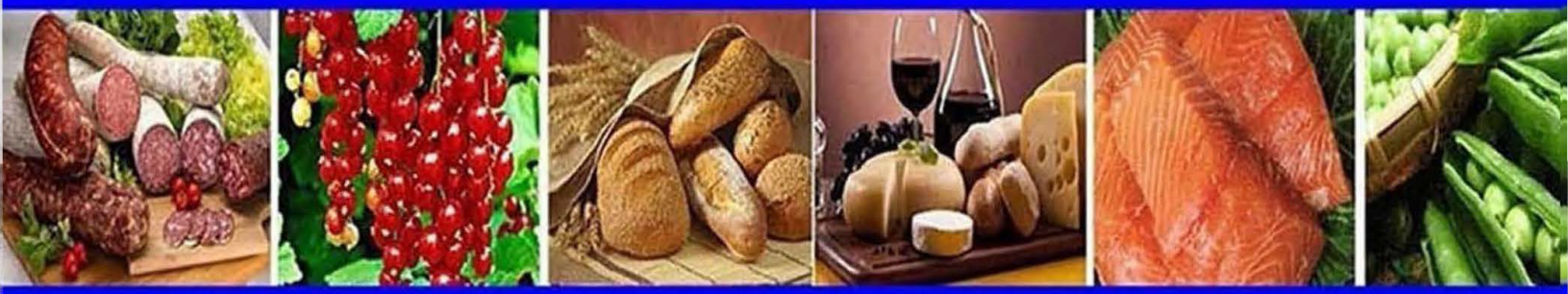

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CONTENT

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THE DEGREE OF RESIDUAL INVASION AFTER INFECTION WITH ANISAKIASIS FISH OF VARIOUS CULINARY PROCESSING

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ABSTRACT

The article presents the results of the study of the degree of residual invasion after infection with anisakiasis of herring *(Clupea harenqus* and *Alosa immaculate)* in various culinary processes and places of catch. During the experiment, the largest extent of the invasion was detected in marinated herring. The intensity of invasion with anisakis in marinated samples exceeded smoked products five times. A similar tendency of parasitic lesion was observed during autopsy. Most of the larvae were found in the abdominal cavity of pickled fish, and the least - in smoked fish. Smoked fish had the largest number of parasites in the wall of the abdominal cavity. In our opinion, the level of damage by fish parasites depends on a complex of external and internal factors. The type of culinary processing affects the intensity of the invasion. The intensity and localization of larvae of anisakis is significantly reduced after removal of the intestines from the fish.

1.Introduction

Fish and fish products with exceptionally high nutritional qualities are an important source of food are widely used in daily nutrition, diet and baby food. It is the source of the necessary protein, vitamins, trace elements and other substances necessary for the human body. According to international medical standards, a person must consume twenty kg of fish and fish products per year (Berezovskiy et al, 2013; Arcos, 2014).

In recent years, the culinary traditions of Japan, Korea, China and other countries of Southeast Asia have been widespread in Europe and in particular in Ukraine, where many dishes are cooked from raw or half-fat, crustaceans, squid and other mollusks. This represents a significant negligence in relation to human anthrop noses, in particular, invasive diseases, which fish carry as an additional or intermediate. Fish-invasive diseases, dangerous to humans, are found in marine and freshwater fish. The sea and the oceans produce on average more than two thirds of the total catch of fish (Gaponenko & Lysenko, 2009; Mikulich, 2013; Mok, 2015). In this case, sea fish is part of a trading network mainly in a chilled or frozen state.

In addition, there are different types of fish processing: marinades, cheeses, pickled, smoked and others. An increase in the supply of fish from abroad to the domestic market of Ukraine has increased the frequency of detection of helminthological pathogens previously diagnosed sporadically, in particular, opisthorchiasis, clonorhosis, metagoniosis, nanofeetosis, diophilobothiosis, coriandrosis, anisakidosis, and others (Berezovskiy et al, 2013; Arcos, 2014).

