Pacific Whiting, *Merluccius productus:* I. Abnormal Muscle Texture Caused by Myxosporidian-Induced Proteolysis

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ABSTRACT—Pacific whiting, Merluccius productus, *has not been widely exploited by the U.S. fishing industry as a food fish because of its variable and often overly soft texture when cooked. During the past 15 years, studies were made to determine the cause of the abnormal texture and to investigate processing techniques and/or product forms that would make it possible to market whiting products having a relatively normal texture. The following conclusions result from these studies: The abnormal muscle texture in Pacific whiting is caused by a myxosporidian-induced proteolysis. The latent potential for proteolytic textural softening in whiting, due to the presence of myxosporidian cysts of variable intensity, appears to be an intrinsic characteristic of the Pacific species. The enzyme appears to be highly localized within the cyst and has little effect on the muscle fibers if the fish are promptly chilled upon catching, maintained chilled during processing, and rapidly cooked. It has been recommended that Pacific whiting be marketed as a fast-cooked or deep-fat-fried, portion-type product, limited to about 3/8 inch in thickness.*

Introduction

Despite the vast abundance of Pacific whiting, *Merluccius productus,* off the west coast of the United States, it has been little exploited by the U.S. industry because it has been considered unsuitable as a food fish due to its variable and often overly soft texture when cooked. Initial work with whiting during the middle-1960's related the visual presence of hairlike cysts containing myxosporidian spores and the accompanying high level of proteolytic activity in the infected flesh with its abnormal, soft texture. This was similar to the condition that was previously reported in Pacific coast species of milky halibut, sole, flounder, and salmon (Patashnik and Groninger, 1964) and to that reported in Mauretanean hake (Fletcher et al., 1951) and in Australian barracouta (Willis, 1949).

Willis described the various developmental stages of the myxosporidian chloromyxum thyrsites after the death of the host fish. He hypothesized that the parasite releases a powerful extracellular enzyme that is removed in the blood stream while the host fish is alive. After the fish dies and the circulation ceases, the enzyme progressively diffuses outward from the infected focus (the cyst) to the noninfected flesh.

The class Myxosporidia[1] is the most numerous group of protozoan parasites in fishes, with almost 300 reported species (Lom, 1970). The protozoan invasion of fish is far more dependent on ecological conditions (type and amount of food, temperature, stress conditions, etc.) than is true of terrestrial animals. These conditions interact with the fine balance between the protective mechanism of fish and the virulence of the protozoa (Lom, 1970). Since man has little control over the infection in nature, the condition must be accepted as an intrinsic property of the fish species.

This type of parasitization occurs in both cold- and warmblooded animals. For example, a similar type of protozoan parasite is found in the skeletal muscle fibers (sarcocysts with masses of spores) in all classes of livestock and may be found in as many as two-thirds of healthy beef carcasses of different grades (American Meat Institute Foundation, 1960). This muscle parasite is of little concern since it does not affect the texture of the meat when cooked and does not affect man otherwise.

The muscle parasite that affects whiting similarly is of little public health concern, but since it degrades flesh texture significantly and limits the utilization of the resource, it is a matter of technological concern. This emphasized the need to broaden our understanding of the parasite and of the related texture problem. As the extent of the problem became clearer, studies on how to control or minimize adverse effects on flesh texture during harvesting and processing of the whiting became increasingly important.

Research on the texture problem in whiting during the middle 1960's and early 1970's was limited due to the absence of industry interest in marketing this species. In recent years the enactment of the 200-mile fishery conservation zone legislation and the need to

[1]Changed to Myxosporea by Levine et al. (1980).

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complement declining traditional fishery resources have awakened industry interest in the use of Pacific whiting for fresh and frozen products and have activated our research efforts.

This paper summarizes our early and more recent research on 1) the nature and extent of the abnormal texture problem and 2) the evaluation of various processing treatments to minimize the problem. Two additional papers are scheduled: 1) the commercial processing procedures necessary for producing a texturally acceptable, domestic frozen product, and 2) the potential utilization of Pacific whiting in the United States and/or Japan for a kamaboko-type fabricated product based on its elastic properties.

Materials and Methods

Fish Samples

During the past 15 years, numerous Pacific whiting samples were obtained from various sources off the Washington and Oregon coasts and, to a lesser extent, off the California coast. The early 1964-65 and many later whiting samples were caught by the NMFS RV *John N. Cobb.* Samples were obtained and examined from charter research vessels, commercial fishing vessels, and Soviet factory ships. During the years 1976-79, sampling and on-board research were carried out aboard the commercial trawler MV *Willapa Bay,* the Polish RV *Profesor Siedlecki,* and the chartered trawlers RV *Forerunner* and MV *Kupreanof.*

Sample lots examined consisted of freshly landed whiting aboard vessel; uniced, iced, and refrigerated seawater-held whiting delivered to commercial fish-processing plants; whiting immediately frozen aboard vessel and later in shore processing plants; whiting variously treated aboard vessel prior to freezing; live whiting frozen in dry ice aboard vessel; and whiting processed and frozen into fillet blocks aboard vessel. The whiting were examined in the round, as frozen and thawed fillet block portions, and as raw and slow- or fast-cooked portions and fillets. The samples were examined with particular reference to the nature of the abnormal texture problem and the severity and incidence of the parasite in the whiting population.

Texture Evaluation

The slow-oven-cooked (oven temperature 400°F) and the fast-cooked, battered-and-breaded, deep-fat-fried (fat at 385°F) samples were examined separately by three project personnel experienced with whiting texture, using the fish flesh only for evaluation. A 5-point rating scale was used for texture as follows: 5 = firm to normal; 4 = normal to soft; 3 = soft; 2 = overly soft; 1 = mushy, pasty. Categories 1 and 2 were considered unacceptable.

Severity and Incidence of Parasitization

The severity of parasitization was estimated by visual observation of parasitic cysts in a section or fillet of the raw fish. The rating scale for cyst intensity was based on the percent area of the fillet affected and density was as follows: 0 = none (0 percent); 1 = trace (up to 5 percent); 2 = slight (5-20 percent); 3 = moderate (20-30 percent); 4 = severe (30-50 percent); 5 = excessive (>50 percent).

Categories 3, 4, and 5 were considered "grossly parasitized" and readily cullable at processing plants from a moving conveyor belt with good lighting. Categories 1 and 2 required careful examination and were not readily cullable. Any fish with observable cysts, from trace to excessive, was considered positive in estimating the incidence of parasitization in whiting population samples. Inevitably, some fish with low-level cyst density are missed; therefore such estimates are on the low side. Data on percentage of fish affected in various samples are measures of the incidence of parasitization; data on the cyst density in an individual fish are measures of the severity of parasitization.

Chemical Determinations

Proteolytic enzyme determinations were made on samples frozen at sea using the method described in Patashnik and Groninger (1964). Whiting muscle was homogenized for 2 minutes in cold water 1:3 (weight:volume) and the homogenate was centrifuged at approximately 17,000 G. The supernatant was used for the enzyme determinations. The pH of muscle and muscle preparations were determined with a glass electrode.

Employing Dyer's protein method (Dyer et al., 1950), flesh samples (below dorsal fin) were extracted in 0.85 M sodium chloride and 0.003 M sodium bicarbonate and centrifuged at 760 G. Total soluble nitrogen and myofibrillar protein nitrogen were determined on an aliquot of the supernatant. Nonprotein nitrogen was determined on the supernatant of another aliquot after precipitation of the protein with 5 percent trichloroacetic acid. All protein and nonprotein determinations were done by the micro-Kjeldahl method of Minari and Zilversmit (1963). Total soluble protein and sarcoplasmic protein were determined by difference.

