ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XIII Fasc. 2 Szczecin 1983

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Parazytologia

STUDIES ON VIABILITY AND INFECTIVITY OF *ANISAKIS SIMPLEX* STAGE III LARVAE IN FRESH SALTED AND SPICED BALTIC HERRING*

BADANIA NAD PRZEŻYWALNOŚCIĄ I INWAZYJNOŚCIĄ LARW III STOPNIA *ANISAKIS SIMPLEX*, WYSTĘPUJĄCYCH W ŚLEDZIACH BAŁTYCKICH ŚWIEŻYCH SOLONYCH KORZENNIE

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The studies involved periodical teste of salted and spiced Baltic herring for the presence of live *Anisakis simplex* larvae through indicing their spontaneous movements when kept in a liver extract – fresh bovine blood medium in a thermostat set at 37°C for 4 days.

The infectivity of the larvae was studies by in vitro observing their development in a medium such as above. A period of time necessery to kill all the *Anisakis* larvae present in salted an spiced herring was determined.

INTRODUCTION

It is very important from the standpoint of food hygiene to determine the variability of *Anisakis simplex* stage III larvae, very common in marine fishes. The larvae are known

* The project financed in part by the M. Skłodowska-Curie Fund trough the Cooperation Agrement No F-05-652-F between the Academy of Agriculture in Szczecin and US Food and Drug Administration.

The paper was sent to the US Food and Drug Administration. In view of the lack of information as to the fate of the manuscript I have decide to publish it – Eugeniusz Grabda.

to cause human anisakiasis, zoonosis discovered for the first time in Holland in 1955 (Van Thiel et al., 1960). The diseases spreads in those countries where raw (Japan) or alightly salted (Holland) fish are traditionally consumed. Single sases of anisakiasis were found also in other countries (England, Denmark, Belgium, USA, Chile, Korea, and Taiwan).

As stated by Margolis (1977), a total of 1200 anisakiasis cases caused by the *Anisakis* larvae were recorded during the last 15 years.

Several studies on the viability of the larvae in salted, smoked, pickled, and frozen fish were carried out in the sixties, adequate technological parameters to ensure the death of the larvae and diseases prevention being established (Ruitenberg, 1970).

On the other hand, there are few studies dealing with the *Anisakis* larvae viability in salted and spiced fish, recently introduced on the market and very popular in Poland, particularly with respect to herring.

Spiced and salted herring are prepared in Poland in accordance to the Production Standard No. ZN-72!ZGR-0915 „Salted and spiced fish" recommending 10–12% salt content in fish flesh; the brine ought to have specific weight of 1.151–1.168 Mg/m^3 equivalent to 20–22°Bé salinity (Podeszewski and Stodolnik, 1973*). The result is slightly salted fish with the addition of sugar (0.5%), spices (0.8%), and preservatives (0.3%).

According to the Production Standard No. ZGR/S–II–4–76, the product is obtained from frozen deep-sea herring in which deep freezing (below –20°C) is assumed to kill the *Anisakis* larvae present, and fresh Baltic herring processed directly after capture and not frozen. The latter can be very strongly invaded depending on the season and fish size (J. Grabda, 1974a).

It is the Baltic herring that were the object of the procent study. So far, no work has been done in Poland on salted and spiced Baltic herring. It was only the North Sea herring (Lubieniecki, 1970) and herring from such fishing grounds off the British Isles as the Irish Sea, English Channel, and Hebrides (J. Grabda, 1974c) that were examined.

Lubieniecki (1970) studied the herring processed according to the Fisheries Central Board Standard issued in 1969, requiring 7–10% salt content in fish flesh and less than 20°Bé brine.

The Standard required the uptake of 9.5 kg salt, 0.405 kg sugar, 0.614 kg spices, and 0.225 kg preservatives per 90 kg fish. Fresh deep-sea herring salted on board were processed.

Lubieniecki recorded several live larvae at 15–19°Bé brine. He found that in most cases the amount of salt required by the Standard produced a 22–23°Bé brine, sufficient to kill nematode larvae over 7 days, on the third or fourth day after salting. However, be made a provision that the herring flesh salt content depended not only on the actual brine salt concentration, but also on the gonad maturity stage, flesh fat content, and salting temperature; all those factors could possibly contribute to elongate the *Anisakis* larvae viability.

* Podeszewski, Z., Stodolnik, L., 1973: Ćwiczenia z technologii zabezpieczenia surowców rybnych (Raw fish protection technology-practicals manual). Akad. Roln. w Szczecinie (in Polish).

