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Title: INFECTION, HISTOPATHOLOGY AND MIGRATION OF *Anisakis* sp. (NEMATODA: HETEROCHEILOIDEA) IN PACIFIC HERRING (*Clupea harengus pallasi*) FROM YAQUINA BAY, OREGON

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Richard A. Tubb

Infection, histopathologic, and migration studies of *Anisakis* sp. larvae Dujardin, 1845 were conducted by examining 235 Pacific herring. The intensity and incidence of *Anisakis* larvae increased with age and size of fish. Histopathological tissue alterations included mechanical compression of the pancreas and liver, liver granulomatous inflamation and necrosis, and trauma to the muscularis externa of the pyloric caeca. Host exudate in most lesions contained macrophages and other inflammatory cell types, indicating a chronic pathological condition in the host. Fish of the migration study were divided into five groups to test the effects of various methods of handling and processing (fresh, frozen, brined, cold smoked, and cold smoked-gibbed) on survival of *Anisakis* larvae and

their occurrence in the flesh. Parasite loads were statistically largest in the musculature of frozen, brined, and smoked fish. Smoked fish harbored the greatest proportions of larvae in the musculature. Human consumption of brined or cold smoked Pacific herring represents a potential public health hazard.

Infection, Histopathology and Migration of *Anisakis* sp. (Nematoda: Heterocheiloidea) in Pacific Herring (*Clupea harengus pallasi*) from Yaquina Bay, Oregon

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INTRODUCTION AND PURPOSE OF STUDY

Anisakis sp. larvae have a wide distribution in many species of marine fishes, which serve as paratenic, intermediate hosts. *Anisakis* larvae are considered a relatively permanent part of the parasitic fauna of herring (*Clupea* sp.) in both Atlantic and Pacific Oceans. Relatively little is known of the inflection of *Anisakis* sp. larvae in benthic and pelagic fishes distributed in waters adjacent to the Pacific and Atlantic coasts of the United States. Information on the abundance of *Anisakis* larvae in host fishes is valuable in assessing the potential for larval infection in North Americans.

Anisakis larvae were first recognized as potential human pathogens in Holland in 1955 (Kuipers et al., 1960; Van Thiel et al., 1960). Later the term "anisakiasis" was proposed for the "herring-worm disease" (Van Thiel, 1962). The severe gastrointestinal syndrome associated with anisakiasis was subsequently reported from Japan (Yokogawa et al., 1962), from other European locations (Davey, 1972), and from the United States (Pinkus and Coolidge, 1975). Alterative changes in human anisakiasis have been classified as eosinophilic phlegmonous enteritis (Sindermann, 1970).

The pathological effect of *Anisakis* larvae accompanying

anisakiasis has been thoroughly documented in man and in both experimental and wild animals (Myers, 1963; Oishi et al., 1969; Young and Lowe, 1969; Migaki et al., 1971; Riley, 1972). However, relatively little is known of the pathological effect of Anisakis larvae upon fish hosts.

The presence of Anisakis larvae in the musculature of fish at capture and the larval migration into the flesh of fish hosts have been controversial subjects documented by a number of investigators (Van Thiel et al., 1960; Roskam, 1960; Vik, 1966; Khalil, 1969; Davey, 1972). Smith and Wooten (1975) subsequently reported a large-scale migration of Anisakis larvae into the musculature of ungutted herring which had been held on ice. Although various investigators have examined the effects of brining and smoking on larval viability (Khalil, 1969; Davey, 1972), percentages of viable and nonviable larvae, migrating into the flesh of either brined or lightly smoked fish, have not been documented.

The purpose of this investigation was to examine the infection, migration, and viability of Anisakis larvae in the musculature of Pacific herring in fresh, frozen, brined, and cold smoked condition. The histopathology of Anisakis in the fish was also documented.

REVIEW OF LITERATURE

Taxonomy and Life History of *Anisakis* Larvae

Anisakis sp. larvae can be most easily distinguished from other genera of nematodes by the division of the esophagus into an anterior muscular portion and a posterior ventriculus. The ventriculus is clearly delineated between the muscular esophagus and the intestine. Yorke and Maplestone (1926) and Oishi et al. (1969) gave accurate anatomical descriptions of *Anisakis* larvae. Chitwood and Lichtenfels (1972) provided important methodology for identifying *Anisakis* larvae in tissue sections.

Although 21 separate species of *Anisakis* have been identified, some descriptions were so inaccurate that specific determination was impossible (Grainger, 1959). Attempts to clarify the taxonomy of the larvae were made by Punt (1941), Baylis (1944), Johnston and Mawson (1945), Dollfus (1953), and Oishi et al. (1969). By successfully rearing the larvae to preadult stage, Grainger (1959) positively identified the larvae as belonging to the genus *Anisakis*. Subsequently, Berland (1961) identified two types of larvae on the basis of ventricular lengths and called them *Anisakis* larva (I) and *Anisakis* larva (II). Van Thiel (1966) studied larvae collected from sea mammals of the North Sea and South Atlantic and proposed that anisakids from these regions

belong to the single species A. marina. Van Thiel did not provide evidence in support of his proposal, and Khalil (1969) believed it should be rejected. Otsuru et al. (1968) reported a third larval type which they designated Anisakis larva (III). And Pippy and Van Banning (1975) successfully identified Anisakis larva (I) as A. simplex. Davey (1971), in revising the genus Anisakis, recognized only three valid species, these being A. simplex (Rudolphi, 1809 det. Krabbe, 1878), A. typica (Diesing, 1860), and A. physeteris (Baylis, 1923). Four species were retained as species inquirendae for lack of sufficient data.

According to Punt (1941), Anisakis larvae have been taken from an unidentified species of copepod. Oishi et al. (1969), quoting Oshima (1966), postulated the life cycle of Anisakis larvae with a crustacean as the first intermediate host and fish and squid as the second intermediate hosts (paratenic). Marine mammals belonging to the suborder Pinnipedia and the order Cetacea have long been recognized as definitive hosts for Anisakis sp. (Dollfus, 1948). Oishi et al. (1969) found that the second stage larva parasitizes crustacean hosts.

In recent years crustaceans from the family Euphausiidae have been implicated as the first intermediate hosts of Anisakis sp. Smith (1971) recorded Thysanoessa inermis and T. longicaudata from waters north of Scotland as having an incidence of infection ranging from

0.5 to 4.0%. Sluiters (1974) found T. inermis, T. raschii, and Meganyctiphanes norvegica of the North Sea to harbor Anisakis larvae.

Oishi et al. (1969) asserted that third stage larvae are parasitic in fish hosts. According to Sindermann (1961), Anisakis larvae may be considered relatively permanent in herring (Clupea sp.). Accounts of incidence of infection of Anisakis larvae in fish hosts are many (Johnston and Mawson, 1945; Rees, 1946 and 1953a; Gusev, 1958; Ichihara, 1968; Kamegali, 1971; Grozdilova, 1974; Yamaguchi, 1966; and Oishi et al., 1969).

