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Identification of *Anisakis* Spp. Infestation and its Effect in Greater Lizardfish (*Saurida tumbil*)

Ruchi S.* and Patricia F.

Smt. Chandibai Himathmal Mansukhani College, University of Mumbai, Thane District, Ulhasnagar 421003, Maharashtra, India

**Corresponding Author*

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Abstract: This study was aimed to identify the type of nematodes that infests the Greater Lizardfish (*Saurida tumbil*). Nematodes, *Anisakis* spp. commonly infests fishes, crustaceans, cephalopods and marine mammals in a wide geographical distribution. *Anisakis* spp. were identified causing parasitic infestation in Greater Lizardfish (*Saurida tumbil*). Greater Lizardfish (*Saurida tumbil*) is an edible fish and is of commercial importance. Proximate composition data given describes the quality of fish pre- and post-parasitic infestation. The study of fish also showed that infestation of parasite were only up to L3 larvae stage. This study has relevance in medicinal and economical aspects as infestation shows that decrease in nutritive content of fish (consumed raw or insufficiently cooked) has adverse effects on human health.

Keywords: Nematodes, *Anisakis* spp., Greater lizardfish, L3 larvae stage, Parasite

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Introduction

Saurida tumbil, the Greater Lizardfish (Bloch, 1795) (Fig. 1) is one of the commercially important fish species caught by demersal trawling. It is the dominant fish species of *Saurida* spp. (Kalhoro *et al.*, 2013). Greater Lizardfishes (family Synodontidae) comprises of 74 species and 4 genera (Eschmeyer, 2018). They are found in soft muddy or sand bottoms up to 200 m of depth and some of the species are in close association with shallow reefs (Russell, 2011). They are found distributed in tropical, subtropical and continental shelves (Golani, 1993). Lizardfishes have descended from a common evolutionary ancestral group within the order Aulopiformes which include species from various regions and bathymetric distributions (Davis, 2010). The measurement of depth of some species ranges from the shallow waters to about 800 m deep (Golani, 1993). It is a marine carnivorous fish that feeds on crusteceans and planktonic

Fig.1: *Saurida tumbil.*

invertebrates (Kadharsha *et al*., 2013; Manojkumar and Pavithran, 2016; Ozyurt *et al*., 2017). Therefore, existence of parasitic infection becomes higher in nature. Host species majorly gets affected by parasitic community because of their diet and feeding habits.

Fish parasites have various negative contributions on the commercial fisheries industries. Nematodes are one of the most common parasites found in fishes, particularly those belonging to the Anisakidae family and are of commercial importance (Anderson,2000, Zhang *et al*., 2007).

Anisakis spp. have a complicated life cycle comprising 5 stages and 4 moults (Mattiucci and Nascetti, 2008; Klimpel and Palm, 2011; Karpiej *et al*., 2013). Fish hosts may become infected through ingestion L3 larvae from second intermediate hosts (crustaceans, small fish, etc.) (Abollo *et al*., 2001). Cetaceans are said to be the definitive hosts for *Anisakis* spp. (Mattiucci and D'Amelio, 2014). These parasites infects human after consumption of raw or insufficiently cooked fish (Audicana and Kennedy,2008; Baird *et al*., 2014). Also dead parasites are capable of producing proteins that induce hypersensitivity and allergic reactions (Anadón *et al*., 2009; Arizono *et al*., 2011; Mattiucci *et al*., 2013). During infestation, other clinical manifestations are important. Some of the symptoms are - epigastric pain, systematic or gastrointestinal symptoms, fever, diarrhoea, vomiting, gastric and duodenal ulcers (Lee *et al*., 2009; Hwang *et al*., 2012; Cho *et al*., 2012) and intestinal obstruction (Kim *et al*., 1991; Takabe *et al*., 1998).

The life cycle (Fig. 2) of *Anisakis* spp. starts in the feces of an infected marine mammal- (i) Marine mammals excrete unembryonated eggs; (ii) Eggs become embryonated in water and L2 larvae stage forms in the eggs; (iii) After the L2 larvae hatch from eggs, they become free-swimming, which are ingested by crustaceans and they mature into L3 larvae form; (iv) Infected crustaceans are eaten by fish like Greater Lizardfish, Rockfish, Herring, Mackerel, Salmon, and Anchovy or Squid; (v) After the host's death, larvae move to the muscle tissue, and through predation, the larvae are transferred from fish to fish; (vi) Marine mammals like dolphins, seals, or humans may become infected from consuming the infected intermediate host; (vii) In humans, these worms don't mature, but the worms can migrate from the GI tract, becoming embedded within the gastrointestinal mucosa and yielding tissue reaction and discomfort such as gastric pain, diarrhoea, vomiting. (Beldsoe and Oria, 2001).

The present study focuses on the identification of the parasite and comparison in the nutritive value of Greater Lizardfish (*Saurida tumbil*) before and after infestation with *Anisakis* species.