Subsequent studies by J. Grabda (1974b,c,d) were carried out during a 3-yr period of 1971–1973 on deep-sea herring processed on board according to the above mentioned Standard. Each year live larvae were being found at 10–19°Bé brine. The shorter herring maturation period and the lower brine salt content, the more larvae were found live.

Therefore an increase in the fish flesh salt content at the expense of certain flavour-enhancing properties was postulated, 20°Bé brine and 20% salt in fish flesh being assumed as the minimum. The maturation period enabling a proper absorption of salt by the flesh, leading to death of all larvae, should be sufficiently long, preferably 2 months before marketing the fish.

It was as late as in 1973 that the Fisheries Central Board implemented the recommended increase in brine salt content; in 1976 a new standard was worked out according to which the salted and spiced Baltic herring are prepared at present.

Both Lubieniecki and Grabda studies the larvae viability only, using the method by Khalil (2-h heating at 37°C), while the studies described below were aimed at finding for how long the *Anisakis simplex* larvae remained viable and whether they retained their infectivity or if salt and spices weakened them so as to render them harmless to man (Cishi et al., 1974).

MATERIALS AND METHODS

The studies were carried out on fresh Baltic herring, salted and spiced in accordance with the 1976 Production Standard No. ZGR/S--II–4–76 „Salted an spiced Baltic herring". The following brine composition is requred per 1000 kg of the final product: 124.00 kg salt, 5.26 kg sugar, 1.30 kg black pepper, 2.22 kg pimento, 0.93 kg cinammon, 0.93 kg clove, 0.47 kg coriander, 1.30 kg bay leaf, 0.47 kg ginger, 0.35 kg nutmeg, 1.17 kg natrium benzoate, 1.75 kg para-oxi-benzoate acid ester.

This brine should yield a final product of 10–12% flesh salt content and 1.151–1.168 Mg/m^3 brine specific weight (Production Standard No. ZN–72/ZGR–09195 „Salted fish. Salted and spiced fish").

Large Baltic herring (25–30 cm l.t.) were caught in the Pomeranian Bay from December through March, i.e., when the *Anisakis* infested western herring migrated here to spawn (J. Grabda, 1974a).

After checking the degree of infestation by opening the fish abdominal cavity, the fishes were headed and placed in jars with salt-spices mixture added. The jars were kept in refrigerator at about +5°C.

In this way 6 batches of herring caught on various dates were prepared in the laboratory. In each batch, the *Anisakis* larvae viability was checked 1, 2, 3, and 4 weeks after salting.

Each time the brine salinity (°Bé) was measured with the Baumé salinometer (J.Long Ltd.London, N.D.:A.2825) at 15°C, while the fish flesh salt content was checked by

$AgNO_3$ titration according to the Polish Standard No. PN–74/A–86739 „Fish and fish products. Salt content determination".

Larvae viability tests The larvae viability was tested in 4 days after removing them from a fish. After 1 week in the brine some larvae showed spontaneous, movements usually on the first day of testing. The remaining larvae, seemingly dead, transferred to the culturing medium consisting of bovine liver extract enriched with fresh bovine blood (J. Grabda, 1976) and placed in a thermostat for 24 h at 37°C. Some larvae became „revived" after 24 hours. The remaining ones were left for further 24 hours in the medium. In this way their viability was checked 3 and 4 days after removal from fish. Some larvae were observed to revive after 72 hours.

The viability after 3,3, and 4 weeks from salting was tested in the some way.

Studies on larvae infectivity The infectivity of the larvae was studied by in vitro cultures (J. Grabda, 1976), assuming that the larval development proceeding in the medium is an evidence of their capability to invade and develop in a live poikilotherm organism, man included, under natural conditions.

The larvae moving spontaneously on the first day and those showing movements on the second and subsequent days were kept separately. The observations were carried out until sexually mature nematodes were obtained, or until the death of the last larva. Usually, 20 larvae of each series were cultured.

RESULTS

The results of viability tests in the *Anisakis simplex* larvae removed from salted and spiced herring and their capability of further development are summarised in tables illustrating 4 series of experiments (Tables 1–4).

After 1 week in salted herring Live, spontaneously moving larvae were being found as early as on the first day of the examination, and also after 24 hours. Generally, almost all (up to 98.2%) of the larvae in the herring tested were mobile. The brine salinity was 17–19°Bé, the viscera salt content ranging within 5.6–8.2%.

