

## Detection of parasites in walleye pollock (*Gadus chalcogrammus* Pallas, 1814) fillets marketed in São Paulo, Brazil

### Detecção de parasitos em filés de polaca do Alasca (*Gadus chalcogrammus*, Pallas, 1814) comercializados em São Paulo, Brasil

#### ABSTRACT

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**Introduction:** Walleye pollock is the most caught fish worldwide and has been imported by Brazil as frozen fillets. This fish has its own parasitic fauna; however, the presence of parasites in the musculature may cause implications for food safety and repulsiveness. **Objective:** This work aimed to investigate and report the presence of parasite groups in commercial samples of frozen fillets of walleye pollock concerning the potential risk to human health and control methods. **Method:** A total of 44 samples of frozen fillets of walleye pollock collected from the retail trade of the metropolitan area of São Paulo were analyzed and the larval forms were isolated by dissection and observed under a stereoscopic and optical microscope. **Results:** One hundred thirty-three dead parasites were found from 68% of the samples. Trypanorhyncha cestodes were detected more frequently (88%), followed by nematodes from the anisakid group (Anisakidae/Raphidascavidae) (11%) and acanthocephalan (1%). Among them, anisakid has zoonotic potential, if ingested alive, and allergenic potential even after thermal processing. **Conclusions:** This is the first report of the presence of parasitic forms in commercial samples of walleye pollock fillets and the results alert to the need for improvements in relation to Good Practices in the production chain and the need for greater attention on the allergenic potential related to the consumption of these parasites on frozen fish fillets.

**KEYWORDS:** Fish; *Gadus chalcogrammus*; *Theragra chalcogramma*; Public Health Surveillance; Food Safety

#### RESUMO

**Introdução:** A polaca do Alasca, considerada a espécie de pescado marinho mais capturada mundialmente, é importada pelo Brasil como filés congelados. Apresenta uma fauna parasitária própria, porém a presença desses parasitos na musculatura pode causar implicações na segurança alimentar e repugnância. **Objetivo:** Este trabalho teve como objetivos investigar e relatar a presença de grupos parasitários em amostras comerciais de filés congelados de polaca do Alasca, considerando o potencial de risco à saúde humana e métodos de controle. **Método:** Foram analisadas 44 amostras de filés congelados de polaca do Alasca coletadas do comércio varejista da região metropolitana de São Paulo e as formas larvares foram isoladas por dissecação e observadas em microscópio estereoscópico e óptico. **Resultados:** Foram encontrados 133 parasitos mortos em 68% das amostras. Os cestódeos da ordem Trypanorhyncha foram detectados com maior frequência (88%), seguidos de nematódeos do grupo dos anisacídeos (Anisakidae/Raphidascarididae) (11%) e acantocéfalo (1%). Dentre eles, os anisacídeos apresentam potencial zoonótico, se ingeridos vivos, e alergênico mesmo após processamento térmico. **Conclusões:** Trata-se do primeiro relato da presença de formas parasitárias em amostras comerciais de filés de polaca do Alasca e os resultados alertam para a necessidade de melhorias com relação às boas práticas na cadeia produtiva e de maior atenção sobre o potencial alergênico relacionado ao consumo desses parasitos nos filés de peixes congelados.

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**PALAVRAS-CHAVE:** Pescado; *Gadus chalcogrammus*; *Theragra chalcogramma*; Vigilância em Saúde Pública; Segurança dos Alimentos



## INTRODUCTION

Walleye pollock, *Gadus chalcogrammus* Pallas, 1814, formerly known as *Theragra chalcogramma* Pallas, 1814, are teleost fish from the northern Pacific Ocean, which span from Alaska to the southern Sea of Japan<sup>1</sup>. Considered in 2017 the world's most caught species of marine fish, *G. chalcogrammus* lives in the wild and is an excellent source of protein, minerals and omega-3<sup>2,3</sup>. In 2018, Brazil imported 15,000 tonnes of frozen Walleye pollock fillets, 99% of which came from China and the remainder from the United States, Vietnam and Portugal<sup>4</sup>.