Studies of the occurrence of Anisakis larvae in the Cetacea and Pinnipedia are extensive. The fourth stage larvae and adults normally parasitize marine mammals of these groups. Schroeder and Wegeforth (1935), Caballero (1940), Campana-Rouget (1955), Kagei et al. (1967), Machida (1969 and 1971), Gembardt et al. (1971), and Dailey and Brownell (1972) are some investigators of Anisakis-pinniped relationships. Studies of cetacean hosts include Crusz (1946), Doan and Douglas (1953), Rees (1953b), Margolis (1955), Bishop and Margolis (1955), Lopez-Neyra (1958), Kagei et al. (1967), Kikuchi et al. (1967), Dollfus (1968), Dailey (1971), Dailey and Brownell (1972), Young (1972), and Machida (1974). Margolis (1954) found A. simplex, A. similis, and A. physeteris to be commonly associated with Cetacea off British Columbia, California, and Alaska, respectively. Oishi et al. (1969) stated that Anisakis larvae are more prevalent in

cetaceans than pinnipeds, and A. simplex is the most common species infesting the order Cetacea. Bisseru (1975), quoting Kagei et al. (1967), considered dolphins to be the most important final host of Anisakis.

Physical Factors Affecting Anisakis Larvae

Reports of temperatures and salinity concentrations affecting the survival of Anisakis larvae are in conflict. Gustafson (1953) discovered that temperatures of -17 C and -30 C effectively killed all larvae in time periods of 24 hrs and 5 min, respectively. Khalil (1969) found them capable of living for 48 hrs at -10 C, and Nygard (1967) disclosed that the larvae survived 4 to 5 days at -20 C. Lee and Chyu (1970) demonstrated a decrease in infectivity with lower temperatures. Davey (1972) observed that the upper temperature limit at which Anisakis could survive was 50 C. This was based on observations of smoked herring. Davey (1972) showed that Anisakis sp. could withstand temperatures up to 60 C during the cooking of fillets. Khalil (1969) discovered Anisakis sp. could withstand 55 C temperatures in saline solutions for 10 sec. But Van Thiel et al. (1960) learned that anisakids were rendered harmless by exposure to 50 C for 10 sec.

Nygard (1967) found Anisakis larvae survived in a brine of 33 g/l NaCl for 7 to 8 days. Khalil (1969) observed that dry salt killed Anisakis larvae in 10 min; in 200 g/l saline concentration, larvae

lived for 2 days. However, survival in 50 g/l saline was indefinite. Khalil also showed that 24 hr immersion in saturated saline solution was necessary to kill 100% of the larvae.

Chitwood (1970) disclosed that Anisakis larvae survived 25 days in a 5%NaCl and 2% acetic acid solution. Lee and Chyu (1970) found Anisakis larvae resistant at a pH ranging between 4-6, whereas they soon died in alkaline solutions of pH 8-11. Ruitenberg (1970), as quoted by Davey (1972), established that some larvae could survive the usual marinating process using 4% acetic acid and 6% NaCl for 26 days. However, all larvae could not be guaranteed dead at the end of this period even when 7% acid and 15% salt were used. He concluded that herring brined in 22% NaCl could be guaranteed free of living anisakids only after an exposure of 10 days. Oishi et al. (1972) showed only 60% larval mortalities with 1.0 Mrad gamma irradiation in 6% saline. Jahnel (1940), as quoted by Khalil (1969), reported live ascarids in a pickled herring which had been recently purchased. He observed that these larvae revived and survived longer than 2 mo when placed in a physiological saline solution. Khalil (1969) asserted that Jahnel's descriptions showed the worms to be identical to Anisakis sp. larvae.

Davey (1972), quoting Ruitenberg (1970), stated that the normal temperature reached in smoking (kippered) herring is only 28 C. Anisakis larvae were reported in 5% of 1,000 cured and smoked herrings by Khalil (1969). Tolerances of Anisakis sp. to acids,

alcohols, alkalies, formalin, temperatures, and medicines were thoroughly summarized by Oishi et al. (1969).

Incidence and Intensity of Infection

Bishop and Margolis (1955) noted an 80-100% incidence of Anisakis sp. in herring (C. harengus pallasi) caught along the coast of British Columbia. Rae (1963) found the incidence of larvae in cod to vary with locality in Scottish waters. Dogiel (1966) investigated the percent infection of Anisakis larvae in different age groups of cod in various tissues. He noted that incidences vary with both age and encystment location in the fish. Kilamby and Delacy (1967) proposed heterogeneity between populations of surf smelt, Hypomesus pretiosus (Girard), in coastal waters off Washington State because of varying levels of infection with Anisakis sp. Okumura (1967) reported incidences of 40% and 25% of Anisakis larva (I) in 30 species of fishes and four species of squid purchased at Osaka (Japan) fish markets. Khalil (1968) asserted that 55% of North Sea herring are infected with Anisakis sp. Oishi et al. (1969) noted intensities ranging from 55-88% in C. pallasi from various Japanese waters. Khalil (1969) noted a larval incidence of 34% in herring of British coastal waters. Quoting Burd (1968, personal communication), Khalil observed a larval incidence of 50% from NW Ireland. Mehl (1970) found the incidence of infection of Anisakis in barracouta (Thyrsites

atun) from eastern Cook Strait to be 6%. Nobel (1970) found 14 species in midwater fishes taken from the eastern Pacific to have a combined Anisakis larval incidence of 8.1%. Saito et al. (1970) surveyed eight species of fishes and cuttlefish of Hokkaido, Japan, and discovered a combined infection ranging from 0-100%. Parsons and Hodder (1971) showed that the incidence of Anisakis sp. in herring from Canadian Atlantic waters increased with northward direction. Sakaguchi and Katamine (1971) found Anisakis larva (I) to have the highest incidence in 68 fish species taken from the East and South China Seas. Rae (1972) revealed that the incidence of Anisakis sp. had increased in North Sea and in offshore waters west of Scotland in recent years. Reimer and Jessen (1972) noted that percentages of infection of Anisakis larvae were higher in western than eastern North Sea herring. They asserted that Anisakis sp. infections have increased considerably in the North Sea during the last decade. Rokicki (1972), reporting on the first-known record of Anisakis sp. in Baltic Sea herring (C. harengus L.), noted an incidence of 31%. Pennell et al. (1973) found the combined incidence of Anisakis and Contracaecum spp. Nematoda in sockeye salmon (Oncorhynchus nerka) of Bristol Bay, Alaska to be 100%. Dailey (1971) reported A. simplex as new to the West Coast of the U.S. His information was based on studies of the distribution of helminths in the dall porpoise (Phocoenoides dalli True).

Bishop and Margolis (1955) noted an increase in infection of Anisakis larvae in British Columbia herring with age. They asserted that the intensity remained the same through winter for any particular age and area and was identical for both sexes. In 1957 the International North Pacific Fisheries Commission reported that western (Pacific) salmon had a higher intensity of infection than the eastern (Pacific) salmon. Vik (1966) examined a number of Scandinavian fishes and found an average load of at least 10 larvae in both herring and mackerel; salmon harbored fewer. Okumura (1967) noted an increase in Anisakis sp. intensity with length in 12 fish species of Japanese waters. Khalil (1968) disclosed that intensity of Anisakis larvae varies with North Sea locality. Quoting Burd (1968, personal communication), Khalil noted larval intensities in NW Ireland herring to be only 1.6 larvae per fish. Hodder and Parsons (1971), analyzing data on intensity and incidence of infection of Anisakis sp. and differences in pectoral fin-ray and gillraker numbers, indicated that herring from southern Gulf of St. Lawrence stocks intermingle, at most, insignificantly with stocks of Chedabucto Bay-Canso Bank and of Banquereau. Parsons and Hodder (1971) found increases in intensity and incidence of infection with fish age (size).