According to the literature (Gaevskaya, 2005; Mok, 2015), virtually all sea fish can be

infected with different types of worms, up to 30 species of which constitute a potential danger to humans or cause unwanted changes in fish, as in technological raw materials. Nematodes of the Anisakidae family are helminths dangerous to humans. Anisakis – pathogenic worms, the representatives of nematodes of the family *Anisakidae* (*Anisakis simplex, Pseudoterranova decipiens, Hysterothylacium aduncum, Contracaecum osculatum*). Localized in the body, on the surface or in the tissues of the internal organs, rarely in the muscles (often below the middle line of the body of fish), sea and passage Pacific fish (cod, mackerel, hake, flounder, knot, herring, pink salmon etc.) (Herrero et al, 2011; Buchmann & Mehrdana, 2016).

The aim of the study is to determine the degree of residual invasion in fish anisakiasis of various culinary processes With a sufficient and comprehensive study of the dynamics and degree of penetration of anisakis into popular types of fish products, it is possible to reduce the amount of low-quality products, break the life cycle of the parasite and reduce the level of infection with helminths population. Thus, our research will be able to participate in the improvement of the population and contribute to improving the sanitary safety of fish products.

2.Materials and Methods

To achieve this goal, the following tasks were set: to make a controlling selection of anisakiasis of fish products (freezing, salting, marinating, smoking Atlantic herring *(Clupea harenqus)* and Black Sea herring *(Alosa immaculata*) in the markets of Vinnitsa; carry out organoleptic and parasitological studies. The study was herring of freezing, salty, smoked, and the subject - the dynamics of lesion with anisakis.

25 samples were selected the 25 of fish products (freezing, salting, marinating, smoking Atlantic herring *(Clupea harenqus)* (the place of catch is the North Atlantic and the Baltic Sea) and Black Sea herring *(Alosa immaculate) (*the place of catch is the Black Sea). The selection was carried in the markets of Vinnitsa (Ukraine) out 2019 year by the work of the expansion network in accordance with the established rules and regulations.

In the course of research, such methods as organoleptic and parasitological are used. In the organoleptic assessment of fish, the appearance, the built in fish, the condition of the outer covers, mucus, eyes, gills (Berezovskiy et al, 2013; Gaevskaya, 2005).

The parasitological examination of fish reveals visible parasites, as well as parasites, muscles, under the skin or shines. Pay attention to the spots and inclusions, which differ in color or consistency from normal tissues, as well as in tumors, and form regions of meat of sparse consistency. To detect parasites in meat use a method of parallel cuts.

Detection of parasites or inclusion, like living parasites, is initially considered under magnifying glass or binocular. Then, if necessary, they are considered under the small and middle levels of the microscope. The vital activity of parasites is determined by the method of irritation.

The following parameters were determined to determine the dynamics of anisakis in different types of culinary processing: the number of damaged specimens, the severity of the invasion, the intensity of the invasion and the index of invasion. The number of specimens affected was determined by simple counting when the fish were opened and examined. Extensiveness of the invasion was determined by dividing the number of damaged specimens by the number of specimens (25 pieces) and multiplying by 100. The intensity of the invasion was determined by counting the parasites of one fish. The invasion index was determined by dividing the number of parasite larvae into the total number of parasites found in the sample (Bogatko et al, 2011).

The digital material was processed statistically. The resulting digital data was processed using the MS EXEL 98 and Windows program, statistically processed by Student. The results were considered statistically significant at $p < 0.1$, $p < 0.01$, $p < 0.001$. In the table material

of the work the following symbols are taken: * p <0.1, ** p <0.01, *** p <0.001.

3. Results and discussion

Experimentally it was established that fish of different culinary procedures had another infection with helmints (Table 1).

Table 1. Dynamics of anisakiasis of herrings for different types of culinary processing *(M ± m, n=25)*

Indicator	Kind of fish processing			
	freezing	salting	marinating	smoking
The number of specimens affected, thing	21.4 ± 0.16	20.9 ± 1.08**	23.7 ± 0.63	5.0 ± 0.09***
Extensiveness of the invasion, %	84.5 ± 3.25	83.6 ± 0.75	94.8 ± 2.87*	2.9 ± 0.13***
The intensity of the invasion, units	1-12	1-17	1-23	1-5
The invasion index, units	6.0 ± 0.34	8.3 ± 0.05***	10.3 ± 0.75***	1.8 ± 0.03***

From the digital material it is evident that the largest number of anisakis larvae was in pickled herring, and the smallest in smoked. The difference was 17.7 units (p <0.001). The experiment showed that the largest extent of anisakis invasion was marinated fish (the difference between frozen samples was 10.3% (p <0.1), and compared to smoked samples - 91.9% (p <0.001). *Anisakis* infestation intensity Herring of different types of culinary processing showed a similar tendency and most of the parasite larvae were found in marinated fish, which was confirmed by the calculation of the index of invasion: the index was greater by 4.3 units (p <0.001) compared with frozen fish, and in smoked fish - by 4.2 units (p <0.001).