Fish, as well as other vertebrates, have their own parasitic fauna in the natural environment, including several species of the main parasite groups<sup>5</sup>. Many authors in ecological studies have reported the presence of several parasites in Walleye pollock caught in Canada, Alaska, and Northern Japan, including the following nematodes: *Anisakis simplex* Rudolphi, 1809<sup>6,7,8,9,10,11</sup>, *Pseudoterranova decipiens* Krabbe, 1878, *Contracaecum* sp. Railliet & Henry, 1912<sup>6,12</sup> and *Hysterothylacium* sp. Ward & Magath, 1917<sup>6,13,12</sup>; cestodes *Nybelinia surmenicola* Okada in Dolfus, 1929<sup>6</sup> and *Phyllobothrium* sp. Van Beneden, 1849<sup>12</sup>; and acanthocephalus *Corynosoma* sp. Luhe, 1904<sup>12</sup> and *Echinorhynchus gadi* Zoega in Müller, 1776<sup>12</sup>.

*Anisakis* nematodes are natural gut parasites of piscivorous marine mammals and have teleost fish, cephalopod mollusks and small crustaceans<sup>14</sup> as intermediate hosts of their infective larval forms (L3). In this group of nematodes, some species of families Anisakidae and Raphidascarididae are capable of causing an accidental infection called anisakiasis<sup>15,16</sup>, characterized by GI symptoms in the form of transient infection, due to the effect of live larvae on the digestive tube wall. Allergic manifestations may also be caused by exposure of susceptible individuals to this nematode's antigens, even with the ingestion of the dead parasite, with symptoms ranging from mild hives to anaphylactic shock<sup>17,18</sup>. Anisakiasis can be acquired by ingesting raw, undercooked, inadequately salted/smoked or insufficiently frozen fish<sup>19</sup> containing the infecting larva, but proper thermal processes like cooking or freezing may render it unviable for infection<sup>20</sup>.

Other fish parasites, like cestodes of the Trypanorhyncha order and acanthocephalus, are not related to human health risk, with rare reports of accidental infection. Trypanorhyncha cestodes have as their definitive hosts stingrays and sharks and use teleost fish and various marine invertebrates as intermediate hosts. These parasites have hygienic and sanitary importance because of their potential to disgust consumers with the presence of larval forms in the muscles of several economically relevant species, causing significant losses<sup>21,22</sup>.

Food surveillance works to preserve and promote the health of the population by controlling food quality. Therefore, this study aimed to investigate and report the presence of parasitic groups in commercial samples of frozen Walleye pollock fillets and to evaluate their potential public health risk, as well as control methods.

## METHOD

We analyzed 44 commercial samples of frozen Walleye pollock fillets of 18 different brands, weighing between 0.5 kg and 2 kg, with about four to nine fillets per sample, totaling 47.8 kg of the product. Among the samples, 26 were collected by the Health Surveillance body for inspection analysis in a monitoring program (São Paulo State Program of Health Surveillance in Food) and 18 were acquired by the laboratory, all in retail stores of the São Paulo metropolitan area. The analyses were performed at the Morphology and Microscopy Center of the Adolfo Lutz Institute, Central Laboratory, from January 2017 to July 2018. According to the information described on the packaging label, 38 samples were from China, five from the United States and one from Vietnam.

The fillets were thawed and observed on a light table. The larval forms were isolated by dissection with the aid of forceps and scalpel. The larval forms were kept in a Petri dish containing distilled water. The nematodes were removed from the cyst and immersed in distilled water at 56°C to enable their distension and plate preparation. For the cestodes, the cysts were torn with tweezers whenever necessary. The parasitic forms were observed in a stereoscopic microscope, by Leica®, model MZ9.5, and a light microscope, by Nikon®, model Eclipse E200, and later preserved in 70% alcohol. The photomicrographs were performed under the Zeiss® Axio Scope.A1 optical microscope with the aid of the Axio Vision LE program.

The nematodes were identified according to Hartwich<sup>23</sup> and Felizardo et al.<sup>24</sup>, the cestodes, according to Campbell and Beveridge<sup>25</sup>, and the acanthocephalus, according to Amin<sup>26</sup>. As a viability criterion for parasites, the following parameters were adopted: physical integrity, spontaneous movement or stimulation, described in the *European Food Safety Authority Journal*<sup>27</sup>.