Proximate composition determinations were made using the standard procedures of the A.O.A.C. (1980).

Microscopy

For microscopic examination, a few muscle fibers from two or three areas of the raw and/or cooked flesh were teased out and placed in one or two drops of water on a glass microslide and covered with a cover glass. The condition of the muscle fibers and the presence of cysts and myxosporidian spores were observed under 450× magnification.

For histology, samples of fresh or frozen material were fixed in Bouin's solution, imbedded in paraffin, and sectioned at a thickness of 6μm. Sections were stained with May-Grunwald Giemsa stain to bring out the polar capsules. Dark staining bodies appearing in some slides were decolorized for 5 minutes with 1 percent acetic acid in order to reveal their relationship to the other spore tetrads present.

In addition, portions of fish tissue from fresh fish were obtained for further light and electron microscopic studies. Some of the tissues were sampled immediately after capturing the fish and others were from fish that had been incubated at 16°C for 0.5, 7, 31, and 48 hours. Cubes of tissue 0.5 × 0.5 × 0.5 cm were initially fixed in a solution containing 0.75 percent glutaraldehyde, 3 percent Formalin, 0.5 percent acrolein, and 0.1 M sodium

cacodylate buffer (pH 7.4) with 0.02 percent $CaCl_2 \cdot H_2O$ and 0.5 percent sucrose (Hawkes, 1974). The cubes were cut into smaller portions, 1.0 mm on each side, washed in buffer (0.1 M sodium cacodylate, 0.02 percent $CaCl_2 \cdot H_2O$, 5.5 percent sucrose), and post-fixed in 1 percent osmium tetroxide in buffer. Dehydration with ethanol and embedding in plastic (Spurr, 1969) completed the preparation of the tissue for sectioning with either glass or diamond knives. Semithin (1.0 μm) sections were prepared with Richardson's stain for light microscopy. Thin sections were triple stained with lead citrate, uranyl acetate, and again lead citrate for electron microscopy.

Results and Discussion

Incidence and Severity of Parasitization

Early in our investigation (May-October 1964-66), using the RV *John N. Cobb*, we extensively sampled the Pacific whiting population of the coastal areas off the Washington-Oregon coast for incidence and severity of myxosporidian infection. Overall, we found an incidence of 20-40 percent of the fish in the samples (10-50 fish per sample) to be infected with visually apparent "hairlike" cysts at the 5-100 percent level. Over three-fourths of the samples had individual fish with a severity of 20 percent cyst density or higher. About 8 percent of the parasitized fish were considered to be "grossly parasitized" and were readily cullable; the balance were uninfected or trace to slightly infected and not readily cullable. In other words, 2-3 percent of the whiting population were grossly parasitized and could be culled by sorting if necessary. Two samples of juveniles (34 g size) among the 38 lots of fish examined during this period were found to be unparasitized, suggesting that the infection may be acquired beyond the juvenile stage.

It was hoped that with the heavy fishing of the resource by foreign fleets since 1966 the incidence of parasitization would be substantially reduced. Nevertheless, periodic sampling during 1967-75 and the heavier sampling during 1976-79 indicated only a slight but insignificant downward trend. In recent years, the percentage of grossly parasitized (cullable) whiting appears to have decreased to the 4-5 percent level of the infected fish, i.e., about 1 percent of the whiting harvested. The visual incidence and severity (cyst density) of parasitization between fish and within fish varies widely from catch to catch and does not seem to be associated with area, depth, or season.

It appears then for developing a fishery one must expect that a relatively small but significant part of the Pacific whiting resource is intrinsically affected with myxosporidian cysts of variable intensity and distribution. This is associated with a relatively higher than normal level of proteolytic activity in the affected flesh areas of the fish, which may adversely affect the texture of both the raw and cooked product. To minimize this texture problem, it was clear that we needed to better understand the relationship between 1) the parasite and the fish muscle, and 2) the related effect of the proteolytic enzyme in the flesh and the time-temperature handling history of the whiting.

Parasitized Condition Involves Enzyme-Induced Proteolysis

Initially it was important to determine whether the texture problem in parasitized whiting was, in fact, proteolytic in nature. The data in Table 1 (variously treated lingcod gels) demonstrated that the parasitized condition in whiting was associated with an enzyme-induced proteolysis. Compared to the lingcod control, the control with added visually normal whiting showed a 30 percent greater proteolytic activity, which indicated a higher proteolytic activity even in normal-appearing whiting.

In contrast, the control with added grossly parasitized whiting showed a 700 percent greater proteolytic activity. However, the control with the parasitized whiting with added mercuric nitrate (enzyme inhibitor) showed a 50 percent lower proteolytic activity than the lingcod control and demonstrated that the enzymic activity of both the lingcod and the grossly parasitized whiting was largely inactivated.

Six control or normal whiting with no visual parasitization were compared with six cyst-infected, parasitized whiting for relative proteolytic activity. Results in Table 2 show a sevenfold higher average proteolytic activity in the parasitized group than the normal-appearing group

Table 1.—Comparison of the degree of protein breakdown of lingcod control gels[1] treated with normal and grossly parasitized Pacific whiting with and without an enzyme inhibitor after incubation at 100°F for 20 hours (whiting obtained by the RV *John N. Cobb* off the Washington coast, October 1967).

Treatment	Relative optical density[2] (degree of protein breakdown)
Lingcod control	1.0
Lingcod + 30 percent normal whiting	1.3
Lingcod + 30 percent grossly parasitized whiting	7.0
Replicates	
Lingcod + 30 percent grossly parasitized whiting	7.4
Lingcod + 30 percent grossly parasitized whiting + 0.1 percent mercuric nitrate (enzyme inhibitor)	0.5

[1]Homogenized fish gels made with 5 percent NaCl solution (1:2 by weight) using lingcod as control substrate.
[2]Optical density was determined at 280 nm on TCA extracts to estimate the relative degree of protein breakdown. The relative optical density was expressed as the ratio of the optical density of the treatments over that of the lingcod control.

Table 2.—Comparison of proteolytic activity in normal and myxosporidian (*Kudoa* sp.) infected Pacific whiting (whiting obtained by the RV *John N. Cobb* off the Washington coast, October 1965).

Group	Parasite[1] cyst intensity	Steam-cooked flesh condition	Proteolytic[2] activity
Normal control	0	Normal and flaking	0.5
"	0	"	0.8
"	0	"	0.9
"	0	"	0.6
"	0	"	0.8
"	0	"	0.4
		Average	0.67
Parasitized	5	Pasty	6.1
"	5	"	[3]1.4
"	1	"	5.3
"	3	"	3.7
"	3	"	5.0
"	4	"	5.7
		Average	4.53

[1]Parasite cyst intensity or density based on visual appearance scale of 0 = none; 1 = trace; 2 = slight; 3 = moderate; 4 = severe; and 5 = excessive.
[2]Activity was determined at pH = 3 using hemoglobin as substrate (g of tyrosine liberated $\times 10^2$ per 100 mg per 30 minutes).
[3]This sample had lysed pockets and an abnormal low pH 6.14. (Proteolytic activity may have been dissipated.)

of samples. The level of proteolytic activity within the parasitized groups did not correlate directly with the visually estimated degree of parasite cyst intensity. The increased proteolytic activity of the parasitized whiting ranged from 2 to 15 times greater than the controls. In subsequent comparisons, we found parasitized whiting with proteolytic activities up to 30 times greater than that in the control.