Migration of Anisakis Larvae and Anisakiasis

Anisakis larvae are histozoic in fishes, living often encapsulated

in body musculature, in internal organs, or on mesenteries (Margolis, 1970). According to Khalil (1968), 6.5% of larvae leave cysts and wander on various organs; 0.7% penetrate musculature and roe where they may become partially or completely embedded. When radioactively labelled Anisakis larvae were fed to haddock (Melanogrammus aeglefinus) and whiting (Merlangius merlangus), Smith (1974) recorded that the larvae were first seen in the body cavity after 24 hrs. He noted that a capsule appeared by 34 hrs. Smith and Wooten (1975) noted higher percentages of Anisakis sp. in the hypaxial musculature of herring (C. harengus L.) which were gutted 14 and 37 hrs after capture than in herring which were gutted immediately.

Myers (1962 and 1963) did not find a definite migrational pattern of Anisakis larvae in guinea pigs. Larvae were recovered from the stomach, liver, mesenteries, pancreas, small intestine, large intestine, caecum, thyroid gland, perirenal fatty tissue, and from subepidermal cysts. He noted that recovered larvae were capable of reinfecting another animal. Asami and Inoshita (1967) reported that larvae approximately 2.4 cm in length had maximum infectivity. However, some degree of infectivity still persisted in larvae which had been cut in half. They maintained that infectivity depends on the larvae, rather than on the susceptibility of the host. Okumura (1967) indicated that while 60% of larvae were excreted in 72 hrs from infected rats, 40% invaded the gastrointestinal wall and migrated

throughout the viscera. No affinity was shown for any specific organs. Nagase (1968) showed that larvae were capable of penetrating the stomach wall of rats within 1 hr and of migrating into the abdominal cavity after 3-4 hrs. Sensitization of the rats with either living larvae or with larval homogenate indicated no influence upon subsequent invasion. Wu (1970) revealed that Anisakis larvae, introduced into the Taiwan monkey (Macaca cyclopis), infected the gastrointestinal tract, migrated to the liver, the omentum, the mesenteries, the diaphragm, and the abdominal cavity. Kikuchi et al. (1970) found that Anisakis larva (II) taken from mackerel differed from those from bonito in affinity to location of infections in puppies. They also noted that larva (II) in infected rabbits had either attached to the stomach wall or were free in the stomach and intestinal lumen. Ruitenberg et al. (1971) noted that only a few larvae penetrated the stomachs of rabbits. Hashiguchi and Takei (1975) reported that 15% of Anisakis larvae, which had been exposed to temperatures of 65 C for 1-5 min. recovered and were still infective.

The public health importance of the herringworm became known when Kuipers et al. (1960) and Van Thiel et al. (1960) correlated Anisakis larvae with a severe abdominal syndrome of man (anisakiasis) in Holland. The initial reports of aniskiasis possibly resulted because of altered fish processing methods in The Netherlands. In 1955, herring were held on ice and gutted and cured on

shore instead of being eviscerated and cured at sea (Van Thiel et al., 1960). After fish were chilled at sea, some of the larvae penetrated the abdominal wall, where they remained after the fish were gutted. Human infections resulted from the custom of eating slightly salted or raw fish (Van Thiel and Van Houten, 1967). According to Chitwood (1970), 10 patients in Holland required surgery between 1955 and 1959 because of sudden, violent gastrointestinal upsets caused by anisakid larvae. Van Thiel and Van Houten (1967), Kuipers (1967), and Merkelbach (1967) reported cases of anisakiasis in Holland that were incorrectly diagnosed as pyloric stenosis.

Aniskiasis had emerged in recent years as a serious public health problem in Japan, where raw fish are commonly consumed (Yokogawa et al., 1962; Yamaguchi et al., 1964; Otsuru et al., 1965; Yoshimura, 1966; Sakurai et al., 1967; Oishi et al., 1969). One known case of anisakiasis was directly attributed to the eating of pickled fish (Yokogawa and Yoshimura, 1967). Yokogawa and Yoshimura (1965) reported four cases where surgical resect was performed because of the clinical misdiagnosis of gastric ulcers or tumor. The same authors noted in 1967 that one of 92 clinical cases of anisakiasis had been incorrectly diagnosed as acute appendicitis. Asami et al. (1965) found that similar cases of these severe epigastric pains had occurred occasionally over the last 30 years and had been erroneously diagnosed. Ishikura et al. (1967), Wu (1970),

and Oishi et al. (1969) indicated cases where anisakiasis was incorrectly diagnosed as gastric polyp, gastric ulcer, peptic ulcer, chronic and atrophic gastritis, cholelithiasis, cholecystitis, stomach cancer, acute abdominal appendicitis, acute peritonitis, pyloric stenosis, regional ileitis, ileus, and duodenal ulcers. Oishi et al. (1969) reported that 67% of the cases of anisakiasis in Japan are of the gastric type; this is different from the situation in Holland where intestinal anisakiasis prevails (Van Thiel et al., 1960 and Van Thiel, 1962). Oishi et al. (1969) implicated Anisakis larva (I) as the pathogen of anisakiasis. Myers (1975) presented morphological variations of nematode spp. which cause anisakiasis.

Following the initial report of the "herring-worm disease" in Holland, other countries became interested in erroneously diagnosed cases of anisakiasis. Ashby et al. (1964) reviewed the literature on eosinophilic granuloma of the human gastrointestinal tract and added several cases from England. Priebe (1971) recorded one known case from Germany. He stated that 300 cases of the illness had been recorded in the world since 1971, mostly, however, in Japan and Holland. Oishi et al. (1969) stated that there were probably more than 700 cases in Japan between 1962 and 1968. Sindermann (1970) stated that although some deaths associated with suspected anisakiasis have occurred in recent times, they were "invariably" due to complications from exploratory surgery, rather

than from anisakid invasion. Bisseru (1975), quoting Polak (1965), reported that human deaths associated with anisakiasis resulted from fecal peritonitis.

Oishi et al. (1969) listed symptoms of anisakiasis under the categories of gastric and intestinal anisakiasis. The main symptoms of gastric anisakiasis are precordial pain, feeling of plenitude in the precordial region, vomiturition, vomiting, loss of appetite, emaciation, general lassitude, heartburn, vomiting blood, hematochezia, anal blood flow, diarrhea, pleuralgia, notalgia, jaundice, phyma in the abdominal region, irritated peritoneum, or no symptoms.

An account of human infection with Anisakis larvae in the U.S. was made by Hitchcock (1950), who, reporting on intestinal parasites in 100 Eskimos in Alaska, found 10% spontaneously passed larval ascaroids. Larvae were identified with reasonable certainty to be Porrocaecum and Anisakis spp. More recent descriptions of anisakiasis in U.S. citizens have been reported (Richman and Lewicki, 1973; Pinkus and Coolidge, 1975; Jackson, 1975). Today aniskiasis is an established disease entity in man (Brown, 1975).

Yokogawa et al. (1962), Yamaguchi et al. (1964), Otsuru et al. (1965), and Yoshimura (1966) implicated Anisakis larvae in in visceral larva migrans in the alimentary tract of man. Little is known, however, of the migratory habits of Anisakis larvae in man. Yokogawa and Yoshimura (1965), quoting Nishimura (1963),

reported on a nematode larva, ostensibly Anisakis sp., which was taken from a mesenteric abscess. Kuipers (1964) hypothesized that anisakiasis only occurs in cases where two larvae perforate the gastrointestinal wall at separate times. The first penetrating larva initiates the sensitizing response; the second larva causes the secondary, more severe response. He asserted that the probability of two larvae penetrating the wall at the same place at the different times might explain the rarity of the condition. However, Ruitenberg et al. (1971) showed conclusive evidence that this "double hit" theory is invalid by experimentally inducing a severe reaction in rabbits which had been infected once. He concluded that anisakiasis in man may be induced by one worm. Oishi et al. (1969) claimed that the consumption of any constituent substance of the larval body results in anisakiasis. His information was based on the supposition that allergic reactions contribute to the pathogenesis of the condition.

