

Original Article

## Distribution of Parasitic Cestod "*Ligula intestinalis*" in Mazandaran Region

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### Abstract

**Background:** *Ligulae intestinalis* is a parasitic cestode, which has the economic-health importance in fishery industries. The aim of this study was to determine the prevalence of this parasite in Mazandaran. The effects of habitat temperature and kind of pool (sandy-cement) were considered as well.

**Methods:** In this study, 103 fish samples were obtained in all stages; the samples (male and female) were divided into 3 groups based on length of fish, temperature, origin of cultured fish, kind of pool, height from sea and sex. Macroscopic and microscopic observations were carried out in all stages of the parasite (proceroid, plerocercoid and adult). Chi-square and Pearson's double square tests ( $P < 0.05$ ) were conducted in order to evaluate the prevalence and determination of reliability in six sampling areas, respectively.

**Results:** Total rate of the parasites were 9.7% in all groups. There was significant difference between parasitism rate and height of sea level, kind of pool (maximum in sandy pools) and high temperature. The multi analyses regarding to above-mentioned three criteria also indicated meaningful difference between these criteria and parasitism rate. Seasonal conditions enhance the prevalence of *ligulae intestinalis*.

**Conclusion:** Flexibility in parasite's life cycle and choosing different hosts makes it challenging case in fishery industry; moreover its prevalence could be predicted according to environmental conditions so choosing the minimal at risk place for salmonids farming. Further studies are recommended for evaluating the problems in fish fertility and probable risk for infected fish consumers.

**Keywords:** *Ligulae intestinalis*, *Salmon Fish*, *Fishery*, *Distribution*, *Cestodes*, *Iran*

### Introduction

Infection with *Ligulae intestinalis* affects cultured or free-living fish of fresh water in all over the world (1, 2). Infection of different kinds of fish with the parasite in plerocercoid stage has wide distribution in Iran and has been reported in pools and local rivers in three ecological areas: Caspian-Sea, Kermanshah and Azerbaijan Province, Hamoun Lake and water pools of the other areas in Iran (3, 4). High prevalence rate of *Cyprinidae* spp. from Hamoun-Hirmand region has been reported. Moreover,

*Ctenopharyngoden* as a new host for the parasite has been detected.

The plerocercoid of *Ligulae* from *Rutilus-rutilus* in the fish of Caspian Sea and salmonids in Ekbatan Dam has been reported (3). This infection is supposed to be one of the most important health-economic problems in culturing-fishery centers and those who consume the infected fish meat. With respect to the effect of parasite in all stages of fish life-cycle (proceroid, plerocercoid, adult) on fish own health (3,4), such as fertility and protein content, the first step in controlling and limiting of infection

is determining the prevalence of infection in different stages of fish life cycle in specified regions.

This study is the first challenge for the primary evaluation of infection in cultured salmon fish in west Mazandaran region.

## **Materials and Methods**

One hundred and three fish samples were obtained from six targeted salmon-culturing pools at seasonally or permanently conditions in standardized (cemented) or sandy pools in Chalous-Noshahr regions, and is concerned with evaluation of different factors affecting the infection.

The studied center and fish farms were classified and coded as follows:

A. Shahid-Bahonar culture center (1650 m. height from sea level)

B. Private fish-farm in Kheyroud (-19 m. height from sea level)

C. Private fish-farm in Noshahr (-19 m. height from sea level)

D. Private fish-farm in Brar-kelardasht(500 m. height from sea level)

E. Private fish-farm in Kandelous (1700 m. height from sea level)

F. Marzan-Abad fish-farm (sandy-cement) (500 m. height from sea level)

The samples were prepared from March 2004 to June 2005 and the temperature of sampling time was recorded about 17 °C. Samples were obtained in random clustering sampling. Sampling was performed from entrance, middle, and terminal edges of feeding places with maximum output in middle feeding area. Age range of fish samples were between 6 to 10 months. After obtaining samples immediately were handled by plastic containers putted into ice box and were sent to reference fishery laboratory and then determination was carried out based on size and sex in a biometry bench. In this process, samples were washed with 5% KOH for clearing the surface of fish body and then were dissected for finding possible parasite. Ambiguous

results were analyzed on slides for morphologically determination and confirmation.