The third moult was observed to start normally on the fourth to sixth day of the cultures; after this moult, in two cases only deaths of 3 and 7 larvae (15–35% of the cultures larvae) were recorded. In other experiments, all the larvae survived. The nematodes underwent the fourth moult, too, to metamorphose into stage 4 proceeding sexual maturity. Saome larvae (5–45%) reached maturity and produced eggs. The nematodes survived for 25 to 67 days.

After 2 weeks in salted herring. The brine salinity amounted to 17–18°Bé, while the flesh salt content was 9.36–12.9%. No motile larvae were found on the first day. After 24 hours 12.6–84.6% of the larvae found were „revived". In one case, motile larvae – seemingly dead initially – were revealed on the third and fourth day. Before the third moult and during it, 11.3–68% of the cultured larvae dies off, only a few reaching maturity in two instances when 3.9–5% survived. The survival time was 14–52 days.

Anisakis simplex larvae viability in salted and spiced herring.
Date of salting: 7 Feb. 1980.

Parameter tested		After 1 week 14 Feb. 1980	After 2 weeks 21 Feb. 1980	After 3 weeks 28 Feb. 1980	After 4 weeks
Brine salinity		18°Bé	17.5°Bé	19° Bé	not studied
Per cent NaCl		5.6	12.9	14.04	
No. of fish chacked		9	17	11	
No. of larvae found		41	71	22	
Motile larvae:	day 1	22 (53.6%)	0	0	
	day 2	not studied	9 (12.6%)	0	
	day 3	„ „	13 (18.3%)	0	
	day 4	„ „	3 (4.2%)	0	
No. of larvae cultured		20	25		
Mortality before 3 rd moult		0	17 (68%)		
Third moult		18–19 Feb.	27–28 Feb.		
Mortality before 4 th moult		12	7		
Fourth moult		6–10 March	not observed		
Mortality after 4 th moult		7			
Culture terminated		1 June	10 March		
Nematode survival		1 ♂ (5%)	1 larvae (4%)		
Period of survival		47 days	19 days		

Table 2

Anisakis simplex larvae viability in salted and spiced herring.
Date of salting: 12 March 1980.

Parameter tested		After 1 week 19 March 1980	After 2 weeks 26 March 1980	After 3 weeks 2 April 1980	After 4 weeks 9 April 1980
Brine salinity		17° Bé	17° Bé	17.5° Bé	20.5° Bé
Per cent Na Cl		7.2	10.6	11.9	14.6
No. of fish checked		10	10	10	23
No. of larvae found		29	93	81	102
Motile larvae:	day 1	4 (13.7%)	0	0	0
	day 2	25 (86.2%)	73 (78.4%)	1(1.2%)	0
	day 3	0	0	7 (8.6%)	0
No. of larvae cultured		20	53	8	
Mortality before 3 rd moult		7 (35%)	6 (11.3%)	7 (87.5%)	
Third moult		24–28 March	1–3 April	8 April	
Mortality before 4th moult		11	35	1	
Fourth moult		14 April	14–21 April		
Mortality after 4th moult		1	10		
Culture terminated		22 April	7 Mai	18 Mai	
Nematode survival		1 (5%)	2 (3.7%)	1 (12.5%)	
Period of survival		35 days	44 days	17 days	

Anisakis simplex larvae viability in salted and spiced herring.
Date of salting: 13 Jan. 1981.

Parameter tested		After 1 week 20 Jan. 1981	After 2 weeks 27 Jan. 1981	After 3 weeks 3 Feb. 1981	After 4 weeks 10 Feb. 1981
Brine salinity		18° Bé	18° Bé	18° Bé	20° Bé
Per cent NaCl		7.1	9.36	11.9	12.6
No. of fish checked		5	6	6	7
No. of larvae found		56	65	71	77
Motile larvae:	day 1	55 (98.2%)	0	0	0
	day 2	0	55 (84.6%)	13 (18.3%)	0
No. of larvae cultured		20	20	13	
Mortality before 3rd moult		3 (15%)	4 (20%)	8 (61.5%)	
Third moult		23–26 Jan.	1–3 Feb.	7–9 Feb.	
Mortality before 4th moult		7	5	4	
Fourth moult		6–11 Feb.	18 Feb.	not observed	
Mortality after 4th moult		1	10	0	
Culture terminated		10 March	16 March	2 March	
Nematode survival		9 (45%) 6 ♂, 3 ♀	1 (5%)	1(7.6%)	
Period of survival		49 days	52 days	28 days	

Table 4

Anisakis simplex larvae viability in salted and spiced herring.
Date of salting: 13 Feb. 1981.