Pathology and Immunological Diagnosis

After migrating through the gastrointestinal tract of fishes, Ascaris capsularia, which later became a synonym for Anisakis salaris (Yamaguti, 1935), was enveloped in a capsule constructed of fibrous tissue (McIntosh, 1864). Berland (1961) and Williams (1967) declared that little is known of the pathological effect of

nematode larvae on fish hosts. Berland (1961) claimed that high intensities of larvae result in a listless, slow host which is less capable of capturing food and more susceptible to predation. Margolis (1970), quoting Prusevich (1964), asserted that the capsule, formed around Anisakis larvae which were experimentally introduced into the sculpin (Myoxocephalus scorpius), was composed of layers of host tissue. This capsule served ostensibly as a barrier in protecting the viable host tissues from products of larval metabolism. Margolis (1970), quoting Mikhaylova et al. (1964), described the capsule as consisting of three layers: a thin innermost layer, a middle layer consisting of degenerative fibroblasts, and an outer layer consisting of loose connective tissue.

Although the majority of larvae are found in the connective tissue capsule, some penetrate deeply into liver parenchyma where extensive destruction of tissue has been reported to occur (Kahl, 1938). Margolis (1970), quoting Brian (1958), did not recognize inflammation or degeneration of infested liver. He asserted that viable Anisakis only cause liver damage through mechanical pressure on the tissue by the parasite. Margolis (1970) quoted Prusevich (1964) as having found an inflammatory reaction to Anisakis larvae which occurred within a few hours after surgically implanting the larvae on the liver of M. scorpius.

Kahl (1938) stated that perforations of the stomach wall might

cause serious results. And Anisakis larvae in body cavities of heavily parasitized Sebastes marinus were purportedly the cause of visceral adhesions which interfered with peristalsis. Arai (1969) found Anisakis larvae in three large ulcerous cavities in the stomach lining of a lingcod, Ophiodon elongatus, taken from British Columbia waters.

Many investigations have been conducted on the pathology of Anisakis larvae in both natural and experimental hosts. Rausch (1953), quoting Hoeppli (1932), described the pathology of larvae in the stomach of a walrus (Odobenus rosmarus L.). Schroeder and Wegeforth (1935) associated A. similis with gastric ulcers in sea mammals (pinnipeds) of the California coast. Gastric ulcers found in sea elephants (Mirounga angustirostris) from Baja California were likewise occupied by A. similis (Caballero, 1940). Machida (1969) noted the attachment of A. similis to the stomach wall of northern fur seals caught in the western Pacific. Gembardt et al. (1971) found that severe inflammation of the stomach wall in a sea lion caused by Anisakis invasion had resulted in pyloric stenosis and death of the animal.

Iwanaga (1970) experimentally demonstrated eosinophilic infiltration in guinea pigs sensitized with Anisakis saline extract. And Wu (1970) showed moderate eosinophilia and no production of eosinophilic granuloma in experimentally infected Taiwan monkeys

(M. cyclopis). Oishi et al. (1969), summarizing the literature of experimental anisakiasis, stated that a local allergic reaction participates in the pathogenesis of anisakiasis in experimental animals. Oishi asserted that intestinal anisakiasis is a secondary allergic reaction caused by reinfection, whereas gastric anisakiasis is a reaction due to a first infection.

Since man is an abnormal host for anisakids, a much more severe pathology may be produced than in the natural host, as was pointed out by Cheng (1965). Sindermann (1970) termed the alterative inflammatory changes of anisakiasis as "eosinophilic phlegmonous enteritis". Oishi et al. (1969), quoting Kojima (1966), further classified the histopathological alterations of anisakiasis into four types: phlegmonous, abscess, abscess-granulomatous, and granulomatous. He maintained that histopathological appearances might suggest an allergic etiology. Oishi et al. (1969) stated that gastric anisakiasis generally appears as the abscess-granuloma type of lesion, while intestinal anisakiasis is mainly a phlegmonous reaction. Oishi compared larval condition with the four histopathological types of anisakiasis. Fairly fresh larvae were found in phlegmonous lesions, slightly degenerated larvae were in abscess lesions, degenerated and destroyed larvae were in abscess, abscess-granulomas, and granulomatous lesions, and moulted parts of larvae were found in abscess and granulomatous-type lesions. He concluded that

these lesions progress from acute exudative inflammation to chronic productive inflammation as time passes.

Immunological diagnosis of Anisakis infection by CF was attempted by Kuipers (1962) and Merkelbach (1964) using Anisakis antigen. Kobayashi et al. (1968a) later correlated positive skin test reactions using both somatic and ES antigens from Anisakis larva (I) in individuals who habitually ate raw marine fish or squid. The somatic antigen consisted of larval constituents; ES antigen was made of secreted materials and excrements in larval rearing liquid. Kobayashi et al. (1968b and 1972) showed the ES antigen to have a higher specificity to antibodies against Anisakis larvae than the somatic antigen. Suzuki (1968) conducted an antigenic analysis of Anisakis larvae using electrophoresis. Morisita et al. (1970) showed a positive Prausnitz-Kuestner (PK) reaction in a patient suspected of anisakiasis. Sumi (1970) discovered that Anisakis sp. antigens reacted positively to Dirofilaria precipitating antibody. Suzuki et al. (1970) described anisakiasis from intradermal tests using purified antigen in 35 cases of confirmed anisakiasis. He divided the infection into fulminant and mild forms; the former was considered an allergic reaction induced by secondary infection, and the latter was possibly due to the primary infection. Taniguchi (1970) made a comparative study of adult and larval antigens. Suzuki et al. (1971) purified the antigen from a crude extract of

Anisakis larvae and identified it as a Hb contained in the worms' body fluids. Woo (1971) compared the CF and immuno-diffusion tests in rabbits and discovered that the former method was probably the more valuable in diagnosing early anisakiasis. Sato et al. (1973) compared three types of antigens prepared from Anisakis larvae to the specificity and sensitivity for use in the fluorescent antibody (FA) test. Shiraki et al. (1973a and 1973b) observed the reactivity of various anti-sera to sections of Anisakis larvae under the fluorescent microscope and concluded that the FA method seemed useful for detection of the anisakid cuticle in histological diagnosis in chromic cases of anisakiasis. Other immunological methods tested for the diagnosis of anisakiasis were agar diffusion, RBC agglutination reaction, starch-gel electrophoresis, and precipitation reaction by the superposition method (Oishi et al., 1969).

MATERIALS AND METHODS

Origin of Fish

Between 24 June and 26 July, 1974, 120 Pacific herring were taken by hook and line from Yaquina Bay, Oregon, and frozen within 2 hrs at -20 C. Between April and July, 1975, 105 live Pacific herring were purchased from commercial bait dealers at Newport, Oregon. Fishes obtained in both years were used in the infection survey; only those specimens purchased in 1975 were used in the histopathology and migration studies.