Data on the parasite groups were analyzed in absolute frequency, relative frequency and range of variation between samples. The average density was obtained per kilo of fillet and comparisons between proportions were made using the chi-square test with a significance level of 95%. Microsoft Excel 2010 and GraphPad Software 2018 programs were used.

## RESULTS AND DISCUSSION

Dead parasites were found in 68% of the samples, totaling 133 parasitic forms. The number of samples that presented any parasitic form (positive samples) and the number of isolated parasites in the total samples by parasite type are shown in the Table.

Trypanorhyncha cestodes (Figure 1) were detected more frequently ( $p < 0.05$ ), with an average density of 3.9 parasites per kilo of fish and range of variation from one to 11 specimens in



the samples. The *Anisakis* nematodes presented average density of one parasite per kilo of fillet, with range of variation of one to four specimens in the samples. Most were spirally encysted, but some were not encysted and others were in encysting process (Figure 2). The only specimen of isolated acanthocephalus was found without its posterior region (Figure 3).

Monoparasitic samples (80%) were significantly predominant ( $p < 0.05$ ) in relation to samples that presented polyparasitism, containing more than one parasitic group (20%). Of these, one contained one larva of acanthocephalus and seven larvae of Trypanorhyncha, while another five contained larvae of Trypanorhyncha and *Anisakis* simultaneously.

The distribution of parasites in the fillets was diversified. They were found both on the surface and inserted in the muscle fibers. Although the location of the parasitic forms in the fillets was not tabulated, predominance was observed in the abdominal

(ventral) region, but this data should be verified in future studies, with more samples. The possibility of detection methodology bias should also be considered, because although the fillets have been completely dissected, this region is thinner and enables better visualization in contrast with light.

The possibility of visual detection was not the object of this study, however, some parasite specimens could be seen with the naked eye, even without the aid of light, especially specimens of *Anisakis* and acanthocephalus. The Trypanorhyncha were visualized mainly by through contrast with white light.

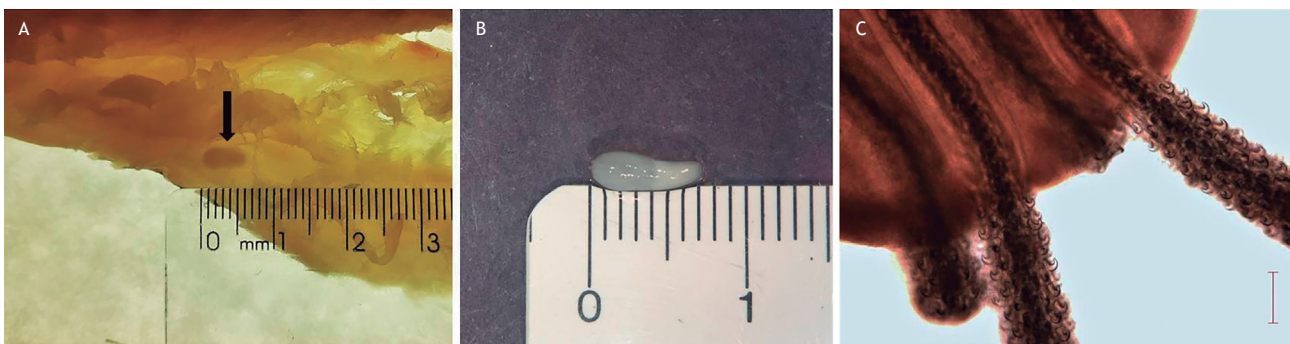
This is the first report of detection of parasitic forms in commercial samples of frozen Walleye pollock fillets. Ecological studies with *G. chalcogrammus* (*T. chalcogramma*) show the natural occurrence of parasites, including the parasitic groups found in the present study, at variable sites of infection, such as mesentery, body cavity, intestinal wall, muscles, among others organs<sup>7,12,8,11</sup>. In the muscles of *G. chalcogrammus* (*T. chalcogramma*), captured in British Columbia and Alaska, researchers have found larvae of *Anisakis* nematodes (*Anisakis* sp.<sup>12</sup>, *Anisakis simplex*<sup>6,7</sup> and *Pseudoterranova* sp.<sup>12</sup>, *Pseudoterranova decipiens*<sup>7</sup>) and cestodes (*Nybelinia surmenicola*<sup>6</sup> and *Phyllobothrium* sp.<sup>12</sup>).