Relation of Parasitization to Flesh pH

In several experiments where parasite-infected fillets were held at 32-34°F for periods of up to several days, we occasionally found enzyme-liquefied pockets on the fillet surface and interior, similar to the occurrence noted in halibut or salmon (Patashnik and Groninger, 1964). The pH of the lysed pockets in halibut and whiting have consistently been measured at 6.1-6.4, which is lower than the pH in other areas on the fillet. The lysed pockets have been found usually in whiting with a lower muscle pH. The average pH of parasitized whiting is usually observed to be slightly lower than that in normal whiting as shown in Table 3 (6.48 vs. 6.67). The lower pH of parasitized whiting may explain the poorer water-holding capacity of the cooked protein of seriously parasitized whiting. This is similar to the poor water-holding capacity found in low pH halibut (Patashnik, 1966).

Relation of Parasitization to Level of Flesh Protein

In utilizing Pacific whiting as a food fish, the level of available protein is important. If the parasite has an adverse effect on the host fish, we might anticipate the myofibrillar proteins to decrease and the sarcoplasmic proteins to increase. The protein content of six visually normal whiting are compared in Table 3 with six significantly parasitized whiting from the same catch. The data show that the myofibrillar protein content of the parasitized group decreased by 11 percent (16.2 vs. 14.4) and the sarcoplasmic protein increased by 15 percent (6.0 vs. 6.9). The total soluble protein nitrogen for the parasitized whiting, however, decreased by only 3 percent (22.2 vs. 21.5), an insignificant change.

Table 3.—Comparison of protein and nonprotein nitrogen and pH in normal and myxosporidian (*Kudoa* sp.) infected Pacific whiting (whiting obtained by the RV *John N. Cobb* off the Washington coast, October 1965).

Group	Parasite[1] cyst intensity	Total soluble nitrogen	Total soluble protein	Myofibrillar	Sarcoplasmic	NPN	pH
				mg N/g			
Normal	0	27.8	24.0	16.3	7.7	3.8	6.56
"	0	27.0	23.0	17.7	5.3	4.0	6.63
"	0	26.0	22.3	17.4	4.9	3.7	6.65
"	0	25.6	21.6	17.7	3.9	4.0	6.56
"	0	28.6	24.7	17.1	7.6	3.9	6.75
"	0	20.0	17.5	11.2	6.3	2.5	6.84
Average		25.8	22.2	16.2	6.0	3.7	6.67
Parasitized	5	23.8	21.5	13.9	6.6	3.3	6.64
"	3	24.0	20.8	16.0	4.8	3.2	6.62
"	5	26.0	22.2	13.6	8.6	3.8	6.14
"	4	24.0	19.6	15.5	4.1	4.4	6.32
"	3	27.6	23.6	14.2	9.4	3.8	6.45
"	1	24.8	21.0	13.1	7.9	3.8	6.73
Average		25.0	21.5	14.4	6.9	3.7	6.48

[1]Parasite cyst intensity or density based on visual appearance scale of 0 = none; 1 = trace; 2 = slight; 3 = moderate; 4 = severe; and 5 = excessive.

In Table 4, an excessively parasitized whiting (raw fillet flesh pasty and full of enzyme-liquefied pockets) was compared with a group of seven slightly to moderately parasitized whiting. The abnormally high moisture, low protein, and low ash for the excessively parasitized whiting indicate a significant adverse effect on its chemical composition. This fish would ordinarily have been culled during processing. Since the protein-water relationships of the slightly to moderately parasitized whiting in Table 4 are well within the normal range, this would indicate a minimal nutritional effect on the host-parasite relationship for most affected whiting.

Table 4.—Comparison of the proximate composition of all excessively parasitized Pacific whiting with seven slightly-to-moderately parasitized whiting.

Degree of parasitization	Moisture (%)	Protein (%)	Oil (%)	Ash (%)
Slightly and moderately parasitized	83.4	15.5	0.29	1.05
Very excessively parasitized	87.0	11.1	0.36	0.72

Inactivation of the Enzyme by Heat

Since the texture of whiting muscle is greatly affected by the parasite-induced proteolysis during cooking, the stability of the enzyme(s) toward heat is of great importance. The heat inactivation of the enzyme(s) at various temperatures is shown in Table 5. The enzyme(s) was completely inactivated by heating at 70°C (158°F) for 10 minutes. This suggested that we must rapidly achieve an internal temperature of 160°F or higher to inactivate the enzyme during cooking.

Various methods of rapid heating to inactivate the enzyme before flesh proteolysis ensued were thus examined. In studies on microwave cooking of fillets, it was determined that parasitized 3-day-iced fillets (frozen and thawed for cooking) were normal in texture when rapidly microwave-cooked to a center-of-fillet temperature of 160°F within 1.5 minutes. In other studies on accelerated

Table 5.—Heat inactivation of proteolytic activity of parasitized Pacific whiting muscle extracts.

Temp. °C	Temp. °F	Activity remaining[1] (%)	Temp. °C	Temp. °F	Activity remaining[1] (%)
2	35.6	100	60	140.0	42
50	122.0	76	65	149.0	10
55	131.0	74	70	158.0	0

[1]Extract was heated for 10 minutes at pH = 6.7. Proteolytic activity was determined at pH = 3 using hemoglobin as a substrate.

heating, it was determined that deep-fat frying at 385°F of battered-and-breaded, 3/8-inch-thick cut sections of parasitized whiting produced similar normal textures. To achieve rapid heat inactivation of the enzyme(s) during cooking, the deep-fat-frying method was found to be the more effective and practical approach. This method will be discussed in greater detail in part II of this series of papers.

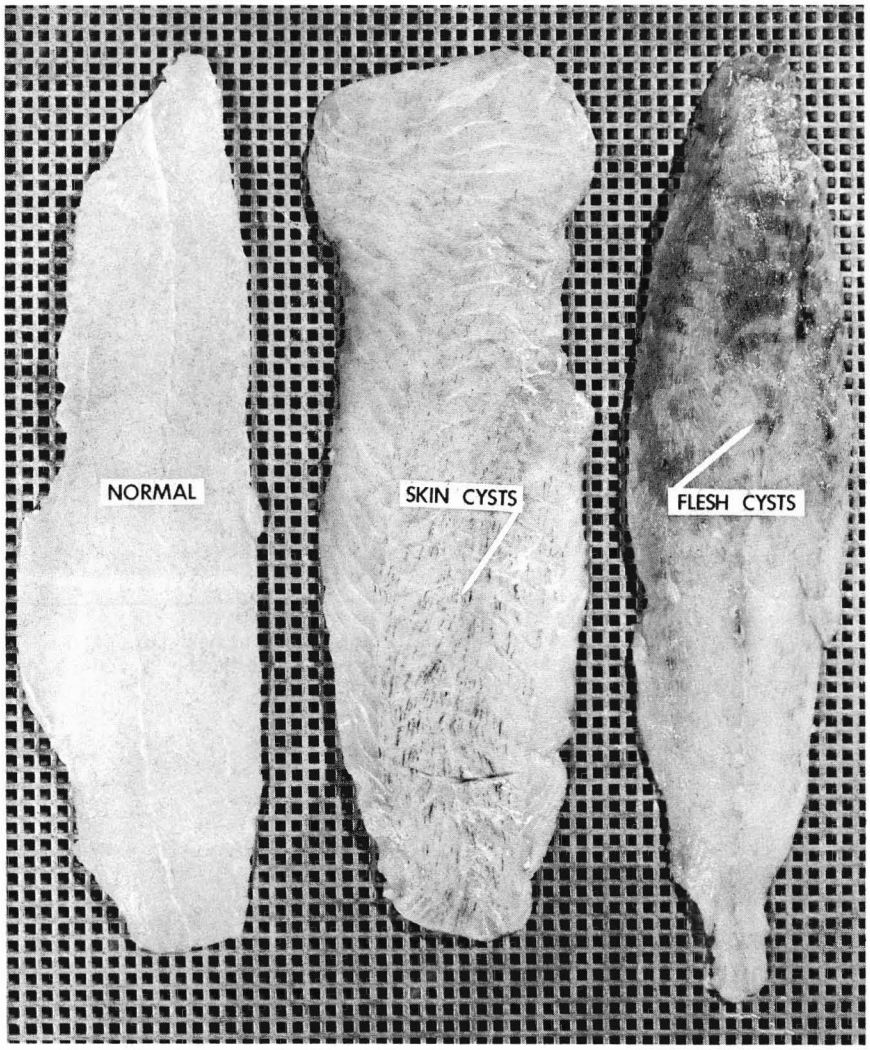

Figure 1.—A normal Pacific whiting fillet in contrast with heavily parasitized whiting fillets showing hairlike white and black cysts.