Only in full mature stages, *Ligulae* has completely segmented strobilla (in bird's intestine), also butheria is completely developed just prior to adulthood. The color is milky-white or creamy-white. The length of adult parasite is between 10-100cm and more than 1.2 cm in width. Because of the nature of infection, the parasite tends to occupy whole of the body cavity. Alive parasite has full movement. Two longitude grooves are observed in back and ventral surface, vaginal pore is opened to ventral groove. This ventral groove is clearly observable in plerocercoid stage. The body is muscular, belt-shaped and scolex is not clear (5, 6).

## **Results**

At first, the prevalence of parasitism in A-F regions was calculated based on total sample groups in 3 sampling areas. In this regard, the distribution of parasite in full length of fish (correspond to age), were as follows: in 5cm length 2.9%, in 10-15 cm 1.9% and in more than 25cm 4.9%. Total prevalence rate was 9.7%. In Chi-Square test, this distribution was more than expected P value ( $P<0.05$ ), so there was no significant difference between this factor and parasitism level (Fig. 1). Same results were obtained in the relation between sex, age and seasonal factors. In evaluation of the parasitism prevalence related to the kind of fishery pools (sandy-cemented), there was 4.9% prevalence for cemented and 4.9% for sandy pools (total 9.7%) (Fig.2). Among 103 samples, 88 pieces were in cemented pool with 5 infected fish (5.7%), 15 pieces in sandy pools with 5 infected fish (33.3%) and in 2×2 double square test so there was significant difference between the kind of pool and infection rate ( $P<0.05$ ). In view of the habitat of cultured-fish, difference between transferred baby fish from the outside of the region (from 5 cm or less) or cultured in the same culture place, there was meaningful

variation between them ( $P < 0.05$ ), and infection rate in transferred fish was much more higher than egg-cultured fish at same pool (Fig. 3).

According to the temperature of regions (above-mentioned A-F groups), the samples were grouped into 3 classes: (e.g., based on moderate annually temperature); group1 (less than 11 °C), group2 (12-14 °C) group 3 (more than 14 °C) were analyzed for parasitism prevalence. Parasitism level was 8.2% (36 cases), 18.8% (25 cases) and 6.1% (42 cases), respectively (Fig.4). Therefore, in  $\chi^2$ -Pearson regression test, there was significant variation between temperature and parasite rate ( $P < 0.05$ ). The other static analyses were calculated based on two parameters: height from sea level and

parasitism rate. In cross evaluation of parameters (contingency coefficient) between height from sea level (in two groups: up to 600 meters from sea level and down to 16-19 meters from sea level) and parasitism rate  $P$  value was equal to 0.048 ( $P < 0.05$ ). In this comparison, in samples cultured in low height, more parasitism was observed. (low altitude) (Fig. 5).

In multi analysis between 3 factors height, temperature and origin of baby fish in selected groups, (lowest height region parasitism, highest temperature region (i.e.; up to 13 °C region) parasitism and parasitism level in transferring baby fish from outside) the difference was significant ( $P < 0.05$ ) (Fig. 6).