Parameter tested		After 1 week 20 Feb. 1981	After 2 weeks 27 Feb. 1981	After 3 weeks 6 March 1981	After 4 weeks 13 March 1981
Brine salinity		19°Bé	18°Bé	18°Bé	18.5°Bé
Per cent NaCl		8.22	10.44	11.6	12.2
No. of fish checked		7	9	9	8
No. of larvae found		73	55	53	72
Motile larvae:	day 1	A. 26 (35.6%)	0	0	0
	day 2	B. 33 (45.2%)	19 (34.5%)	2 (3.7%)	0
No. of larvae cultured		A. 20 B. 20	17	2	
Mortality before 3rd moult		A. 0 B. 0	6 (35.2%)	2	
Third moult		A. 24 Feb. B. 24–26 Feb.	3–4 March		
Mortality before 4th moult		A. 19 B. 19	11		
Fourth moult		A. 14–16 March B. dead 16 March			
Culture terminated		A. 27 April B. 16 March	12 March	8 March	
Nematode survival		A. 1 (5%) B. 1 (5%)	1 (5.8%)	0	
Period of survival		A. 67 days B. 25 days	14 days	3 days	

After 3 weeks in salted herring. The brine salinity was 17.5–19°Bé, the salt content in flesh ranged within 11.6–14.04%. Notile larvae were found in two cultures, One of them (18°Bé brine, 11.9% salt in flesh) contained 13 (18.3%) motile larvae of which 8 died before the third moult and only 1 survived 26 days. The third moult took place at a normal time. This larva then, in our opinion, was fully capable of surviving in the human intestine and of penetrating to the intestinal mucosa, thus inducing pethological changes. The fourth moult was not observed. The other culture (18°Bé brine, 11.6% flesh salt content) was found to contain 2 larvae only which performed very weak spontaneous movements and died on the second day.

After 4 week in salt herring. The brine salinity ranged within 18.5–20.5°Bé, 12.2–14.6% being the flesh salt content range. No motile larvae were found in the cultures.

The experiments show the *Anisakis* larvae to retain their full viability during fish maturation in the first week after salting; thus the larvae capability of a further development and thus of invading is retained as well (obviously, the natural mortality of larvae, affecting also the cultures of fresh larvae not exposed to salt (J. Grabda, 1976) should be taken into account). This is the period when, as should be supposed, the larvae are most harmful to man.

After 2 weeks the danger is considerably smaller, while after 3 weeks from salting any posibility of the larvae invading humans is virtusally non-existent. The larval mortality rate is very high, single individuals only proceeding through the third moult and none reaching sexual maturity owing to their considerable weakening. Nevertheless, it is only after 4 weeks from the salting date that the salted and spiced Baltic herring can be regarded as entirely safe, parasitologically, for human consumption and should be allowed time to reach their full flavour properties.

DISCUSSION

When performing the experimented described above, it was seemed necessary to find appropriate criteria of larval viability.

Van Thiel et al. (1960) regarded the larvae as dead when no visible movements could be observed under x20 magnification for a week after transferring the larvae to 50%-diluted sea water at 10°C.

Khail (1969) differentiated between spontaneous larval movements and those induced by mechanical stimuli; he regarded non-motile larvae as dead. The author placed the larvae, removed from fish, at the Tyrode fluid at room temperature for an hour and then transferred them for 2 hours to a pepsin-HCl solution at 36°C in thermostat and observed them under a stereomicroscope.

Khalil's method, slightly modified, has been in common use (Lubieniecki, 1970; Oishi et al., 1574; Hanek, 1977; Lee and Chyu, 1570; and others).

In view of the present results, the assumption that those larvae remaining motionless after 2 hours in a thermostat are dead dose not suffice to detect live larvae in salted and

spiced herring and should not be applied when checking larval viability. The examination should continue for 4 days as motile larvae removed 3 weeks after salting were observed as late as after 72 hours, the larvae shoeing at first no trace of any movement.

It is not necessary to use a pepsin-HCl digesting medium; a liver extract-blood medium and a storage at 37°C, i.e., the conditions corresponding to those under which the parasite's life cycle proceds should be applied instead.

Another issue of importance for the *Anisakis* invasiology is the infectivity of those larvae considered live. Pathogenic properties of the larvae are determined not only by their viability but also by their ability to penetrate the host's intestinal mucosa.