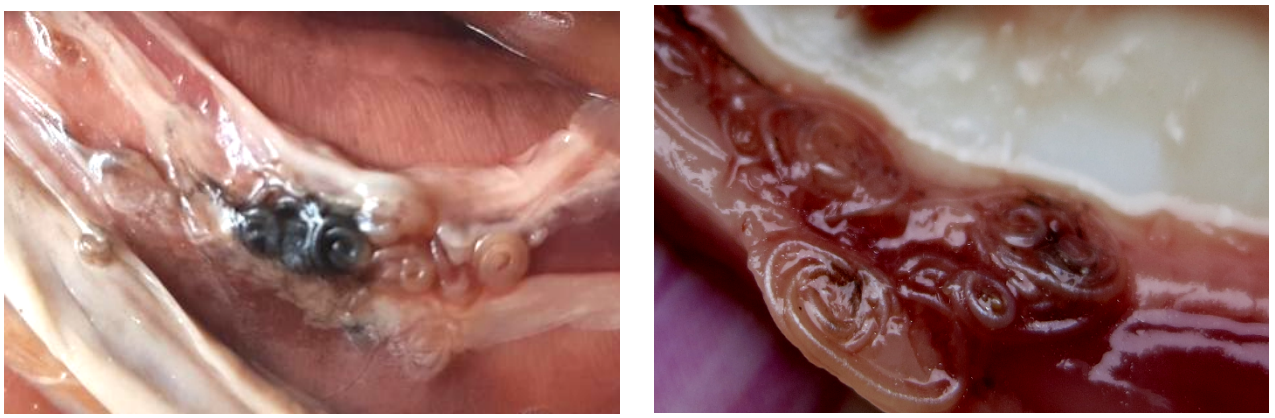
The study of the localization of helmints in fish of various types of culinary processing yielded similar results (Table 2).

Table 2. Placement of larvae of *A.simplex* in herring carcasses of different types of culinary processing *(M ± m, n=25)*

Indicator	Kind of fish processing			
	freezing	salting	marinating	smoking
Number of helmints in fish body, units	6.0 ± 0.34	8.3 ± 0.05***	10.3 ± 0.75***	1.8 ± 0.03***
Localization of parasites in the abdominal cavity, units	5.2 ± 0.05	6.6 ± 0.09***	9.2 ± 1.35**	0.2 ± 0.09***
% of the total number of larvae in one fish	86.67	79.51	89.32	11.11
Localization of parasites in the abdominal wall, units	0.7 ± 0.03	1.2 ± 0.01***	1.0 ± 0.83	0.8 ± 0.05*
% of the total number of larvae in one fish	11.67	14.46	9.71	44.44

Localization of parasites in the muscles of the back, units	0.3 ± 0.01	0.2 ± 0.08*	0.2 ± 0.03**	0.8 ± 0.08***
% of the total number of larvae in one fish	5.00	2.41	1.94	44.44

As the digital material shows, the largest number of larvae was found in pickled fish. At the autopsy it was found that marinated fish had 1.77 times more intruders in the abdominal cavity compared with frost fish, 1.39 times more than salty fish and more than 46 times more than smoked fish. At the same time, the difference was significant (p <0.1-0.001) (Fig. 1).



a **b**

Fig. 1 - Localization of parasite larvae in the abdominal cavity of saline (a) and marinated (b) herring

The largest larvae in the abdominal wall and the muscles of the back were smoked specimens (44.44%, p <0.1-0.001).

Experimental material indicates the connection of the type of culinary processing of fish with the degree of damage to its anisakis. Thus, the smallest extensiveness, intensity and index of damage were detected in smoked herring. In addition, she had the smallest larvae of the parasite in the abdominal cavity. Probably this is due to the fact that before the smoke the fish was subjected to the removal of the inwards and thorough cleansing.

The features of the invasion of herring anisakis from different places of capture were studied. At the same time, imported and domestic fish products were investigated. The research results are presented in table 3.