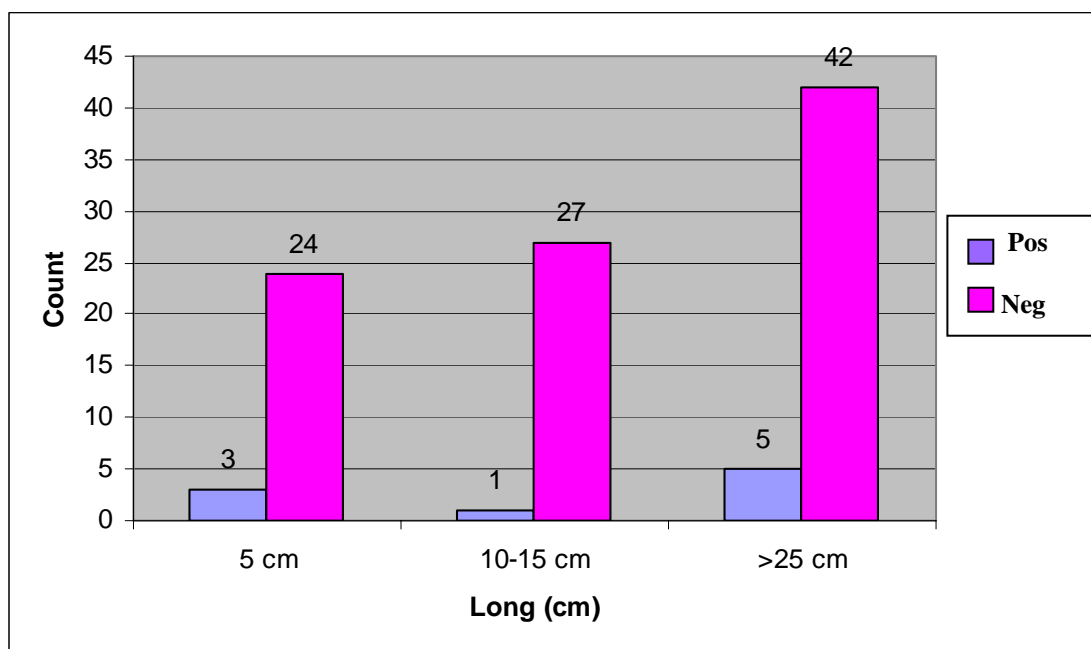


Fig 1: Parasitism rate in order of length (cm) in three grouped fish

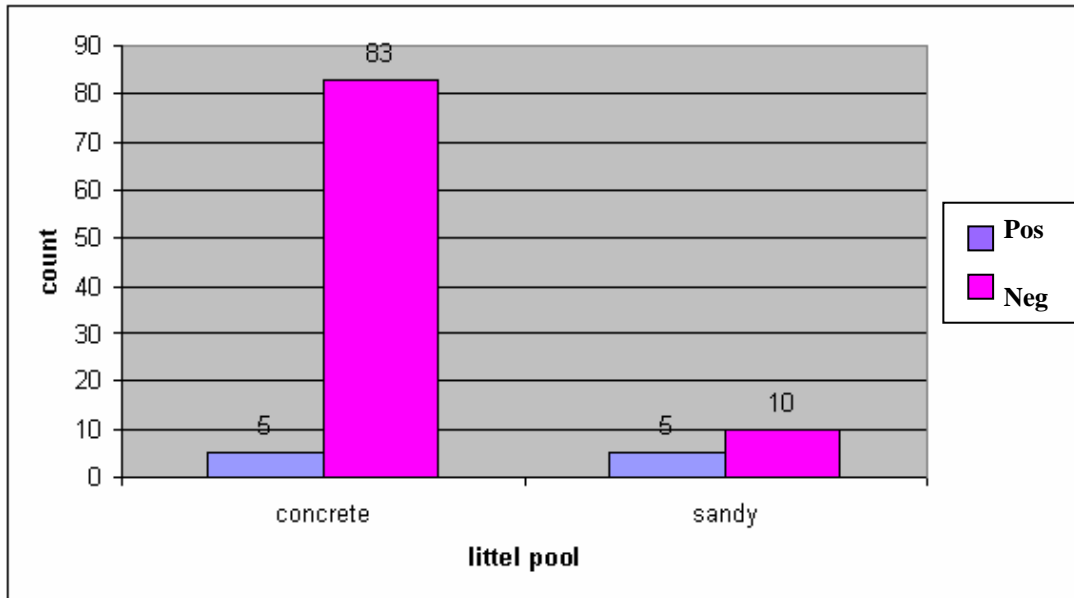


Fig 2: Parasitism in accordance to the kind of pool (cement-sand)

