.

The results with the gaspereau fillets were similar to those of herring but the accumulation of histamine was more extreme (table 2, figure 2). High levels of histamine had been found in survey samples of gaspereau products so some accumulation was expected. The very high accumulation was not expected. Three of the individual fillets in fact exceeded the level where, in Canada, they are considered to be toxic due to histamine (100 mg %). If these samples were encountered in commercial products during an inspection the product would be rejected and no re-inspection would be permitted. The sample averages are also higher than expected (table 2) and approach levels sometimes found in scombroid fish damaged by mistreatment. While the potential for high histamine accumulations quite apparently exists for gaspereau it seems probable that those observed are artificially high due to the large inocculum of a particularly effective histamine-forming bacterium. In commercial samples held under abusive conditions the fish would be contaminated with a mixed culture of bacteria which would compete for the free histidine and metabolize the histamine formed. The result would be less histamine formation and accumulation.

The rate of increase in histamine in the gaspereau was very rapid (figure 2). The acceptance level of 10 mg % was exceeded in less than one day after exposure to P. morganii when held at room temperature.

While the scombroid fish have been extensively studied due to their spectacular accumulations of histamine under bad conditions and their resultant toxicity, the herring-like fish have not been studied in such detail. Presumably this is because the incidence of poisonings attributed to herring and herring-like fish is small.

The susceptibility of herring to histamine formation due to free histidine in the tissues, was reported as early as 1955 (5) when histidine was tentatively identified in extracts from herring (Clupea harengus) and Twaite shad (Clupea finta). Free histidine was measured by amino acid analysis technique in sardine, Sardinops caerulea, menhaden, Brevoortia tyrannus and shad, Alosa sapidissima at 49.2, 15.0 and 7.3 umole/g (14). (One umole/g is equivalent to 155 ug/g or 15.5 mg %.) The smelt, Atherinopsis californiensis, on the other hand contained only 0.1 umole histidine/g. Free histidine levels were determined in herring (Clupea harengus) extracts by paper chromatography and colorimetry to vary seasonally from a low of 26.5 to a high of 160.2 mg % (6). The sardine, Sardinea melanosticta, was reported to contain 606 mg % free histidine (15).

Histamine was tentatively identified in herring extracts in 1955 (5). It was reported to be 300.9 mg/kg (30.09 mg %) in dried herring (species not identified), 98.3 mg/kg in salted herring and 345.2 mg/kg in smoked herring (16) obtained from the local market. Sardines (species not identified) contained 124.3, 15.0 and 398.9 mg/kg for dried, salted dried and broiled, seasoned dried fish, respectively, from the market(16). The accumulation of histamine during spoilage with the normal bacterial contamination at 10-13 C was determined by Hughes (6). The maximum accumulation found was ca. 50 mg %, somewhat less than that found in the present trials.

CONCLUSIONS

Icing appears to prevent histamine formation in herring and gaspereau fillets contaminated with the "natural" microflora found on previously frozen fish. The greatest histamine levels in herring, under conditions which probably favor the accumulation more than those of commercial mistreatment of herring products, can be as high as 100 mg %. Under the same conditions the average histamine accumulation in five replicates can exceed the Canadian acceptance level of 10 mg % in less than 24 hours. The greatest histamine accumulation in gaspereau was even higher, 125 mg %. Again under these conditions favorable to histamine formation, the acceptance level can be exceeded in less than 24 hours. Both of these extreme accumulations would be considered toxic in Canada (greater than 100 mg %). These results show that the histamine accumulations in these products can be much greater than had been generally considered possible, and that it is possible that toxic product can result from severe mistreatment of products of both fish.