Table 3. Comparative characteristics of invasion of anisakiasis of Atlantic herring *(Clupea harenqus)* and Black Sea herring *(Alosa immaculata)* depending on the place of catch and the method of preparation *(M ± m, n = 10)*

View and culinary processing of fish	Place of catch (importing country)	Extensiveness of the invasion, %	The intensity of the invasion, units	The invasion index, units
Atlantic herring *(Clupea harenqus)* (freezing)	North Atlantic (Norway)	93.5 ± 3.78***	5-19	11.5 ± 6.83**
Atlantic herring *(Clupea harenqus)* (freezing)	Baltic Sea (Latvia)	85.3 ± 11.35***	6-37	21.5 ± 9.75*

Black Sea herring *(Alosa immaculata)* (freezing)	Black Sea (Ukraine)	15.6 ± 5.33	1-10	5.7 ± 3.01
Atlantic herring *(Clupea harenqus)* (salting)	Baltic Sea (Latvia)	80.2 ± 7.25*	3-20	12.7 ± 7.85**
Black Sea herring *(Alosa immaculata)* **(**salting)	Black Sea (Ukraine)	70.3 ± 15.35	1-17	7.6 ± 3.33
Atlantic herring *(Clupea harenqus)* (marinating)	North Atlantic (Norway)	100	7-19	8.6 ± 5.67
Black Sea herring *(Alosa immaculata)* (marinating)	Black Sea (Ukraine)	5.3 ± 2.61***	3-15	7.3 ± 5.94
Atlantic herring *(Clupea harenqus)* (smoking)	North Atlantic (Norway)	8.3 ± 1.34*	0-9	4.3 ± 3.59
Atlantic herring *(Clupea harenqus)* (smoking)	Baltic Sea (Latvia)	9.1 ±3.25***	3-6	3.2 ± 1.26*
Black Sea herring *(Alosa immaculata)* (smoking)	Black Sea (Ukraine)	2.6 ±0.95	0-5	2.6 ± 0.87

Tabular materials suggest that imported products were more affected by larvae of *A. simplex* than domestic ones. Thus, in the analysis of fresh frozen herring imported from abroad, the difference in the degree of invasion was 69.7 - 77.9%, and the index of invasion - by 5.8 - 15.8%. A similar tendency of helmosis was found in fish of other types of cooking.

3.1.Discussions

Anisakiasis was first detected in the Netherlands in 1955 after eating slightly salted herring. Every year in many countries new cases are registered. According to studies of various scientists, the invasiveness of fish with anisakiasis reaches high rates: Atlantic herring (*Clupea)* is infected with larvae of anisakis by 41%, *Gadus* - by 25%, *Micromesistius* - by 41%, *Clupea harengus membras* - by 20%, *Theragra chalcogramma* - by 34%, *Scomberomorus niphonius* - 28 %, *Sprattus sprattus* - 16%, *Cololabis saira* - 28% etc (Berezovskiy et al, 2013; Carballeda-Sangiao, 2014).

Anisakis is a zoonotic helminthiasis characterized by a defeat of the gastrointestinal tract as a result of parasitism in the human body at the stages of the larvae of the worm family *Anisakidae*. The causative agents of human anisakiasis are the larval stages of the development of worms of the following groups: *Anisakis, Contracaecum, Pseudoterranova, Hysterothylacium*, belonging under the line *Ascaridida Skrjabin et Schikhobalova, 1940*, to the family *Anisakidae Skrjabin et Korokhin, 1945* (Gaevskaya, 2005; Faeste, 2014).

Additional mosquitoes of parasites have many species of sea fish, mollusks, large crustaceans, eating small crustaceans. The larvae of anisakis in the organism of intermediate hosts are localized in the body cavity, on the surface or within the various internal organs and muscle of the fish. They are inside semitransparent capsules - cysts or without them. The size of the cysts ranges from 1 to 7 mm on average. Anisakis larvae that do not form capsules, for example *P. dicipiens*, have a length of 1.5 to 6 cm (Gavryuschenko, 2016; Sondak & Gritsik, 2006).

Infection of end-owners occurs when they use infected intermediate hosts: fish, crustaceans and mollusks. If large intermediate hosts feed on small, invasive larvae of anisakis, then these larvae accumulate in the body of a larger, predatory fish.