Several authors carried out in vivo studies by artifical infested experimental animals (rabbits, guinea pigs, rate, pigleta). On the other hand, Ruitenberg (1970) used in vitro laboratory techniques in addition to his studies in vivo. He was using 188 mm high 23 mm diameter vials, filled with agar-agar to 3/4 of their volume. In a half of the vials, the medium had a amooth surface; in the other half, the medium surface was roughened by scrubbing with forceps. A 1 cm thick layer of herring blood was poured on to the agar-agar and 5 *Anisakis* larvae placed there. The vials were kept in a thermostat at 37°C; the larvae penetrating into the medium were counted after 1, 2, and 3 days. Most larvae were fiund to have penetrated after 1 night (18 hours), more larvae penetrating the rough-surface agar-agar medium.

Although possessing many advantages, Ruitenberg's technique is not sufficient to check the infectivity of larvae of differing viability. Therefore, during the present work, the larval life cycle was followed in an in vitro culture kept in a blood-enriched liver extract at 37°C, with corresponds most closely to the conditions created by the internal milieu of a poikllitherm, the definits host for *Anisakis simplex*.

Having penetrated their definite hosts, the larvae become very active as early as during the first 24 hours and penetrate the intestinal mucosa, thus inducing pathological changes in a patient (Van Thiel et al., 1960; Ruitenberg, 1970; and others). It is then sufficient to continue the culture until the 3rd moult occurs, i.e., for about 5 days.

In the present work, only the effects of salt content in fish flesh and in brine on the *Anisakis* larvae viability and infectivity were studies, no consideration being given to effects of spice essential cils and preservatives using in the salted and spiced herring production, the effects undoubtedly existing as shown by Japanese authors (Oishi et al., 1974). They found all the spice essential cils, particularly the nutmeg and nutmeg flower ones as well as those of coriander, dill, cummin, clove, allspice, and cinnamon to affect the *Anisakis* larvae. Of the synthetic preservatives, particularly strong, deterimental for *Anisakis*, effects were obtained when using sorbic, benzoic, dehydroacetic, and salicylic acids. Effects of salts of those acids are, however, much weaker.

Effects of those substances are certainly additive with respect to salt and provide an additional factor detrimental to the *Anisakis* larvae.

CONCLUSIONS

1. The viability criterion for *Anisakis simplex* larvae occurring in fresh salted and spiced Baltic herring was a spontaneous movement of the apparently dead larvae removed from herring, placed in a cattle blood-enriched liver extract and kept for 4 days in a thermostat. The „revival" of those apparently dead larvae can occur on the 1st, 2nd, 3rd, and even 4th day.
2. The infectivity of the larvae removed from salted and spiced herring can be checked in an in vitro culture (fresh cattle blood-enriched liver extract as a medium, 37°C) instead of an artificial infested of laboratory animals or Ruitenberg's agar-agar technique.
3. It is sufficient to keep the culture until the third moult, i.e., for 5 days.
4. A period of 4 weeks from salting is sufficient to kill all the *Anisakis simplex* larvae occurring in salted and spiced Baltic herring produced in Poland according to the Production Standard No. ZGR/s-II–4–76.

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BADANIA NAD PRZEŻYWALNOŚCIĄ I INWAZYJNOŚCIĄ LARW III STOPNIA *ANIASKIS SIMPLEX*, WYSTĘPUJĄCYCH W ŚLEDZIACH BAŁTYCKICH ŚWIEŻYCH SOLONYCH KORZENNIE

STRESZCZENIE

Większość dotychczasowych badań nad anisakidozą dotyczyła badań nad parametrami technologicznymi doprowadzającymi do zabicia larw *Anisakis simplex* w produktach rybnych a tym samym zabezpieczenie ludzi przed zarażeniem.

Mało jest badań nad żywotnością larw w śledziach mało solnych, tzw. korzennych, ostatnio bardzo preferowanych w przetwórstwie rybnym.

Śledzie korzenne przygotowywano wg normy polskiej Zn-72/ZGR–09195 „Ryby solone" w zaprawie 10–12% soli kuchennej w mięsie (20–22 Bé) z dodatkiem cukru (0,5%) i korzeni (0,8%) oraz środków konserwujących (0,3%).

Dotychczas przebadano śledzie z Morza Północnego (Lubieniecki, 1970), wokół Wysp Brytyjskich i M. Irlandzkiego (J. Grabda, 1974). Obecna praca dotyczy po raz pierwszy śledzi bałtyckich. Ponieważ niejednokrotnie mimo tych zabiegów trafiały się larwy żywe, wzbudziło to niepokój. Trzeba nadmienić, że do badań nad żywotnością larw używano testu Khalila, polegającego na stwierdzeniu ruchu larw po 2-godzinnym ogrzaniu w temperaturze 37°C.