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TABLE 1

COMPARISON OF HISTAMINE ACCUMULATION IN THAWED HERRING FILLETS HELD AT ROOM VS. ICE TEMPERATURES, AND WITH NATURAL BACTERIA VS. CONTAMINATED WITH PROTEUS MORGANII

	HISTAMINE (MG %)				
DAYS	Ø	1	2	4	8
TREATMENT					
		NATURAL BACTERIA			
ICED	Ø.7Ø	Ø.87	2.4	1.6	Ø.ØØ1
	Ø.7Ø	1.Ø	2.7	1.5	Ø.Ø13
	Ø.71	1.Ø	2.7	1.6	Ø.Ø13
	Ø.7Ø	Ø.63	2.7	1.6	Ø.ØØ1
	Ø.7Ø	Ø.61	2.7	1.6	Ø.Ø15
(MEAN +- SD)	Ø.7Ø +-Ø.Ø1	Ø.83 +-Ø.2Ø	2.6 +-Ø.1Ø	1.6 +-Ø.Ø7	Ø.Ø1 +-Ø.Ø1
ROOM TEMP		Ø.72	Ø.26	2.7	Ø.47
		Ø.72	Ø.31	2.7	Ø.21
		Ø.7Ø	Ø.31	2.7	Ø.3Ø
		Ø.7Ø	Ø.31	2.7	Ø.6Ø
		Ø.55	Ø.31	2.7	1.2
(MEAN +- SD)		Ø.68 +-Ø.Ø7	Ø.3Ø +-Ø.Ø2	2.7 +-Ø.Ø1	Ø.56 +-Ø.4Ø
ROOM TEMP (2Ø C) NATURAL BACTERIA		Ø.13	Ø.Ø8	Ø.19	Ø.92
		Ø.13	Ø.Ø8	Ø.24	1.2
		Ø.13	Ø.Ø8	Ø.24	1.Ø
		Ø.17	Ø.Ø8	Ø.21	1.1
		Ø.13	Ø.Ø8	Ø.24	Ø.84
(MEAN +- SD)		Ø.14 +-Ø.Ø2	Ø.Ø8 +-Ø	Ø.22 +-Ø.Ø2	1.Ø +-Ø.15
ROOM TEMP (2Ø C) PROTEUS CONTAMINATED		24.1	24.5	82.7	58.9
		26.5	2Ø.3	58.Ø	6Ø.5
		26.2	27.9	68.9	51.7
		16.1	51.6	3Ø.5	66.Ø
		32.6	23.4	1Ø5	51.5
(MEAN +- SD)		25.1 +-6.Ø	29.5 +-13	69.1 +-28	57.5 +-6.2

TABLE 2

COMPARISON OF HISTAMINE ACCUMULATION IN THAWED GASPEREAU FILLETS HELD AT ROOM VS. ICE TEMPERATUTRES WITH ONLY NATURAL BACTERIAL CONTAMINATION, AND AT ROOM TEMPERATURE WITH ACTIVATED AND UNACTIVATED PROTEUS MORGANII CONTAMINATION

	HISTAMINE (MG %)				
DAYS	Ø	1	2	4	8
TREATMENT					
		NATURAL BACTERIA			
ICED	Ø.Ø5	Ø.12	Ø.Ø7	Ø.28	Ø.23
	Ø.14	Ø.21	Ø.Ø7	Ø.11	Ø.43
	Ø.Ø2	Ø.2Ø	Ø.Ø7	Ø.Ø7	Ø.33
	Ø.2Ø	Ø.Ø7	Ø.Ø7	Ø.ØØ	Ø.25
	Ø.Ø9	Ø.21	Ø.Ø7	Ø.ØØ	Ø.29
(MEAN +- SD)	Ø.1Ø	Ø.16	Ø.Ø7	Ø.Ø9	Ø.3Ø
	+-Ø.Ø7	+-Ø.Ø6	+-Ø	+-Ø.12	+-Ø.Ø8
ROOM TEMP (2Ø C)		Ø.Ø34	Ø.11	Ø.44	61.7
		Ø.Ø9	Ø.11	Ø.41	31.4
		Ø.14	Ø.17	3.4	32.7
		Ø.12	Ø.11	1.6	47.6
		Ø.13	Ø.11	Ø.32	112
(MEAN +- SD)		Ø.1Ø	Ø.12	1.2	57.3
		+-Ø.Ø4	+-Ø.Ø3	+-1.3	+-33
	PROTEUS MORGANII CONTAMINATED				
UNACTIVATED CULTURE		41.7	51.6	49.4	63.6
ROOM TEMP (2Ø C)		26.2	1Ø1	56.8	77.4
		33.2	87.8	54.2	74.3
		42.1	33.6	8Ø.8	49.7
		3Ø.6	72.7	61.6	125
(MEAN +- SD)		34.8	69.5	6Ø.6	78.2
		+-7.Ø	+-27	+-12	+-29
ACTIVATED CULTURE		47.5	54.7	63.1	6Ø.4
ROOM TEMP (2Ø C)		4Ø.7	49.1	73.5	74.9
		62.5	57.7	64.8	76.3
		53.9	72.9	8Ø.2	61.1
		63.6	81.6	81.7	1Ø5
(MEAN +- SD)		53.6	63.2	72.7	75.7
		+-9.8	+-14	+-8.5	+-18