Materials and Methods

Collection of Fishes:

Approximately 100 Greater Lizardfish (*Saurida*

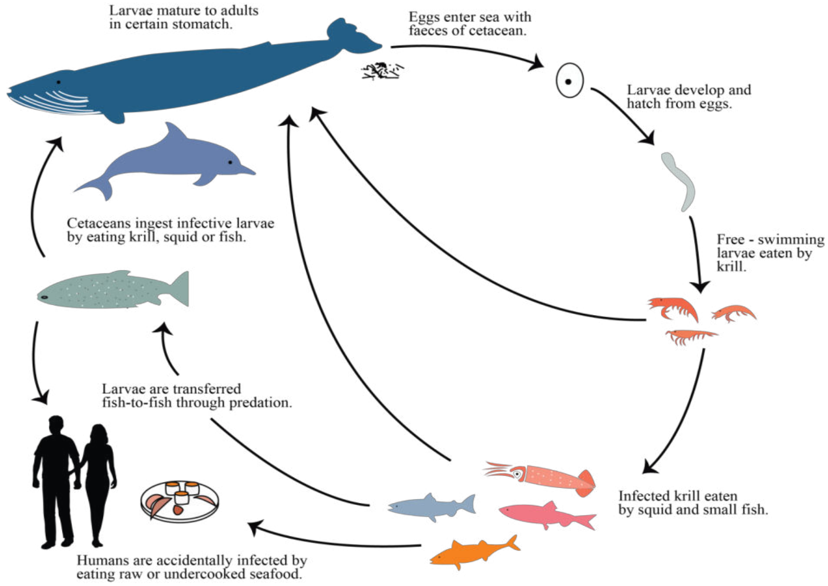

Fig. 2: Life cycle of *Anisakis* spp.

tumbil) were collected in different season's from local fish markets of Kalyan, Dombivili, Thane of Maharashtra from March, 2018-March, 2020. Fishes were transferred to the Zoology Laboratory of Smt. CHM College, Unr-3, Thane, Maharashtra, India where clinical and parasitological examinations were performed.

Length and weight measurement:

Total length and weight of fishes were measured as follow:

- Total length of the fish was measured by using measuring tape by placing the fish on flat surface.
- Total weight of the fish was measured by using;

a) Table top weighing balance

b) Table balance.

Clinical and Post-mortem examination:

The collected fishes were subjected to clinical and post-mortem examination. The part of the visceral organs (intestine, stomach and liver) were separated in the petri dish and checked for parasitic infections. Specimens were washed in water and then fixed in 70% alcohol. Collected nematodes were identified by using Scanning Electron Microscopy (SEM) technique.

Proximate analysis/ Nutritive analysis:

Proximate analysis/ Nutritive analysis of Greater Lizardfish (*Saurida tumbil*)) like total protein content was estimated by Lowry's (1951) method, carbohydrate by Anthrone reagent method (Hedge and Hofreiter, 1962), lipid by SPV method (Knight *et al*., 1972; Johnson *et al*., 1977) and moisture content was estimated by AOAC (2000) method.

Results and Discussion

Anisakid nematodes are parasites of commercially significant marine fish, and their presence is a major problem for both human health and the economy. The occurrence of some Anisakis larvae in marine fishes has been studied, and a number of fish species have been discovered to be susceptible to Anisakid infection (Smith, 1984; Manfredi *et al*., 2000; Luque and Poulin, 2004; Cruz *et al*., 2007; Abd-Al-Aal *et al*., 2008; Ahmed *et al*., 2010).

Anisakis larvae were discovered in the muscles, body cavity, mesenteries, liver surface, and gonads of infected marine fish in our investigation (Fig. 3). The morphological structure of the anterior and posterior end of Anisakis

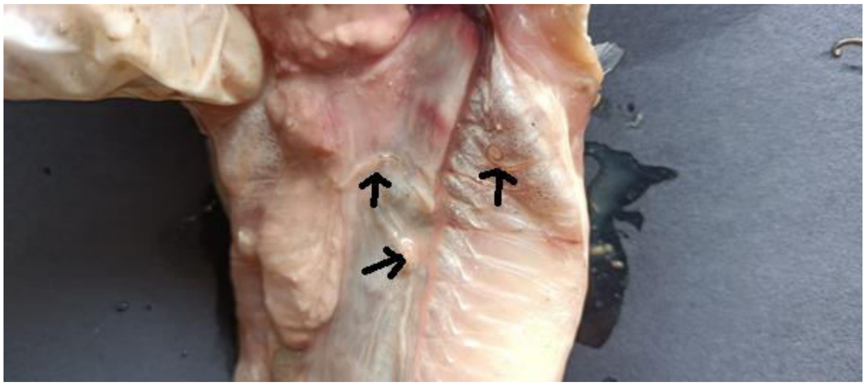

Fig. 3: Gut and visceral muscles of *Saurida tumbil* showing infection of larva of *Anisakis* spp.

Fig. 4: Anterior end of Larva of *Anisakis* spp. under Light Microscope.