Severity of Parasitization and Relation to Abnormal Texture

Three criteria were used for estimating the severity of parasitization: 1) the visual presence of dark or white hairlike cysts, 2) microscopic examination of muscle for typical myxosporidian spores, and 3) sensory detection of abnormally soft or mushy texture of samples prepared by slow oven cooking.

The visual presence of parasitic cysts in a fillet (Fig. 1) provides an estimate of the severity of parasitization in a fish, but this figure may not correlate with the degree of proteolytic softening in the cooked product, as not all the visually parasitized fish develop an abnormal texture on cooking. Microscopic examination more accurately reflects the severity and developmental stage of parasitization in a sample; it is usually the best indicator of the likely predisposition to abnormal texture on cooking. Sensory evaluation of the slowly cooked flesh determines the actual portion of the parasitized fish samples that are sufficiently parasitized to become overly soft or mushy. Occasionally, a severely parasitized fish (all black cysts) will have a normal, chewable cooked texture, whereas a normal-appearing fish (visually free of parasitic cysts) but one which contains a significant quantity of microscopically identifiable cysts or low-contrast white cysts will become mushy on cooking.

On microscopic examination, we have commonly found samples of Pacific whiting to be 90-100 percent parasitized in varying degrees from trace to severe. When these lots were examined visually — the criteria most commonly used because of its speed — the incidence of parasitization was found to be much lower. We have found the severity of parasitization with white cysts (visual or subvisual) to be the best indicator of the predisposition to abnormal cooked texture. This was statistically confirmed ($P<0.001$) in our collaborative work with Polish research scientists aboard the RV *Profesor Siedlecki* in August 1977 (Schwartz et al., 1978).

Macroscopic and Microscopic Appearance of Parasite

Macroscopically, the cysts appear as dark and/or white filaments between myotomes (Fig. 1) and may be closely packed or widely separated depending on the severity of the infection. The cysts may be either uniformly or unevenly distributed through the interior flesh, on the flesh surface below the skin, or clustered in localized areas only (Fig. 2). The nape end of the fish fillet is usually found to be more heavily parasitized than the tail (Fig. 1).

Microscopically, in wet mounts, the cyst is commonly seen within the muscle fiber and is packed with a myriad of myxosporidian spores. In the thousands of samples examined, we have observed

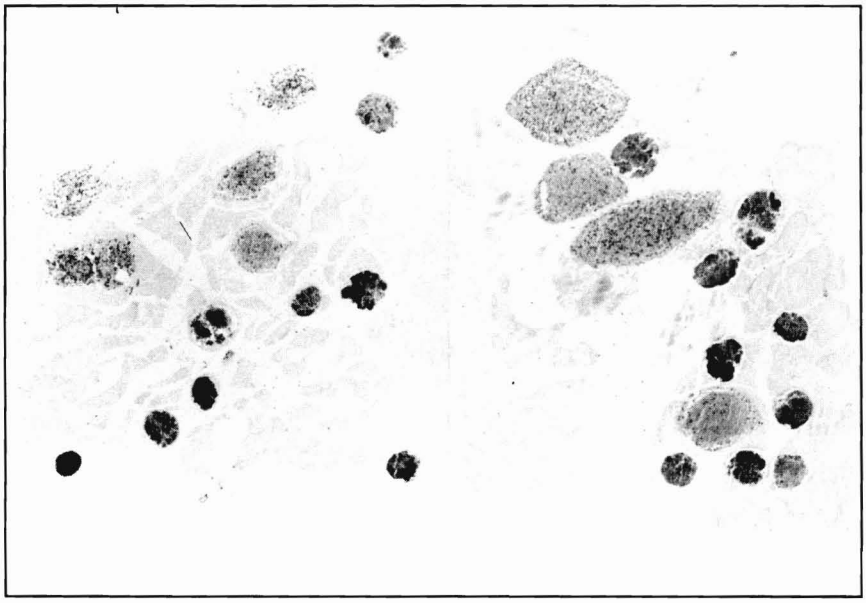

Figure 2.—A histological section through the muscle of a heavily parasitized Pacific whiting showing the distribution of various size and shape cysts within the muscle fiber (16×).

cysts ranging from subvisual size both in diameter and in length along the muscle fiber to those occupying the entire muscle fiber (Fig. 3).

The black or darker cysts are the older, more mature ones and are colored with a light- to dark-brown pigment. As the cyst ages, the granular, brownish pigmented material, which was initially localized (Fig. 4), spreads throughout the cyst. Very old cysts are pigmented throughout, devoid of spores, and no longer proteolytically active. This explains why some fillets with dark cysts may have low proteolytic activity and be normal in texture. Other dark cysts may still retain part or much of their proteolytic activity and be abnormal in texture to varying degrees.

The white cyst is the younger and more proteolytically active form and the main source of texture problems. Being white and in poor color contrast with the surrounding flesh, it is less readily

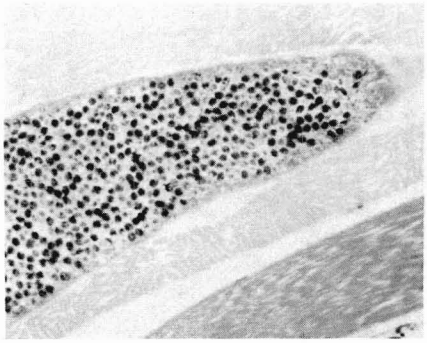

Figure 3.—A histological section through an infected muscle fiber showing an elongated cyst packed with myxosporidian spores (190×).

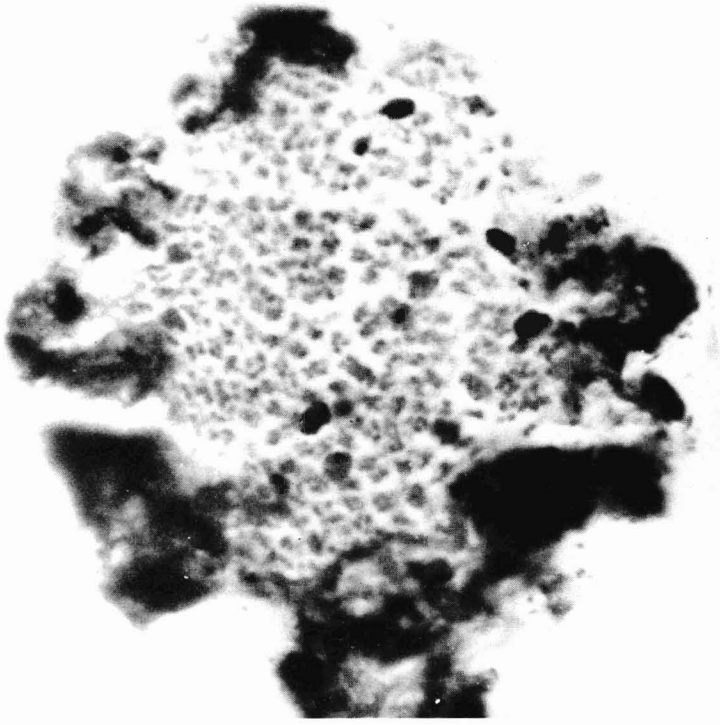

Figure 4.—A histological section through an individual cyst showing numerous myxosporidian spores within and the deposition of dark pigmented material on the periphery (300×).

apparent on visual inspection; thus such fillets are more difficult to cull than those containing black cysts. The microscopic cysts are usually found to be young, active, and growing.