Autorka przeprowadziła badania nad zdolnością inwazyjną larw po przeprowadzonych zabiegach przewidzianych normą: Żywotność larw sprawdzano w ciągu 4 dni od chwili wyjęcia z zalewy solnej: Po tygodniu trzymania w solance, zwykle już pierwszego dnia, część larw wykazywała ruch spontaniczny. Reszta larw pozornie martwych umieszczano w pożywce do hodowli larw *Anisakis*, składającej się z ekstraktu wołowej wątroby z dodatkiem świeżej krwi bydlęcej (J. Grabda, 1976) w termostacie 37°C na 24 godziny. Część larw ożywała a pozostałe zostawiano na dalsze 24 godziny. Tak czyniono aż do 4 dnia. Obserwacje prowadzono do chwili uzyskania stadium dojrzałości płciowej ew. śmierci ostatniego osobnika.

Kryterium badania ruchów larw po przebyciu kąpieli przygotowującej śledzie korzenne jest niewystarczające. Dopiero zbadanie zdolności inwazyjnej daje pełną gwarancję nieszkodliwości larw dla konsumenta. Do celów rozpoznawczych wystarczy prowadzić hodowlę do czasu wystąpienia III linki tj. przez 5 dni.

W śledziach bałtyckich solonych korzennie wg polskiej normy dopiero okres 4 tygodni przebywania w zalewie od dnia zasolenia daje gwarancję nieszkodliwości.

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ИССЛЕДОВАНИЯ ВЫЖИВАЕМОСТИ И ИНВАЗИОННОСТИ ЛИЧИНОК III СТЕПЕНИ ANISAKIS SIMPLEX, ПРИСУТСТВУЮЩИХ В СВЕЖЕМ БАЛТИЙСКОМ СЕЛЬДИ ПРЯНОГО СОЛЕНИЯ

Р Е З Ю М Е

Большинство имеющихся исследований анисакидоза касалось определения технологических параметров, необходимых для уничтожения Anisakis simplex в рыбных продуктах и тем самым обеспечения людей от заражения.

Немного имеется исследований, касавшихся жизнеспособности личинок в малосолёной сельди, т.н. пряного соления, в последнее время особенно предпочтительной в переработке рыбы.

Пряную сельдь изготовляли по польскому стандарту Zn-72/ZGR-09195 „Солёная рыба" в 10-12% растворе поваренной соли в мясе (2%-22 Bé) с добавкой сахара (0,5%) и пряностей (0,8%) а также консервантов (0,3%).

До этого времени были проведены исследования сельди происходящей из Северного моря (Lubieniecki , 1970), вокруг Британского острова и Ирландского моря (J.Grabda, 1974). Настоящая работа главным образом касается балтийской сельди. Несмотря на применявшиеся мероприятия, много раз встречались в сельди живые личинки, что представляет собой опасение. Жизнеспособность личинок исследовали с помощью теста Khalil , заключавшегося в установлении движения личинок после нагрева при 37°C на протяжении 2 часов.

Автором исследовалась инвазионная способность личинок после выполнения мероприятий предвиденных стандартом. Жизнеспособность личинок проверяли через 4 дня с момента окончания соления сельди. При однонедельном выдерживании в соляном растворе, обычно уже с первого дня часть личинок проявляла спонтанное движение. С виду мёртвых остальных личинок помещали в питательную среду для выращивания личинок Anisakis , состоящую из вытяжки говяжей печени с добавкой свежей крови скота (J. Grabda, 1976) и инкубировали в течение суток при 37°C. У части личинок наблюдались признаки жизнеспособности, остальных снова помещали в термостат на протяжение суток. Эти мероприятия повторяли до 4 дня. Наблюдения продолжались до достижения личинками половой спелости, или смерти последних особей.

Исследование движения личинок после выдерживания рыбы в рассоле для подготовки сельди пряного соления - это недостаточный критерий. Полную гарантию безопасности можно получить при определении инвазионной способности личинок. Для опознавательных целей исследования должны продолжаться до III линьки, т.е. на протяжении 5 дней.

Для балтийской сельди пряного соления по польском стандартам полную гарантию безопасности можно получить при выдерживании в рассоле на протяжении 4 недель.

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Received: 1981