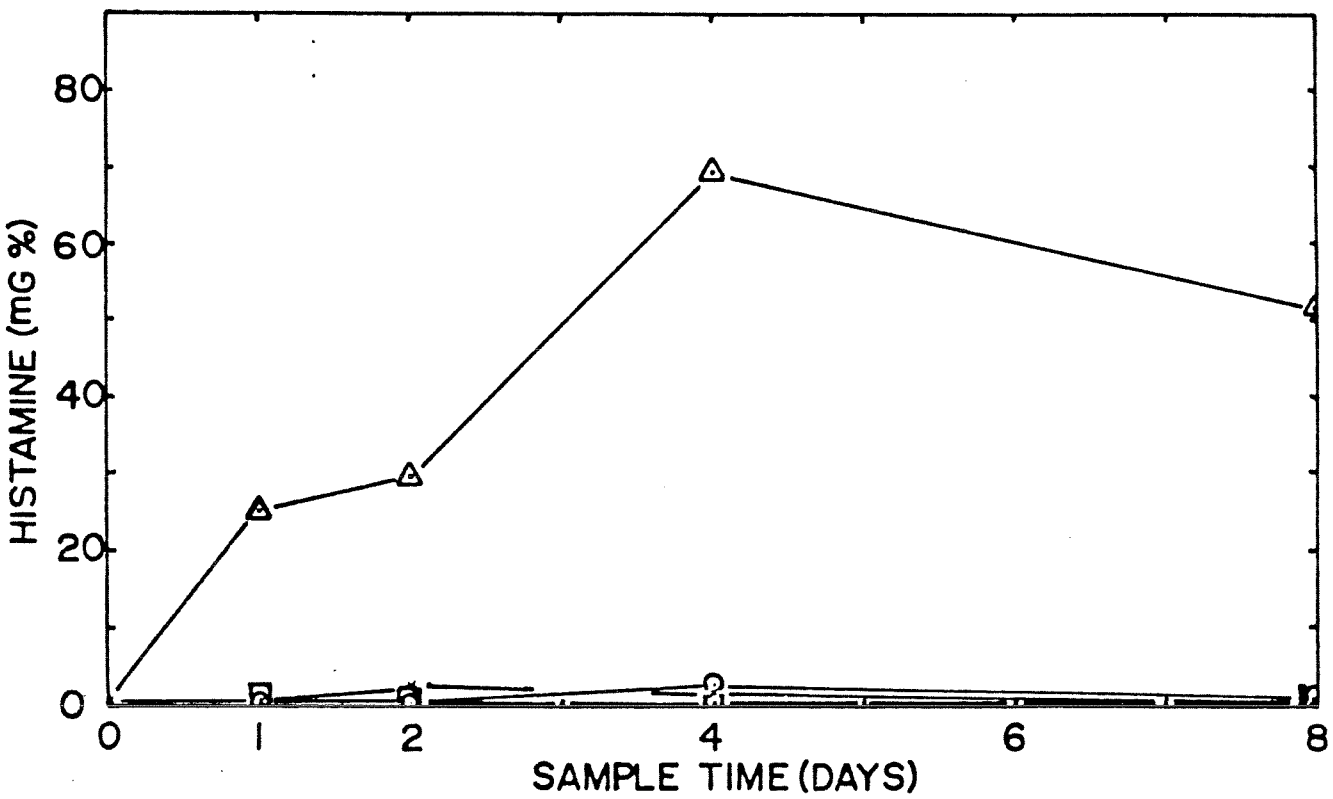

Figure 1. Histamine accumulation in herring (Clupea harengus harengus) fillets held at room temperature (—⊙—) or on ice (—X—) with natural bacterial contamination, or at room temperature with natural bacteria (—□—) or contaminated with P. morganii (—△—).