Since it is impractical to cull whiting fillets containing either white-colored or microscopic cysts, they may be present in most processed whiting. This is essentially the nature of the texture problem in whiting and the basis for our research on control through handling and processing. The problem will have to be controlled by time-temperature handling by the catcher vessel, processor, and consumer to minimize the enzymatic activities that result in the adverse texture in the cooked product. Inspection and elimination of fillets with readily apparent cysts should be done largely for esthetic reasons and to eliminate severely parasitized, potential problem fish.

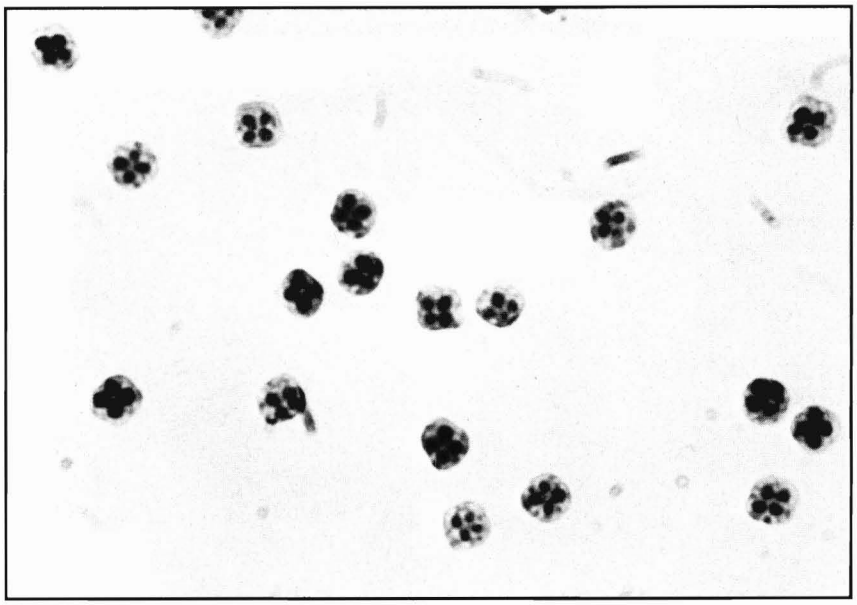

Figure 5.—The small myxosporidian spore in Pacific whiting (*Kudoa* sp.) as seen in aqueous wet mount made from cooked or raw flesh (700×).

Morphology and Structure of Myxosporidia

The classification and identification of the myxosporidia are based almost entirely upon the structure of the spore. The myxosporidian spore in whiting (*Kudoa* sp.) is best seen in an aqueous wet mount containing a small quantity of flesh fibers teased from either cooked or raw flesh and viewed at 450× or 1,000× magnification. Samples of mushy cooked flesh invariably show copious quantities of typical spores. The typical spore commonly seen associated with Pacific whiting is a small 4-6 μm spore containing four oval-shaped polar capsules. Microscopically, it appears as a square with rounded corners for four equally sized ovoid capsules (Fig. 5). About 30 percent of the whiting samples may display a coinfection with a large myxosporidian spore 8-18 μm in size containing 4 subequal-sized elongated capsules encapsulated in a starlike 4-pointed sheath. Histological sections of the two types of myxosporidia found in Pacific whiting, one being about twice the size of the other, are shown in Figure 6.

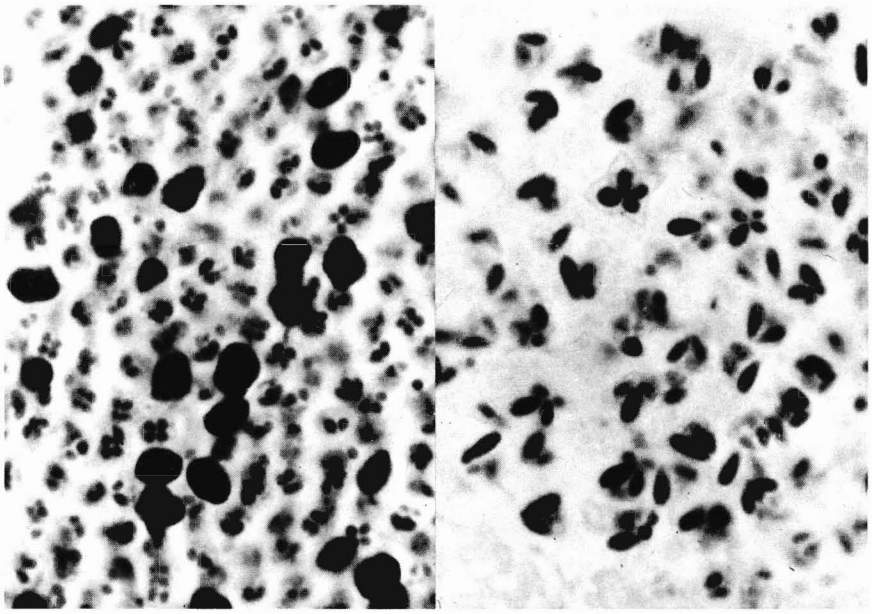

Figure 6.—Histological sections through highly parasitized Pacific whiting showing the two types of myxosporidia: The small *Kudoa* sp. (4-6 μm) and the large *Kudoa* sp. (8-18 μm). The larger, dark-stained bodies associated with small spore are likely a transition or presporulation stage which is periodically seen (540×).

Structural details of the small spore are shown in the electron microscopic enlargement in Figures 7 and 8. The sectional views show two of the four polar capsules, the coiled filaments, and

Figure 7.—Electron microscope enlargement of small spore showing two of four capsules and other structural details (37,500×).