Atlantic and Black Sea herring are the most popular among the domestic population. Atlantic herring inhabit the North Atlantic: from the Bay of Biscay to Iceland and southern Greenland and east to Spitsbergen and New Zemlya, including the Baltic Sea. In the western

part of the Atlantic Ocean, it is distributed from the southwestern shores of Greenland and Labrador to South Carolina, USA. This schooling bentopelagic oceanodrom fish keeps near the sea surface, winters and spawns in the near-bottom horizons (Herrero et al, 2011). It occurs at a positive temperature and high salinity and suffers considerable desalination. There are several separate subpopulations with different spawning periods. By the time of spawning, spring, summer, autumn and winter herring are distinguished. Spring and autumn herring are most numerous. The life cycle of herring of these populations occurs in the North Sea. Small, immature herring is distributed in three areas: the coastal waters of Norway and the Kola Peninsula, including fjords, bays, open waters of the Barents and White seas; the central and northeastern part of the Norwegian Sea; southeastern part of the Greenland Sea (Alekseenko, 2009; Daschner et al, 2012). The degree and dynamics of invasion by anisakis is determined by the place of catch. Thus, N.M. Bogatko, V. V. Vlasenko, O.Yu. Golub (Bogatko et al, 2011) reports that one hundred fish with a one-time spawning (for example, some salmon fish), after which they die, apparently, and anisakis larvae die. In fishes that spawn more than once, in particular herring ones, anisakis larvae do not perish and may eventually accumulate in the host organism. Changes in the hydrological conditions (when fish move from seawater to freshwater) and, above all, the salinity of the water do not have a detrimental effect on the larvae of the parasite.

For example, it indicates differences in the degree of invasion of herring from different places of catch and species specificity. For example, Dolinskaya herring (Southern Caspian, eastern coast) was infected with worms by 76.9%, Sarinskaya - by 33.3%, Eastern and Hasankulinskaya - by 50%, Krasnovodskaya - 76.5%, white-headed - by 93.3% Volgskaya - by 13.2 - 142%. The intensity of invasion of herring from different subpopulations also turned out to be different: there were up to 18 larvae in Dolinskaya herring, up to 4 larvae in Sarinskaya, 32 in eastern, 60 in Krasankovskaya, 51 in white-headed, Volgskaya, up to 21 larva (Alekseenko, 2009; Bogatko et al, 2011; Faeste, 2014).

Ye.L. Mikulich (Mikulich, 2013) reports that the main exporters of frozen fish to Belarus are: Russia, Norway, the Baltic countries, Denmark, Spain, the United Kingdom, Iceland, the USA, Canada, the countries of Latin America and Indochina. As a result of studies conducted on the internal organs of frozen fish, single larval stages of the *Anisakis simplex* were found, as well as *Nybelinia surminicola* larvae (the extensiveness of invasion was 70%, and the intensity of invasion was 2–6 parasites per fish). Scratches of the genus *Echinorhynchus* were found in gutted and decapitated codfish (the extensiveness of invasion was 50%, and the intensity was 2–4 parasites per fish).

Disinfection of seafood from larvae of anisakis can be freezing and heating. In the usual saline and acetic solutions used for cooking fish, lizards of anisakis can remain viable for many days or even months. Freezing of fish up to -18°C leads to the death of all larvae of the 14 days, at - 20°C they die within 4-5 days, at -30°C die for 10 minutes (Gaevskaya, 2005; Buchmann & Mehrdana, 2016; Baptista-Fernandes, 2017).

In Europe and the United States, sanitary rules regulate the freezing of fish, which can not be subjected to further heat treatment at -20°C for 5 days. Larvae can tolerate a temperature rise of up to 45°C, but at 60°C and above they die within 10 minutes. Thus, the thermal processing of fish in the range of temperatures 45-60°C does not guarantee its decontamination from the anisotropic larva (Mikulich, 2013; Nieuwenhuizen & Lopata, 2013).

In studying the localization of larvae of anisakis, it was established that the determining factor is not a kind of culinary processing, but a kind of processing of raw materials. Most of the invasions were found on the internal organs of fish. Therefore, in our opinion, removal of insects from the carcass of fish can reduce the level of invasion. The larvae may be twisted (shape of a spiral, wide ring) or elongated, in translucent capsules or without them. The size

of the cyst in the width - 3,5-5 mm, thickness 1,0-1,5 mm (*A. simplex*). The larvae obtained from cysts reach up to 4 cm in length, with a thickness of 0.4-0.9 mm. *Anisakis* genera are white to yellowish. Mature nematodes parasitize in the gastrointestinal channel of vertebrate animals living in an aqueous medium (Berezovskiy et al, 2013; Carballeda-Sangiao, 2014; Buchmann & Mehrdana, 2016).