Age Determination

Ages of 160 Pacific herring were determined by scale readings. All fishes captured in 1974 (frozen) and those fishes purchased fresh in 1975 were used for age studies. Herring subjected to brining and smoking were not aged. Scales were taken from an area on both sides of the fish below the lateral line; the preferable site was the posterior tip of the pectoral fins. Scales were read with a Bausch and Lomb 120 volt Microprojector and a Recordak Easamatic Microfiche Reader (Eastman Kodak model PFCD).

Brining and Cold Smoking

The fish were brined and smoked following methods described by Berg (1971) and the following modifications. Fish were washed thoroughly and placed in a brine made of 59.6 g NaCl per liter water for 30 to 45 min. At the end of this period, the fish were rinsed in fresh, cold water, drained for approximately 15 min, then dredged in fine salt for 1 to 1.5 hrs. Fish which were to be smoked were first brined as described above, then they were dried for 3 hrs on drying racks. Smoking was conducted using a Koch Smoke Tender Unit No. 1779 for 24 hrs. Cold smoked-whole fish sustained an average smoking temperature of 21 C with a range of 18 to 41 C. Cold smoked-gibbed fish sustained an average smoking temperature of 30 C with a range of 26 to 57 C.

Autopsy Methodology

Fish were weighed to the nearest gram and measured to the nearest mm, SL. All fish specimens used in this study, with the exception of those specifically used in the histopathology section, were digested following a modification of a technique described by Stern et al. (1958). The digest solution consisted of 2.5 g of 1:3, 000 pepsin powder and 1 liter of 1% HCl. Digestions were performed in a temperature-controlled water bath shaker (American Optical No. 2156) for 1 hr at 37 C. Viscera were removed and

placed in 150 ml bottles containing the digest solution, and musculature surrounding the body cavity up to the vertebral column was separated from the fish and digested separately (a few larvae were found partially embedded in the body wall near the vertebrae; therefore, the hypaxial and epaxial musculature up to the spinal column was used). The digesting tissues were shaken continuously for 1 hr at 140 oscillations per min. Contents of the bottles were then poured into a No. 70 U.S.A. standard testing sieve (212 μm mesh), and the residual fish tissues were sprayed with a jet of water to separate host tissues from the larvae. The remaining larvae and host tissues were washed into a petri dish, and larvae were counted on a standard laboratory counter.

Larvae were identified according to methods outlined by Hoffman (1970) and Millemann (1970). When difficulties in identification occurred, the larvae were cleared in lactophenol and identified using the stereomicroscope. When generic identification of nematodes, which had been frozen within their respective hosts, was hindered, larvae were observed against a dark background using a 4 watt longwave UV minerallamp (U-V Prod. UVSL 25) and identified following methods outlined by Pippy (1970).

The viscera from the smoked-gibbed specimens were digested separately to determine the percentage of larvae lost from the herring body cavity to the gibbed tissues. Larval viability was

determined following the method of Khalil (1969), who used both spontaneous movement and movement in response to mechanical stimulus as standards.

Infection Study

The incidence of infection was determined as a percentage of the number of herring sampled, and the intensity of infection was determined by the mean number of larvae per age group and length group of fish.

Histology

Fifteen Pacific herring of varying Anisakis infections were selected for the histopathologic study; these were killed and fixed immediately after purchase. Since the larvae excysted and continued to migrate from fish which were being fixed in 10% buffered formalin, Dietrich's fixative was used to fix the larvae in situ. To facilitate fixation of the gastrointestinal tract, a substantial amount of Dietrich's fixative was injected into the esophagus using a 50 cc syringe. An incision was made along the ventral side of the fish from the anus to the isthmus, and the visceral organs were immersed in the fixative. Tissues were routinely processed according to standard histological methods (Luna, 1968), and 6 μm sections were stained with Harris' hematoxylin-eosin for conventional light

microscopy. Anisakis larvae were identified in tissue sections after descriptions outlined by Chitwood and Lichtenfels (1972).

Migration Study

Fish used in the experiment to determine relative migration and viability of Anisakis larvae in Pacific herring were divided into five experimental groups: Fresh-whole, frozen-whole, brined-whole, cold smoked-whole, and cold smoked-gibbed. Twenty fish were tested in each experimental group with the exception of the frozen-whole group, in which 120 fish were used. (Gibbing is the extraction of gills, heart, liver, and viscera through an incision made ventrally to the gills, leaving the belly uncut (Berg, 1971).)

RESULTS

Infection Study

Anisakis larvae were recovered from all herring age classes with the exception of yearling fish, which were inadequately sampled (Table 1). Of the 160 Pacific herring examined, 147 (91.9%) were infected with Anisakis sp. The incidence of infection ranged between 81 to 100% for infected herring of all ages.

The degree of infection (intensity) varied considerably with the age of the herring (Table 1). Herring in their second year harbored only very few larvae, and a substantial increase in degree of infection was first seen in fish of age class 4. The maximum number of Anisakis larvae recovered from a single herring was 90, and infections of 25 to 50 larvae were very frequent in fishes of ages 5 to 8+. Intensity of infection increased with the age and length of the fish (Fig. 1) and averaged 23.9 larvae per fish.

Histopathologic Study

Anisakis larvae were associated with the pyloric caeca, pancreatic tissue, and liver of Pacific herring. The larvae were concentrated in the pancreatic tissue, with fewer larvae located directly adjacent to the pyloric caeca and liver between the mesenteries and serosa of visceral organs.

Table 1. Results of Infection of Anisakis sp. Larvae in 160 Pacific Herring from Yaquina Bay

Age class	Range of fish length (cm)	No. of fish examined	No. of fish infected	Incidence %	Range of larvae per fish	Mean no. of larvae per fish
1	8	1	0	0.0	--	0.0
2	14-20	10	9	90.0	1-11	3.5
3	15-20	36	29	80.6	1-21	3.7
4	17-22	28	26	92.9	1-32	8.4
5	19-25	34	32	94.1	2-65	18.4
6	21-27	20	20	100.0	18-84	33.7
7	23-27	22	22	100.0	13-60	34.4
8+	24-27	9	9	100.0	14-90	44.6

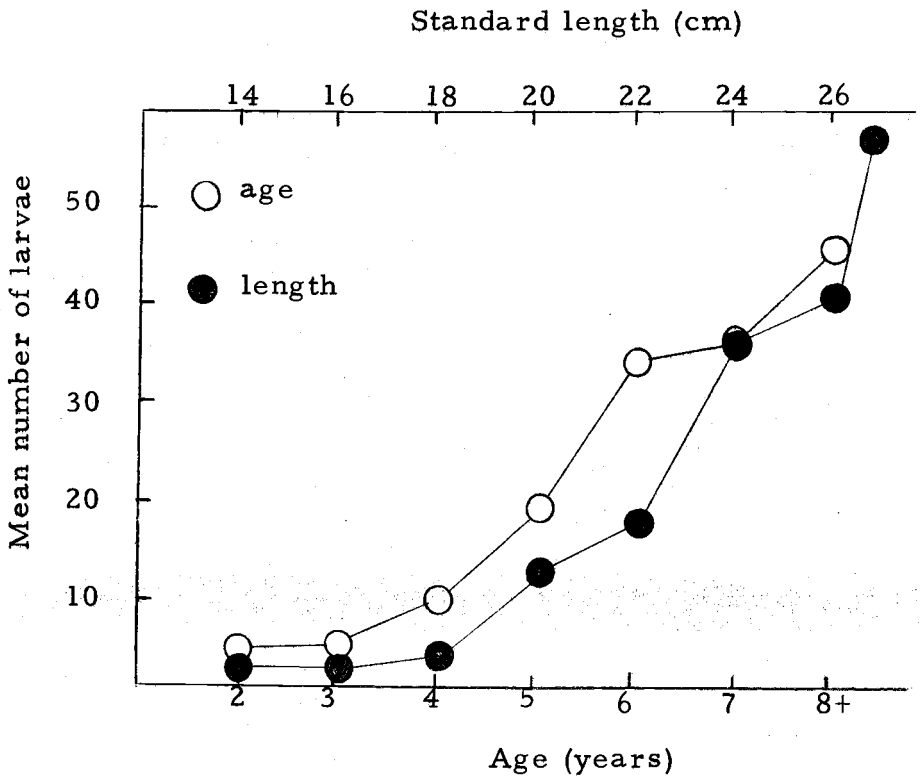


Figure 1. Relation between the mean intensity of infection of Anisakis larvae and the age and standard length of Pacific herring