In Brazil, these same parasitic groups have been reported in several species of river and coastal fish like flounder, triggerfish, lookdown, among other<sup>28,29,30</sup>. Larvae of *Anisakis* nematodes have also been found in samples of dried and salted gutted cod sold in Brazil<sup>31,32</sup> and in Portugal<sup>33</sup>.

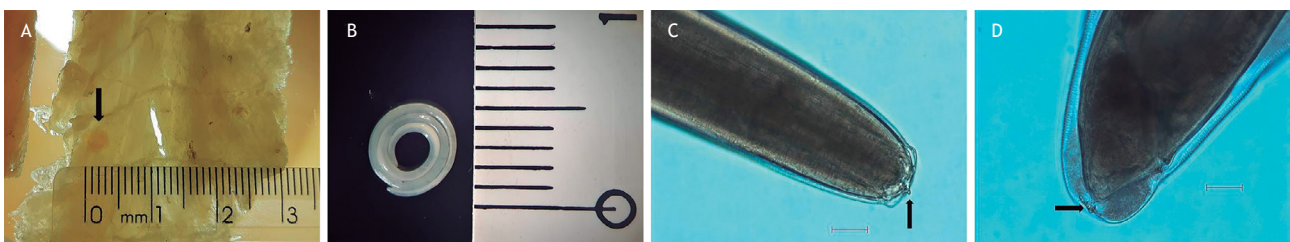
**Table.** Frequency of positive samples and isolated parasites in Walleye pollock fillet samples, analyzed in the city of São Paulo, Brazil, between 2017 and 2018.

Type of parasite	Positive samples		Isolated parasites	
	n.	%	n.	%
<i>Anisakis</i> nematodes	10	23	15	11
Trypanorhyncha cestodes	25	57	117	88
Acanthocephalus	1	2	1	1
TOTAL	30*	68	133	100

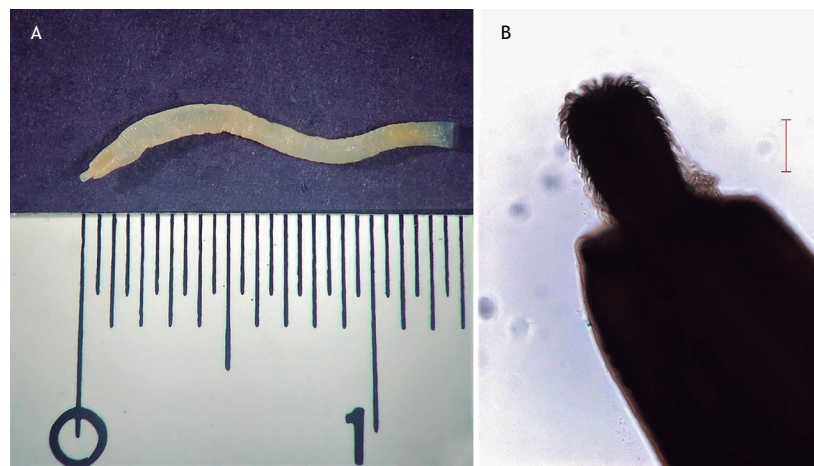
\* Six samples presented polyparasitism.



**Figure 1.** Cestodes of the Trypanorhyncha order isolated from *Gadus chalcogrammus*. (A) Macroscopic view of a specimen in a fillet on a light table. (B) Metacestoid observed under stereoscopic microscope. (C) Microscopic detail of the tentacle with hooks. Scale bar C = 100 µm.



**Figure 2.** L3 Larvae of *Anisakis* nematodes isolated from *Gadus chalcogrammus*. (A) Macroscopic view of the larva in a fillet on a light table. (B) Larva removed from the cyst observed under stereoscopic microscope. (C) Microscopic detail of the anterior region with larval tooth (arrow). (D) Microscopic detail of the posterior region showing mucron (arrow). Scale bar C,D = 50 µm.



**Figure 3.** *Acantocephalus* larva isolated from *Gadus chalcogrammus*. (A) Stereomicroscope visualization. (B) Microscopic detail of proboscis with hooks at anterior extremity. Scale bar B = 100  $\mu$ m.