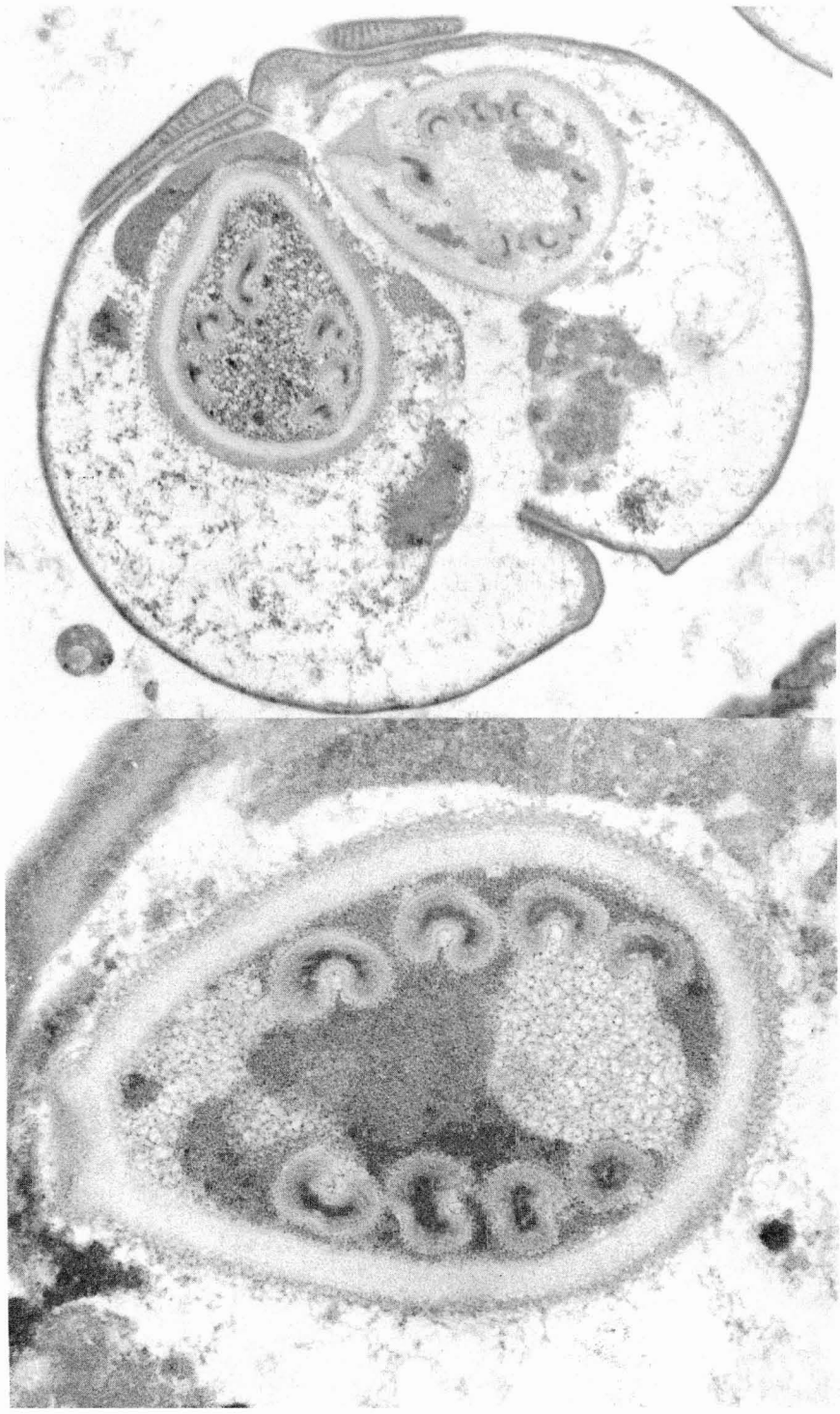

Figure 8.—Electron microscope enlargement of small spore showing two of four capsules and spiral filaments (35,000×).

a very heavy, protective outer spore wall. Exposure of the spore to 5 percent KOH in a wet mount causes a vigorous extrusion of the coiled filament. The spore may extrude one, two, three, or all four of its filaments, one for each capsule. Spores exposed to long-wave ultraviolet light for 2½ hours or to 0.2 Mrad irradiation still retain the ability to extrude filaments on treatment with 5 percent KOH. Similarly, spores frozen over extended periods of time (over 1 year) retain this capability. The spores of cooked whiting lose this capability. Whether this phenomenon is related to spore viability is not known.

Heavily parasitized raw whiting fed to mice over a 6-week period were not found to invade the musculature or any vital organs. The excreta showed the typical spore. Spores from the excreta subjected to 5 percent KOH displayed the typical filament extrusion characteristic it did prior to feeding.

Muscle destruction in parasitized whiting proceeds more rapidly in unrefrigerated than in refrigerated whiting held at 32°F. To examine the various stages of muscle destruction, severely parasitized whiting were held at ambient temperature about 16°C (61°F) and were sampled at 0.5, 7, 31, and 48 hours of storage aboard the RV *Profesor Siedlecki* (August 1977) and preserved for later microscopic and photographic examination.

In samples taken at 0.5 hour of ambient storage, cysts are located intramuscularly and are intact (Fig. 9). Most of the surrounding muscle is normal in appearance.

The samples taken after 7 hours of ambient storage (Fig. 10) still show intact cysts. However, the surrounding muscle is beginning to show structural disorientation.

The samples taken after 31 hours of ambient storage (Fig. 11) show the cyst wall breaking down and discharging its spores, with extensive damage of the surrounding muscle. Spores discharged from the cyst are seen in the intermuscular spaces.

The samples taken after 48 hours (Fig. 12) show few intact, walled-in cysts and the associated vast muscle damage.

We must note, however, that cysts in

Figure 9.—Histological section showing the initial normal relation of the intact cyst and the muscle fiber in severely parasitized Pacific whiting after 0.5-hour storage at ambient temperature of 16°C (61°F) (295×).

Figure 10.—Histological section showing the early phase of muscle disorientation and damage (arrow) with the cyst relatively intact in severely parasitized Pacific whiting after 7 hours of storage at ambient temperature of 16°C (61°F) (295×).