Most larvae found in microscopic examination were encapsulated in a concentrically layered fibrous capsule. In some cases the capsule adhered to the tissue serosa, but it was generally separated by a layer of host exudate containing free macrophages and other inflammatory cells, thought possibly to be lymphocytes and basophils (Fig. 2).

The organ most commonly affected by larvae was the pancreas, where tissue alterations resulted from mechanical displacement of acinar cells by invading larvae (Fig. 3). All specimens examined demonstrated mechanical compression of the pancreas as well as host exudate at sites of close or direct larval contact. Large numbers of inflammatory cells were found infiltrating the same region, but they were not found within the pancreatic tissue.

Four of the fish had high larval infections; these specimens had Anisakis involvement near the liver. In moderate to light infections, larval concentrations near the liver did not exist. All four specimens had parenchymal granulomas (Fig. 4) of an undetermined nature. Necrosis, often associated within granulomatous inflammation in mammals, was not observed. Mechanical compression of the liver by Anisakis larvae was found in all four fish, with the hepatic lobules losing their normal polyhedral pattern and sinusoidal spaces. In general, necrosis was not associated with this compression, even in the most severe instances of parenchymal

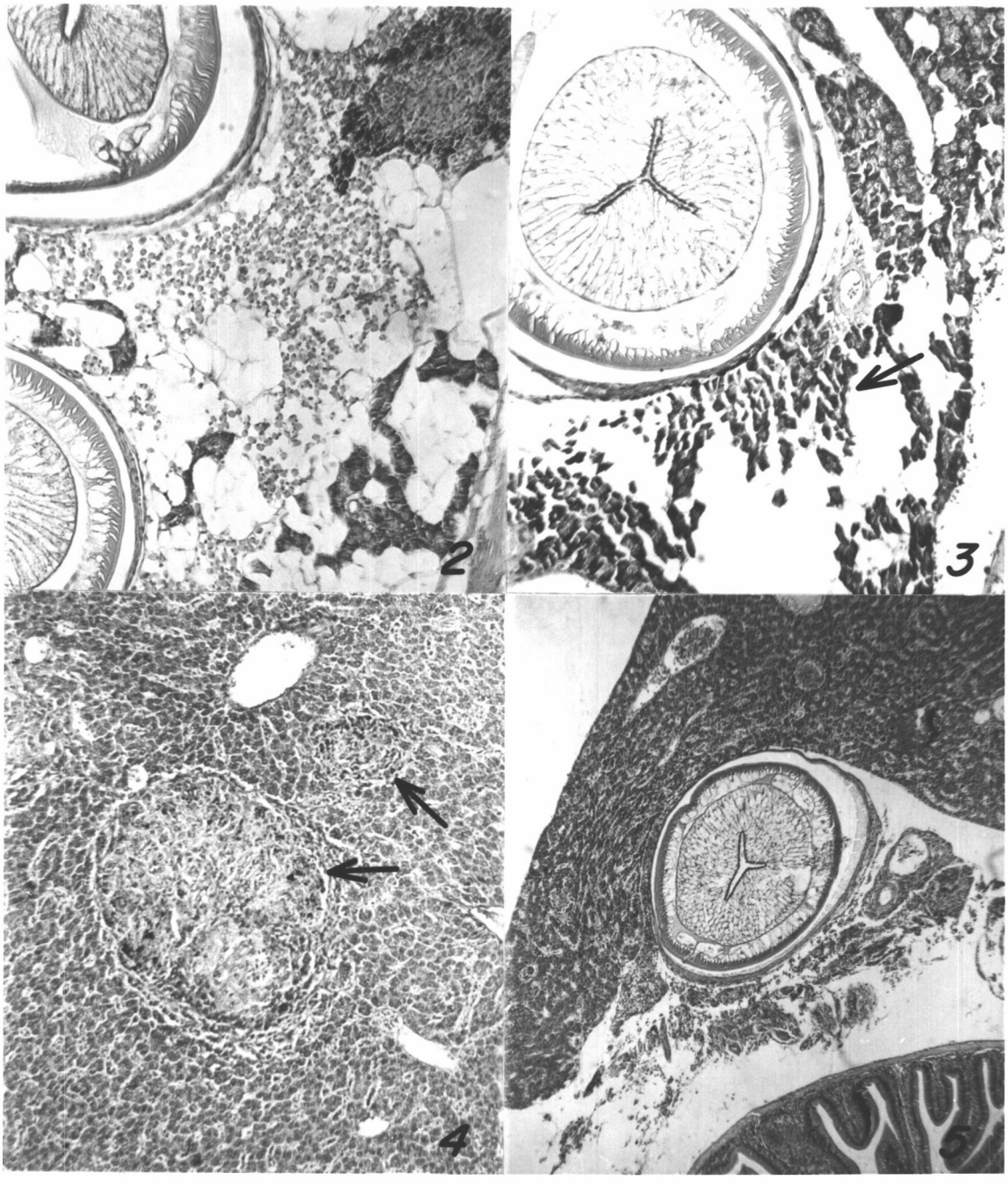
Legends for Figures 2-5:

Figure 2. Encapsulated larvae and host exudative cells. H&E x 128.

Figure 3. Mechanical displacement of pancreatic acinar cells (arrow). H&E x 128.

Figure 4. Granulomatous hepatic tissue (arrows). H&E x 128.

Figure 5. Mechanical compression of hepatic tissue. H&E x 50.4.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alterations (Fig. 5). One fish of the heavily infected group had severe, diffuse liver necrosis (Fig. 6). Since compression of the parenchyma was not evident in this case, it was ruled out as the cause. Larval penetration of the parenchyma was nonexistent in all fishes examined; one specimen, however, showed larval penetration deep in interlobular spaces (Fig. 7).

Evidence of new lesions or old scar tissue was not found in the stomach or intestine proper, which would be the expected sites of larval penetration and migration to the peritoneal cavity. Mechanical damage to the muscularis externa of the pyloric caeca was noted in one fish (Fig. 8); a single autolytic larva was found in the muscularis externa of a second specimen (Fig. 9).

Migration Study

The majority of larvae found in this study anatomically resembled the third-stage larvae designated as Anisakis larva (I) by Berland (1961) and Oishi et al. (1969).

Highly significant differences ($P < 0.005$) were found between the test groups in percentages of fish with infected and uninfected flesh (Table 2). The highest proportions of fish having larvae in the flesh were found in the smoked groups (Table 2). In the five test groups, the percentages of viable larvae in the flesh decreased as the percentage of total larval burden, represented by larvae in the

Legends for Figures 6-9:

Figure 6. Diffuse necrosis of hepatic tissue.
H&E x 128.