The samples of Walleye pollock we analyzed underwent industrial evisceration, filleting, freezing processing and were subsequently selected for packaging. No information was found about the measures adopted to eliminate parasitic contamination in this product, but the results of the present study indicate that whatever was done was not enough to eliminate it completely, given the high percentage of positive samples.

The environment of wild fish like pollocks cannot be controlled, nor can their eating habits, so it is impossible to prevent them from acquiring lifelong parasites. However, the presence of parasites in the musculature is an aggravating factor, because it increases the possibility of ingestion. In this sense, good practices measures are necessary in the production chain, after the stage of capture, to reduce and/or eliminate parasitic contamination in the product that is provided to consumers, such as fillets. Many of these measures are contained in the Codex Alimentarius International Food Standards<sup>34,35</sup> and the Food and Drug Administration<sup>36</sup>, as well as the Industrial and Sanitary Inspection Regulation for Animal Products<sup>37</sup> and the Technical Regulations of the Brazilian Ministry of Agriculture. Among them, fish evisceration is recommended right after capture to prevent the migration of *Anisakis* larvae to the musculature<sup>38</sup> and abdomen (belly) clippings, combined with visual inspection techniques using of light sources for the physical removal of parasites<sup>34,35</sup>. Cutting the ventral region may contribute to the reduction of parasitic contamination in Walleye pollock fillets, but the predominance of larval forms localization needs to be confirmed in further studies.

When fish still have parasitic forms even after processing and with the adoption of best practices, as may have occurred in the analyzed samples, thermal processes like freezing or cooking to inactivate the remaining larval forms and minimize the risk of transmission of parasitic infections are indicated. Nevertheless, the efficacy of these methods depends on the combination between appropriate time and temperature<sup>36</sup>, since there are

reports in scientific literature of survival of the L3 larva of *Anisakis* during the freezing process due to the use of insufficiently low temperatures and lack of temperature homogeneity within the freezer<sup>39,40,41</sup>.

According to the Food and Drug Administration, freezing at at least  $-20^{\circ}\text{C}$  for 7 days or at  $-35^{\circ}\text{C}$  until solidification and maintenance of that temperature for a minimum of 15 h or after solidification and maintenance at  $-20^{\circ}\text{C}$  for 24 h<sup>36</sup> would be sufficient to inactivate the parasites. According to the World Health Organization, cooking at temperatures above  $70^{\circ}\text{C}$  makes the fish safe for consumption<sup>42</sup>. Since the analyzed Walleye pollock fillets are imported and kept frozen for a long time until they are marketed, this process was enough to make the parasites unviable.

Although the larvae of the parasites found in the present study were dead, the risks of consuming Walleye pollock fillets containing *Anisakis* nematodes should be considered, since it has been found that proteins of nematodes like *A. simplex* are highly resistant to thermal processing and maintain their allergenic properties even after freezing<sup>43,44,39</sup>. Therefore, the findings may pose some risk due to the possibility of ingestion by sensitized individuals, who could have allergic reactions regardless of larval viability<sup>45,46,47</sup>. Furthermore, both exposure to small doses of antigens and repeated exposure to these allergens increase the risk of developing this condition<sup>18</sup>. In some species of cestodes of the Trypanorhyncha order, an allergenic potential has also been observed in mice with induction of immune sensitization and manifestation of allergic reactions, including anaphylaxis<sup>48,49,50</sup>.

In Brazil there is only one case of anisakiasis reported in a patient who may have consumed raw seafood, with manifestation of gastrointestinal lesions<sup>51</sup>, and there are no medical reports of allergic reactions caused by *Anisakis*. It is difficult, however, to determine if there were no cases or if they were not diagnosed. In addition to the issues related to human health arising from the consumption of fish fillets



containing parasites, one must also consider the impact that this may have on consumers who get to see them, because, although dead, they have a disgusting appearance when present in food.

To prevent these occurrences, techniques for reducing parasitic contamination in processed fish must be improved and adopted to the maximum, thus contributing to the supply of high quality products in sanitary terms. Public sharing of information about the possibility of natural occurrence of parasites in extractive sea fish and about potential health risks also contribute to the understanding and prevention of any events.

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#### Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.



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