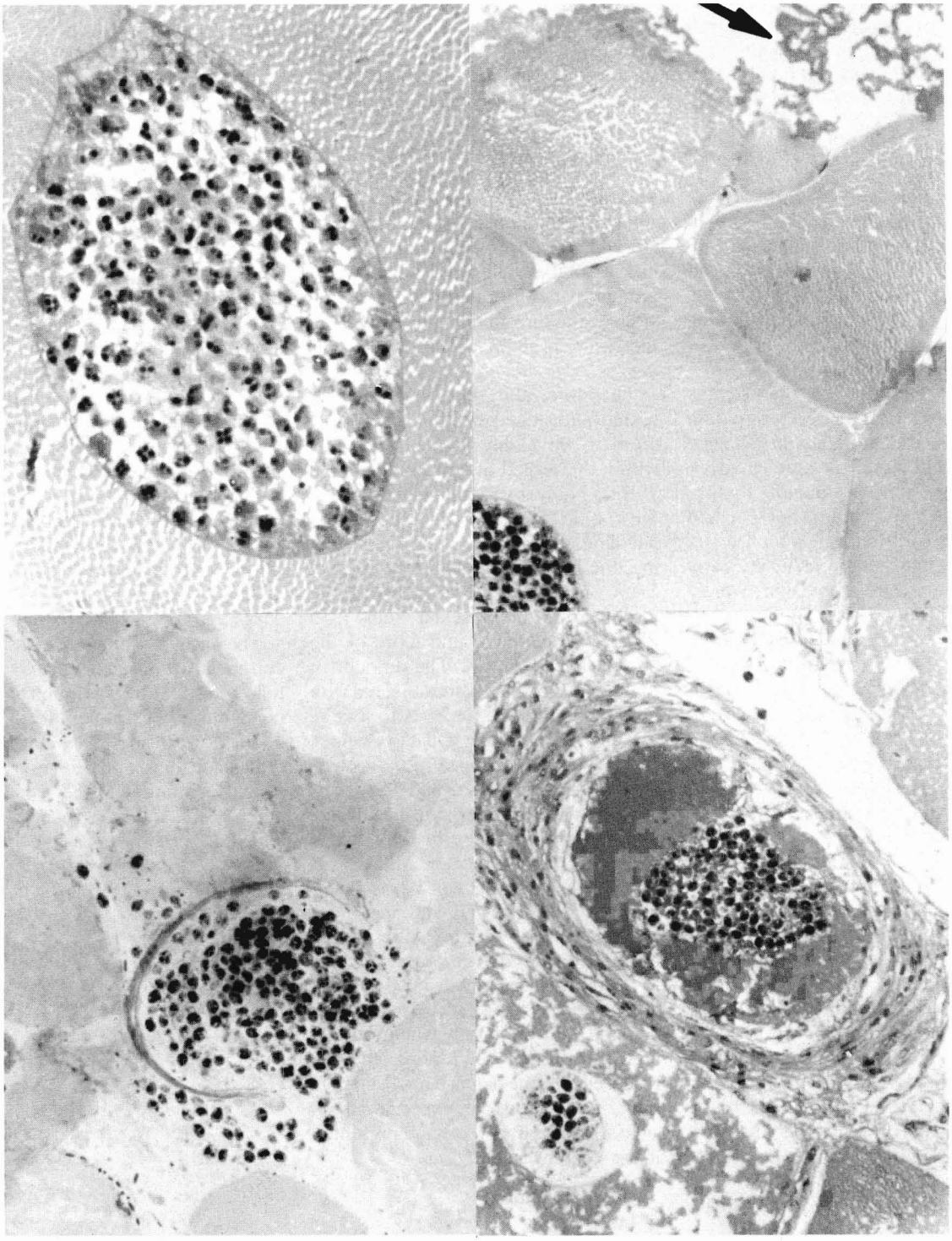

Figure 11.—Histological section showing the cyst wall breaking down with extensive damage of the surrounding muscle and the spores discharged from the cyst in the intermuscular spaces in severely parasitized Pacific whiting after 31 hours of storage at ambient temperature of 16°C (61°F) (295×).

Figure 12.—Histological section showing few intact walled-in cysts with the associated vast muscle damage in severely parasitized Pacific whiting after 48 hours of storage at ambient temperature of 16°C (61°F) (295×).

individual fish are not of the same developmental stage or age and therefore are not uniform in their proteolytic activity, nor are they uniformly distributed. Thus, deterioration and muscle damage will proceed at different rates in different parts of the fish. This can be observed microscopically.

Mechanism of Abnormal Texture Development

Observations over 15 years on the development of abnormal texture in Pacific whiting that has been given various treatments and processed fresh and frozen at sea have permitted us to formulate a working hypothesis. The following findings may be set forth in support of this hypothesis:

1) Early tests showed that steam-cooked subsamples of severely parasitized whiting landed aboard the RV *John N. Cobb* and stored at ambient deck temperature were normal in texture during the first 3 hours but became abnormally soft to mushy after 4 hours of storage, indicating a relatively early onset of the texture problem in severely parasitized fish.

2) Proteolytic activity was demonstrated in severely parasitized whiting aboard the RV *Profesor Siedlecki* in September 1977 within the localized, walled-off parasitic cyst containing the spores and was detected in the blood of severely parasitized whiting but not in the adjacent parasite-free muscle fibers. This suggests that the enzyme is removed from the parasitized muscle fiber by the blood during life, but after death this capacity is lost.

3) Using fresh, severely parasitized whiting with white cysts only and 3-hour iced fish with both white and dark cysts, we observed in collaborative work aboard the RV *Profesor Siedlecki* in August 1977 that the myxosporidian spores separated out by bacterial filter did not generate significant proteolytic enzymic activity during incubated storage at 40°C (104°F) for 6 hours. We concluded that the spores per se were not the source of proteolytic activity after the death of the host fish.

4) The initial level of proteolytic activity per unit of flesh in parasitized whiting was determined in collaborative work aboard the RV *Profesor Siedlecki* in August 1977. We found, after samples were stored in ice for 60 hours or at ambient deck temperature for 36 hours (Schwartz et al., 1978), that proteolytic activity per unit of flesh did not increase significantly. Rather, it appeared to be a fixed amount which spread through the flesh during storage.

5) Microscopic examination of parasitized whiting stored at ambient temperature (RV *Profesor Siedlecki*, September 1977 sampling) showed the muscle and walled-off cysts to be initially intact. As the walled cyst containment gradually deteriorated with time of storage, the surrounding muscle showed increasing proteolytic breakdown (Fig. 9-12).

Therefore, the proteolytic activity within parasitized whiting is hypothesized to be highly localized initially within the walled-in cyst containment, affecting only a very minute portion of the muscle biomass. During subsequent handling, storage, processing, and cooking, the active proteolytic enzyme diffuses out of the cyst, and with the sarcoplasmic and vascular fluids as a carrier, affects more and more of the muscle fiber biomass. The amount of muscle biomass ultimately affected with proteolytic enzyme depends to a large extent on the type of cyst (dark vs. white), the cyst density (severity of parasitization) of the host fish, and the time-temperature handling of the fish. In general, the more rapidly the whiting are chilled, processed, frozen, and cooked, the less fish muscle will be adversely affected with enzyme and the more normal will be its texture. After muscle texture is damaged by enzyme action due to poor storage, extremely rapid cooking is of little value to protect texture. Rapid cooking is of value only when refrigerated storage has been good, and the localized enzyme damage has been minimal. A few typical experiments in support of this hypothesis are presented in the next few subsections.

Texture Evaluation of Live Whiting Frozen in Dry Ice

One of the difficult problems in research on abnormal texture of Pacific whiting has been the wide variance within fish and between fish in the treatment groups, making it difficult to replicate and interpret results. The differences of greatest concern in whiting have been due to the wide variation in parasite incidence and severity of the infection between lots.

To minimize the variance, 18 live whiting were frozen aboard vessel between slabs of dry ice to serve as a quality baseline for superimposing test variables. Each whiting was cut into two lateral halves: One half for microscopic classification of severity of parasitization and the other half for imposing test variables. The half sides were cut into 24-28 3/8-inch slices and placed sequentially into treatment groups as shown in Figure 13. The individual whiting were classified on the basis of severity of infection as follows: 1) None to slight, 2) slight to severe, and 3) severe. Test variables included preparation by the slow oven-cooked and the fast deep-fat-fried methods of whiting portions for texture evaluation. Portions were cooked from the frozen state and after thawing and holding for 1 and 2 days at 33°F.

The results (Table 6), classified according to these categories, show the following: Texture was found to be independent of the treatment for the none-to-slightly parasitized whiting. Texture ratings for the slightly-to-severely parasitized whiting showed insignificant decreases for the various treatments including the slow or oven-cooked and the fast-cooked, deep-fat-fried portions.

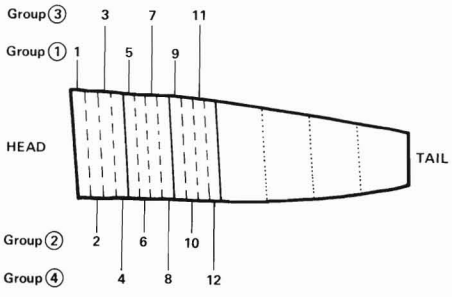

Figure 13.—Sampling sequence for dry-ice frozen Pacific whiting (24- to 28⅜-inch slices, sequentially placed in 4 treatment groups of 6 or 7 slices per whiting per treatment.

Table 6.—Effect of various treatments on the average texture of live Pacific whiting frozen in dry ice (unparasitized to severely parasitized).

Parasite spore[2] intensity	Texture[1]				
	Dry-ice frozen control, not thawed		Thawed, held 1 day at 33°F refrozen		Thawed, held 2 days at 33°F, refrozen
	Oven[3] cooked	Deep-fat[4] fried	Oven[3] cooked	Deep-fat[4] fried	Deep-fat[4] fried
None to slight: 1-3 (average for 6 hake)	4.5	5.0	4.8	4 8	4.8
Slight to severe: 3-5 (average for 6 hake)	4.4	4.8	4.3	4.8	4.5
Severe: 5 (average for 6 hake)	3.5	4.7	2.0	3.3	3.0

[1]Texture: 5 = normal to firm; 4 = normal to soft; 3 = soft; 2 = overly soft; 1 = mushy, pasty.
[2]Parasite spore intensity (microscopic wet mount examination of cooked flesh): 1 = none; 2 = trace; 3 = slight; 4 = moderate; 5 = severe
[3]Oven-cooked samples were heated at 400°F for 18 minutes (slow cooked).
[4]Battered-and-breaded, ⅜-inch-thick frozen slices were deep-fat fried at 385°F for 3 minutes (fast cooked).