Figure 7. Larval penetration of interlobular hepatic spaces.
H&E x 50.4.

Figure 8. Trauma in muscularis externa of pyloric caecum (arrow).
H&E x 128.

Figure 9. Autolytic larva at muscularis externa of pyloric caecum.
H&E x 128.

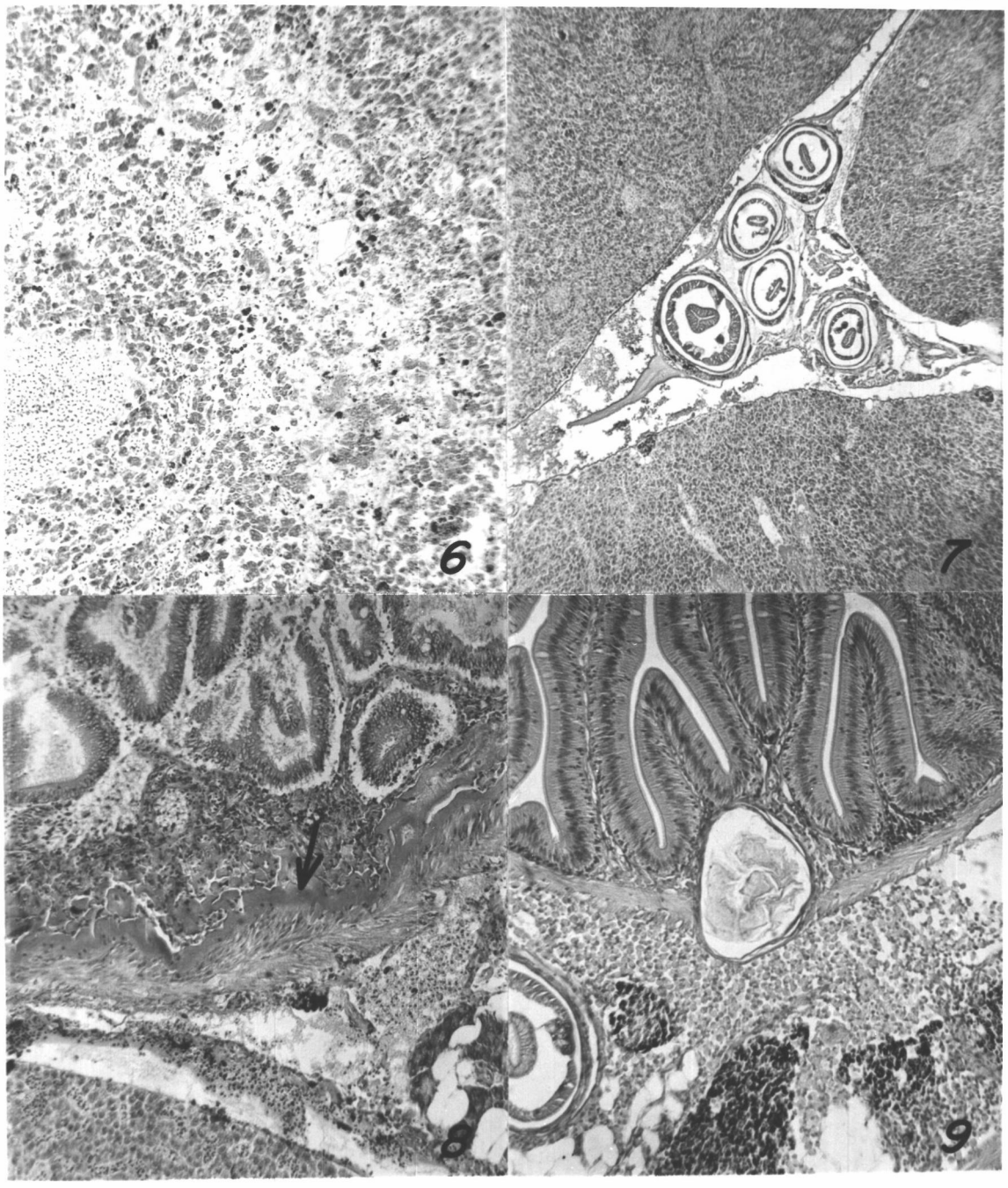
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Table 2. Proportions of 220 Pacific Herring with *Anisakis* Larvae in the Flesh, Time Between Death and Autopsy, and Average Processing Temperatures for Various Test Groups.

	Fresh	Frozen	Brined	Cold smoked	Cold smoked-gibbed
No. of fish	39	120	20	21	20
Flesh infected (%)	38.5	42.5	50.0	57.1	95.0
Flesh uninfected (%)	61.5	57.5	50.0	42.9	5.0
Time (hr)	8	2*	8	26	26
Average processing temperature (C)	20	-20	18	21	30

*Time between death and being frozen.

flesh, increased (Table 3).

The frequency distributions of the numbers of Anisakis larvae in the musculature of herring for each experimental group are presented in Table 4. The mean intensities of infection were compared in a one-way analysis of variance and transformed using the following model

$$y = (x + \tfrac{1}{2})^{\frac{1}{2}}$$

No significant differences were found between transformed mean intensities of larvae in the flesh of fresh and brined or of brined and cold smoked-whole groups ($P < 0.05$) (Table 5). Transformed mean intensities of fresh and cold smoked-whole groups were significantly different ($P < 0.05$), as were those of brined and cold smoked-gibbed and of cold smoked-whole and cold smoked-gibbed ($P < 0.005$) (Table 5).

Forty-eight percent of the larval burden was lost from the fish through the gibbing process. After gibbing, 46% of the larval burden was located in the flesh; 14% of these larvae (in the flesh) were viable at autopsy (Table 6).

Table 3. Total Numbers of Anisakis Larvae in Herring (T); Number of Larvae in Viscera to Number in Flesh (V:F); Anisakis Burden in the Flesh (%F); Percent of Viable Larvae in the Flesh (%Vf).

	Fresh	Frozen	Brined	Cold smoked	Cold smoked-gibbed
T	753	2099	542	232	285
V:F	727:26	1988:111	506:36	184:48	153:132
%F	3.5	5.3	6.6	20.7	46.3
%Vf	96.2	---	100.0	87.5	14.4

Table 4. Frequency Distribution of *Anisakis* Larvae in Herring Musculature and Mean Intensity of Infection.

No. of larvae	Frequency				
	Fresh	Frozen	Brined	Cold smoked	Cold smoked-gibbed
0	24	69	10	9	1
1	7	29	2	5	3
2	5	11	3		2
3	3	3	1	2	2
4		1	2	2	
5		2		1	1
6		1			2
7		2	1		1
8		1			2
9		1		1	2
10			1		1
11					
12					
13					
14					1
15				1	1
16					
17					
18					
19					
20					
21					
22					1
Total	39	120	20	21	20
Mean intensity	0.667	0.925	1.800	2.286	6.600

Table 5. Mean Intensity of Infection with Anisakis Larvae After Transformation.
$(y = (x + \frac{1}{2})^{\frac{1}{2}})$

Fresh	Frozen	Brined	Cold smoked	Cold smoked-gibbed
1.0016	1.0679	1.3178	1.4208	2.4596

Table 6. Total Numbers of Anisakis Larvae in Herring (T); Number of Larvae in Viscera to Number in Gibbed Fish (V:G); Parasite Burden in Gibbed Fish (%G); Percent of Viable Larvae in the Gibbed Fish (%Vg).