Fig.5: Posterior end of Larva of *Anisakis* spp. under Light Microscope.

spp. Is illustrated by light microscope (Figs. 4, 5) and electron microscope (Figs. 6, 7). The morphological description of Anisakis larvae acquired from The Greater Lizardfish (*Saurida tumbil*) in the current study is consistent with Shih (2004). Smith (1984) proposed that the dispersal of anisakid larvae is mostly determined by conditions encountered within host tissues and

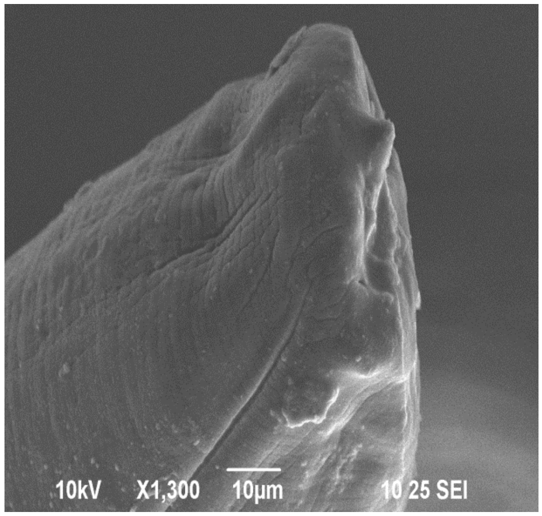

Fig. 6: Anterior end of Larva of *Anisakis* spp. under Scanning Electron Microscope (SEM).

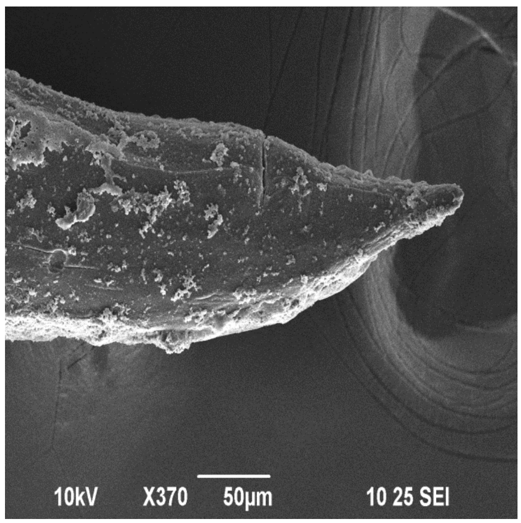

Fig. 7: Posterior end of Larva of *Anisakis* spp. under Scanning Electron Microscope (SEM).

may be connected to nutrition availability. Although the majority of the larvae were found connected to the viscera or free in the body cavity of infected fish, their significance as a possible cause of human infection cannot be discounted (Smith and Wootten, 1975). It is worth noting that the findings show that the larvae of anisakids invade the human body through the digestive tract and settle in the liver, intestine, spleen, heart, and muscle, causing pathological illness, as well as the type of *Anisakis simplex*. Furthermore, it causes severe urticaria when it comes into contact with human skin while cleaning fish or consuming them through the digestive tract.

Literature study of proximate composition of uninfected Greater Lizard fish (*Saurida tumbil*) shows that the protein content in the fish is 19.01%, carbohydrate content is 0.40%, lipid content is 1.13%, moisture content is 76.99%, and ash content is 1.13% which shows that the Greater Lizardfish (*Saurida tumbil*) is highly nutritive.

In the current study, the seasonal variation of anisakis larvae infection rate peaked in the post-monsoon season in The Greater Lizardfish (*Saurida tumbil*), resulting in a degradation in the

Table 1: Proximate composition of The Greater Lizardfish (*Saurida tumbil*) infested with Larva of *Anisakis* spp.

	PROTEIN	CARBOHYDRATE		LIPIDS	MOISTURE	ASH
		MUSCLE	LIVER			
PRE-MONSOON	17.143 ± 0.459	0.352 ± 0.026	0.988 ± 0.007	1.123 ± 0.004	70.580 ± 0.766	0.678 ± 0.011
MONSOON	18.047 ± 0.554	0.398 ± 0.020	1.052 ± 0.183	1.148 ± 0.011	73.023 ± 0.316	0.723 ± 0.019
POST-MONSOON	16.874 ± 0.497	0.346 ± 0.023	0.981 ±0.017	1.114 ± 0.005	68.583 ± 0.826	0.653 ± 0.043

Values are Mean ± SD

fish's nutritional composition (Table 1). Eissa (2002) discovered that the thin-shelled eggs were laid and passed out into seawater through the faeces of infected final hosts such as dolphins or whales (Anisakis), and that the first stage larva undergoes the first moult within the egg capsule, where its development is strongly influenced by water temperature. Thus, water temperature plays a significant impact in boosting the life cycle and increasing post-monsoon prevalence. These findings coincided with those of Choi *et al*. (2011) and Li *et al*. (2011).

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