Table 7.—Effect of various treatments on the average texture of live Pacific whiting frozen in dry ice (severely parasitized).

Parasite[2] spore intensity	Texture[1]				
	Dry-ice frozen control, not thawed		Thawed, held 1 day at 33°F, refrozen		Thawed, held 2 days at 33°F, refrozen
	Oven[3] cooked	Deep-fat[4] fried	Oven[3] cooked	Deep-fat[4] fried	Deep-fat[4] fried
Severe: 5	3 5	5 0	2.0-2.5[5]	4.0	3.8
"	1.0-3.0	5 0	1.0	2.0-2.5	1.0-2.5
"	2.0-3.0	4 8	1.0-1.5	2.8	2.0-3.5
"	4.3	5 0	2.0-3.0	3.0	2.0-3.0
"	4 3	4 8	2.0-2.5	3.5	1.0-4.0
"	4.3	3 5	2.0-3.0	4.0	3.8
Overall average	3.5	4 7	2.0	3.3	3.0

[1]Texture: 5 = normal to firm; 4 = normal to soft; 3 = soft; 2 = overly soft; 1 = mushy, pasty.
[2]Parasite spore intensity (microscopic wet mount examination of cooked flesh): 1 = none; 2 = trace; 3 = slight; 4 = moderate; 5 = severe.
[3]Oven-cooked samples were heated at 400°F for 18 minutes (slow cooked).
[4]Battered-and-breaded, ⅜-inch-thick frozen slices were deep-fat fried at 385°F for 3 minutes (fast cooked)
[5]Underscore = unacceptable texture in one or more slices; range indicated.

Texture ratings for the severely parasitized whiting varied significantly according to the treatment and clearly showed the advantage of fast cooking from the frozen state and the texture degradation caused by holding the thawed portions.

Since the severely parasitized whiting are the main source of the texture problem, the data are shown for each of the individual fish (Table 7). The slow oven-cooking of the frozen control produced one or more slices with unacceptable textures in two of the six severely parasitized whiting, but none in the fast, deep-fat-fried whiting. When the whiting were thawed for only 1 day at 33°F, the number of whiting with unacceptable textures increased to six of six when slow oven-cooked, but to one of six when fast cooked. When the whiting were thawed and held for 2 days at 33°F, the number of portions with unacceptable textures increased to four of six, even when deep-fat fried. It is apparent that severely parasitized whiting are highly prone to proteolytic softening depending on their treatment, but that not all severely parasitized whiting are equally affected.

The tests clearly show that variability in the severity of the infection within and between fish will determine their actual predisposition to proteolytic softening during either slow or fast cooking. Poor handling practice will intensify this predisposition, while good handling practice will overcome or moderate it.

Texture Evaluation of Frozen Fillet Block Portions

In this experiment, fillet block portions were prepared at sea from whiting obtained under commercial catching and handling conditions. The whiting were taken between Astoria and Coos Bay, Oreg., about 12 miles off the coast, and over one-third of the fish were found to be 100 percent parasitized on microscopic examination. The objective was to compare 1) the effect of thawing and 1-day storage at 33°F on the texture of portions obtained from whiting filleted and frozen 3-4 hours after catching, and 2) the effect of slow vs. fast cooking. Paired test portions were cut 3/8-inch thick from the frozen fillet block for the controls and treatment (Table 8).

Comparison of 38 portions prepared and cooked from the frozen state by the slow oven-cooked method with 38 portions allowed to thaw for 1 day at 33°F prior to cooking showed a significant increase from one to six portions with unacceptable texture. A similar comparison with paired lots of 39 portions that were fast cooked by deep-fat-frying showed no problems with unacceptable texture in either lot. In spite of the high level of parasitization of these whiting, the portions prepared by fast cooking before and after thawing had textures within the acceptable range in both cases. The results with the slow-cooked portions again demonstrated that the potential to develop the unacceptable texture was present and is a limiting characteristic in holding and cooking the portions.

Table 8.—Texture of cooked fillet block portions prepared from 3-4 hour commercially landed Pacific whiting.

Item	Texture treatments			
	Oven cooked[2]		B/B[3], deep-fat fried	
	Not thawed (control)	Thawed, held 1 day at 33°F	Not thawed (control)	Thawed, held 1 day at 33°F
No. of paired portions	38	38	39	39
Texture range[1]	2-5	1-5	4-5	3-5
Avg. texture	4.4	3 6	4.9	4.4
No. of unacceptable textures of 1 and 2	1	6	0	0

[1]Texture: 5 = normal to firm; 4 = normal to soft; 3 = soft; 2 = overly soft; 1 = mushy, pasty.
[2]Oven-cooked samples were heated at 400°F for 18 minutes (slow cooked).
[3]Battered-and-breaded ⅜-inch-thick frozen slices were deep-fat fried at 385°F for 3 minutes (fast cooked).

Effect of Refrigerated Storage on the Texture of Commercial Fillets

Some processors have considered fresh fillet marketing of whiting. Typical data from one study using 2-day-iced commercial fillets is presented to indicate the texture problem in fresh fillet marketing. The fillets were carefully screened and divided into visually normal and parasitized fillets and frozen.

Paired subsections were cut from each fillet and slow oven-cooked initially and, to simulate marketing conditions, after thawing and holding for 5 days at 34°F. The texture ratings of the normal and parasitized fillets (Table 9) show clearly the extent of the problem. The normal fillets had acceptable texture initially and after 5 days of refrigerated storage at 34°F. In contrast, the parasitized fillets initially had 9 of 42 fillets with unacceptable soft texture and, after 5 days of storage at 34°F, had 16 fillets with unacceptable texture. This experiment emphasizes the risk of marketing whiting in fresh fillet form, since it is impractical to cull out the severely parasitized whiting. As shown earlier, the fast-cooked or deep-fat-fried portion-type product appears to be the only feasible option to eliminate a high percentage of product rejected for unacceptable, soft to mushy texture.

Table 9.—Effect of refrigerated storage on the texture of 2-day-iced commercial fillets (normal vs. parasitized).

	Number in each texture category			
	Normal fillets		Parasitized fillets	
Oven-cooked[1] texture ratings	Initial	After 5 days at 34°F, refrozen	Initial	After 5 days at 34°F, refrozen
5 = Normal to firm	32	28	8	7
4 = Normal to soft	19	21	21	18
3 = Soft	0	2	4	1
2 = Overly soft	0	0	3	5
1 = Mushy, pasty	0	0	6	11
Total fillets[2]	51	51	42	42

[1]Oven-cooked samples were heated at 400°F for 20 minutes.
[2]Each of the 51 normal and the 42 parasitized fillets were cut into 3 or 4 paired subsections for the initial and 5-day test groups.

Conclusions

The abnormal muscle texture in Pacific whiting is caused by a myxosporidian-induced proteolysis. The latent potential for proteolytic textural softening in whiting, due to the presence of myxosporidian cysts of variable intensity, appears to be an intrinsic characteristic of the Pacific species. The enzyme appears to be highly localized within the cyst and has little effect on the muscle fibers if the fish have been rapidly chilled, processed, and rapidly cooked. We recommended that whiting be marketed as a fast-cooked or deep-fat-fried, portion-type product, limited to about 3/8-inch in thickness.

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