The final hosts of these worms are marine mammals (cetaceans), predatory sea fish and violent birds that parasitize men and women with anisakis. The average length of a female is 60-65 mm, male - 50-55 mm. The fertilized eggs fall into the water, where the larva comes out of them, which is swallowed by the first intermediate hosts - crustaceans, often belonging to the family *Euphausiidae.* The relevance of the study of anisakiasis is obvious, since the danger of infection by these parasites is proven (Herrero et al, 2011). According to the results of our research, the largest number of larvae was found in the intestines of pickled fish, and the least - in cartridges of smoked fish. Ye.L.Mikulich. V.V. Sondak and et. there are cases of invasion not only in frozen fish, but even in fish canned fish from whole fish (Mikulich, 2013; Sondak & Gritsik, 2006; Buchmann & Mehrdana, 2016).

Samples of pickled fish were the most invasive. In our opinion, this is due to the selection of low-quality raw materials that have not been properly treated with cold. Another reason may be wrongly prepared marinade. Therefore, in the muscles of ready-to-eat pickled fish, the salt content was less than 14%. Such conditions, according to scientists, are favorable for the life of larvae. Therefore, pickled herring could have invasive larvae, dangerous for humans. It is dangerous that in most cases anisakiasis remains unnoticed. For example, in Japan 60% of lesions of worms were mistakenly diagnosed as appendicitis, cholecystitis, diverticulitis, tuberculosis peritonitis, and others (Daschner et al, 2012). According to S. A. Alekseyenko (Alekseenko, 2009), during the summer and autumn spawning of salmon in Khabarovsk there is an increase in the number of patients with acute abdominal pains. Helmints larvae were then detected in patients and fish products.

The total volume of herring samples of different types of culinary processing in Vinnitsa was somewhat higher than the data on the loss of imported fish, as shown in the article Ye.L.Mikulich according to their data, the degree of damage by parasites of fish was within 59%, the intensity of the invasion - up to 38, and the index of invasion - up to 12 (Mikulich, 2013). However, according to experimental data V. V. Grigoryeva (Gaponenko & Lysenko, 2009) the extensiveness of the invasion - the herring was 89%, the intensity of the invasion - 7-22 units. Apparently, discrepancies in the data are related not only to the type of culinary processing, but also to the observance of sanitary norms for the storage of fish raw materials and finished products. In our opinion, an important role in this is played by the place of catching fish.

For example, M. G. Gaponenko i S. Ye. Lisenko (Gaponenko & Lysenko, 2009) made the fish from the Azov-Black Sea basin anisakiasis mainly affect the abdominal cavity and internal organs (91.3%), less often the muscles of the abdominal wall (6.4%) and back muscles (2,3). %). The intensity of the invasion of the Black Sea herring was 1–12 individuals. In the case of imported fish, the abdominal cavity and internal organs (82.2%) are most often affected, less frequently the muscles of the abdominal wall (13.3%) and the back muscles (4.5%). The prevalence of herring invasion was highest at 59%, the intensity of Atlantic herring invasion was 1–38 individuals, and the invasion index was 12 larvae per fish carcass. Our studies have confirmed this tendency of contamination of herring with Atlantic and Black Sea anisakiasas. During our experiment, it was revealed that imported fresh-frozen fish from Norway and Latvia, regardless of the method of preparation, had higher levels of extensiveness, intensity and invasion than domestic fish.

4.Conclusions

The type of culinary processing of fish determines the degree of invasion of anisakis. The highest intensity, intensity and index of invasion were found in pickled fish. The analysis of carcasses showed that up to 89% of all larvae are localized in the abdominal cavity. Smoked fish had the slightest damage with anisakis. The removal of the intestines from the fish before smoking caused a decrease in the degree of invasion of the body by 78%. The practical significance of this study is to increase the efficiency of sanitary and epidemiological control of fish products, increase public awareness of the risk of anisakiasis and social responsibility for non-proliferation and detection of parasites.

Further research will be aimed at finding an optimal and quick method for the detection of living anisakis larvae and its use for the study of samples of fishery products of various types of culinary processing.

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