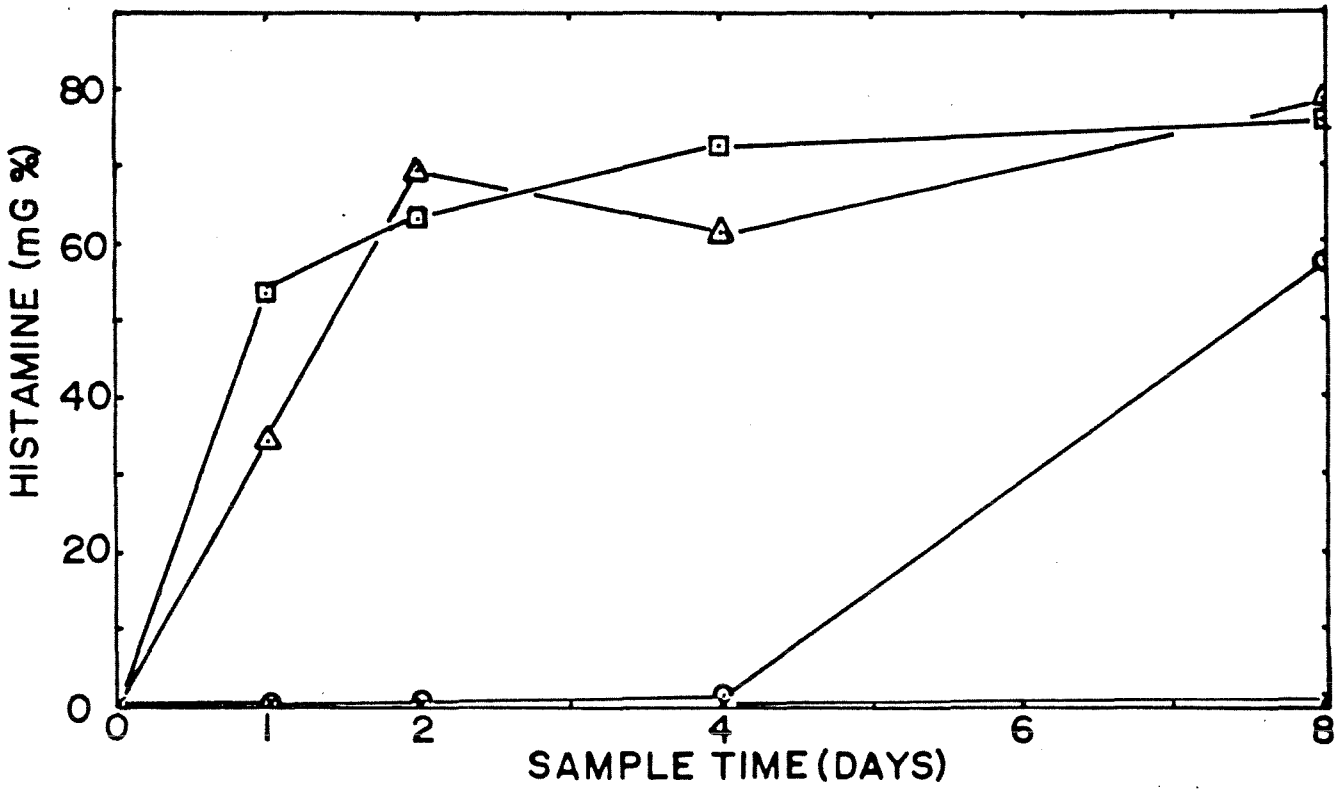

Figure 2. Histamine accumulation in gaspereau (Alosa pseudoharengus) held at ice temperature (—X—) or at room temperature (—⊙—) with normal bacterial contamination, or at room temperature after contamination with unactived (—⊡—) or activated (—△—) P. morganii.