	T	V:G	%G	%Vg
Cold smoked-gibbed fish	444	159:285	64.20	19.60

DISCUSSION

Infection Study

Bishop and Margolis (1955) noted an 80 to 100% incidence of Anisakis larvae in Pacific herring caught along the coast of British Columbia. They discovered that the intensity of infection increased after the first year, yearling fish being uninfected. My findings compare favorably with the results of this study.

Some controversy exists as to whether Pacific herring are infected with Anisakis larvae during their first year. Sindermann (1963) reported that Anisakis infection begins in Atlantic herring before the first year, peaks at age 1+, and lives past the sixth year. Bishop and Margolis (1955) based their evidence of uninfected yearling fish on 297 fish of the same age. Khalil (1969) suggested that Bishop and Margolis did not find infected yearling fish because this age group was only lightly represented in their catches. Further, Bishop and Margolis hypothesized that yearling fish were probably uninfected because their diet and inshore feeding habits differ from those of older fish. My studies did not provide sufficient evidence to clarify this problem.

Incidences of infection of Anisakis larvae vary considerably with geographic location of Clupea sp. hosts. Khalil (1969)

reported that 55% of North Sea herring, 34% of herring from British coastal waters, and 50% of herring from NW Ireland harbor Anisakis larvae. Rokicki (1972) noted a 31% incidence of Anisakis larvae in Baltic Sea herring. Oishi et al. (1969) noted that 55 to 88% of Pacific herring from various Japanese waters were infected with Anisakis sp.

Some investigators believe that the incidence of Anisakis larvae increases with northward direction in Canadian Pacific and Canadian Atlantic waters (Bishop and Margolis, 1955; Parsons and Hodder, 1971). My findings indicated that the incidence of infection in Oregon waters is consistent with that found in British Columbia waters. Therefore, either the incidence of infection does not increase with northward direction, or infections have increased considerably in Pacific herring during the last two decades.

Histopathologic Study

Helminthic infections in higher vertebrates will generally elicit an eosinophilic inflammatory response in host tissues. Such response is common in human anisakiasis, but was not present in the fish I examined. This corroborates the findings of Mawdesley-Thomas (1975), who asserted that extensive eosinophilia is not the usual inflammatory reaction of fish hosts against parasites. The presence of host exudate and inflammatory cells suggest an

immunological response of the host to Anisakis larvae. Host exudate and inflammatory cells were especially evident in severely infected fish, indicating a probable chronic pathology.

Capsule formation around Anisakis larvae appears similar to that described in other fish species (Margolis, 1970). For example, a thin, hyalinized layer of degenerative connective tissue was located adjacent to the larva. The next layer contained loose connective tissue; this was followed by free inflammatory cells. The capsule, as characterized above, was particularly distinct when associated with larvae located near the pancreatic tissues.

Mechanical compression of pancreatic tissue does not appear to cause a serious pathological condition. Compression of the liver, however, may result in pressure atrophy in severe cases.

The specific route of parasitic migration from the gastrointestinal tract to the final position in the peritoneal cavity was undetermined. The reaction in two fish indicated that the pyloric caeca may be a pathway to the peritoneal cavity. Both fish showed traumatic response and actual larval presence in the muscularia externa, but more fish must be examined before the migration route can be determined. Further, the origin of the liver granulomas and necrosis remains unclear. Granulomas were associated with high infections, but the absence of repair tissue indicated no direct mechanical injury.

The presence of the exudate and the associated inflammatory cells would suggest a chronic pathology caused by Anisakis sp. larvae in Pacific herring. The larvae are encapsulated in mesenteries and may cause compression damage to adjacent host tissues, with a significant pathology occurring only in heavily infected fishes.

Migration Study

Various investigators have reported that larvae were not found in the flesh of herring or mackerel which were eviscerated immediately after capture (Van Thiel et al., 1960; Vik, 1966). However, Khalil (1969) reported that 2% of the total anisakid burden was partly embedded in the flesh of herring at capture, and Smith and Wootten (1975) indicated that 4% of the total worm burden was distributed in the flesh of recently-captured herring. The latter figure compares favorably with the findings of my study, which indicated that 3.5% of the larval burden was located in the flesh of fresh fish.

Smith and Wootten (1975) reported a large-scale migration of Anisakis larvae from the fiscera to the flesh of herring; they found that 20% of the total worm burden was present in the flesh after 37 hrs. Results of my study indicate that almost 50% of the total burden of Anisakis larvae was located in the musculature in the herring of the cold smoked-gibbed group. Reports by other investigators on the migration phenomenon conflicted with the findings of this study

and those of Smith and Wootten (1975), who summarized and attributed these discrepancies to different larval detection techniques. In many of these conflicting reports, investigators used the more conventional methods to examine fish musculature for nematode larvae, such as using the unaided eye, candling, or slicing. Stern et al. (1958) reported a 21.9% increase in the recovery of Anisakis larvae from salmon musculature when a pepsin-HCl digest method was used to detect larvae. Smith and Wootten (1975) employed the pepsin-HCl technique; their findings are comparable to the results of my study.

Factors leading to the excystment and migration of Anisakis larvae into the musculature of the dead host are not understood. The post-mortem changes in the decomposing viscera may contribute to this phenomenon. My findings indicated that the mean larval intensities in the flesh of fresh and frozen herrings are not significantly different, possibly precluding any effect of decomposing viscera on larval migration in herring held for about 8 hrs. This period compares favorably with information reported by Cheng (1976), who stated that total decomposition of viscera from cod (Gadus callarias) and summer flounder (Paralichthys dentatus) occurred after 6 to 8 hrs. The combination of time between capture and processing and the exposure of the larvae to the saline and smoking temperatures probably stimulated the larvae to migrate into the flesh where more acceptable surroundings might exist.

The ability of Anisakis larvae to withstand extremes in temperatures used during the cooking of fillets is well known. Davey (1972) found larval mortalities to be 82% in fillets cooked to 60 C in 2 to 5 min, and Khalil (1969) reported that 5% of 1, 000 cured and smoked herring contained Anisakis larvae, all of which were reported viable (temperatures used in the smoking process were not reported). My study showed that the initial temperature sustained for 1 min during the smoking of the gibbed group was 57 C. Approximately 14. 4% of the larvae in the musculature of this group were viable. The cold smoked group, which sustained temperatures between 18 to 41 C for 24 hrs, had larval viabilities of approximately 87. 5%. The temperature of 21. 1 C was suggested by Berg (1971) as appropriate for the cold smoking process. My findings indicate that the majority of larvae in fish smoked at this temperature would be viable and probably capable of infecting man.

The third-stage larva of Anisakis sp. was reared to adult form and identified as A. simplex by Pippy and Van Banning (1975). A. simplex has been implicated as the pathogen of anisakiasis (Oishi et al., 1969). They also noted that Pacific herring from Japanese waters were predominantly infected with Anisakis larva (I). The larvae examined in this study were morphologically similar to the type (I) larva which Oishi described. My investigation of Anisakis infection in Pacific herring may not apply to other Pacific Coast fish

or to Atlantic Coast herring. Nevertheless, the consumption of Pacific herring in the brined or cold smoked condition represents a potential public health hazard. This is particularly important in light of recent reports by the U. S. Department of Health, Education, and Welfare (1975), which stated that the American appeal for raw and exotic fish delicacies is increasing.

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