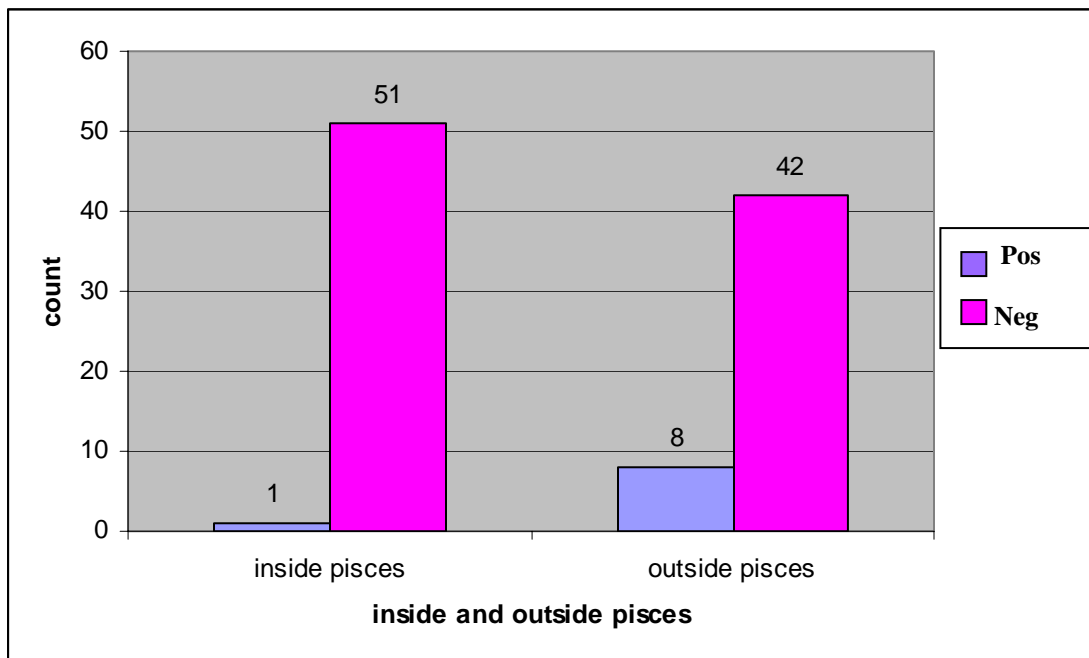


Fig 3: Distribution between parasitism and origin of transferred baby fish (outside - inside)

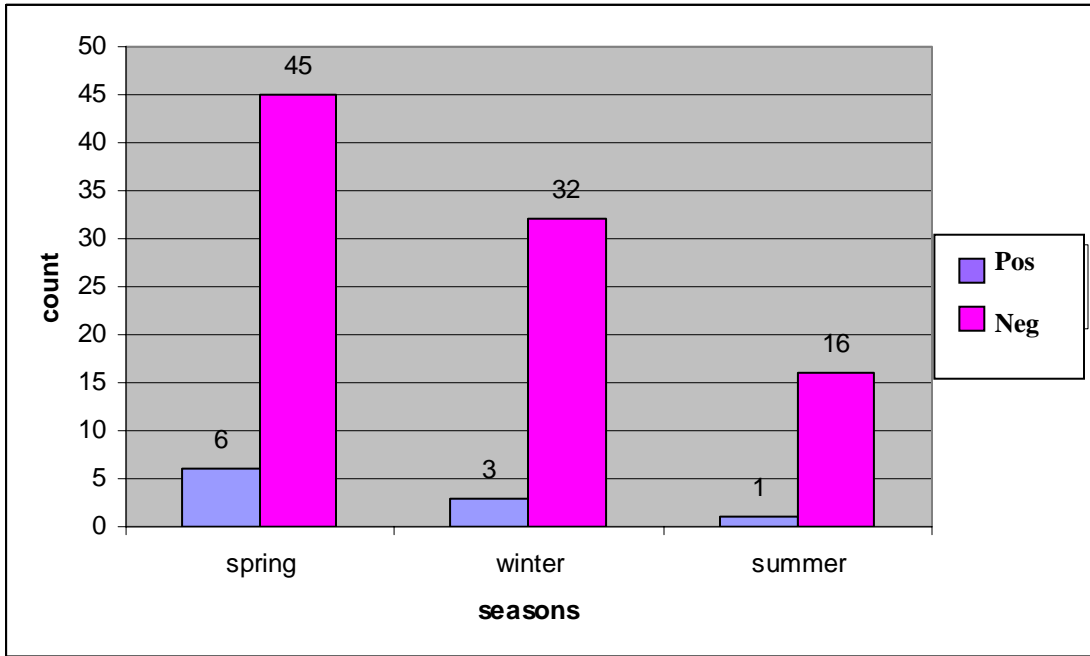


Fig 4: Parasitism rate in accordance to the season (temperature)

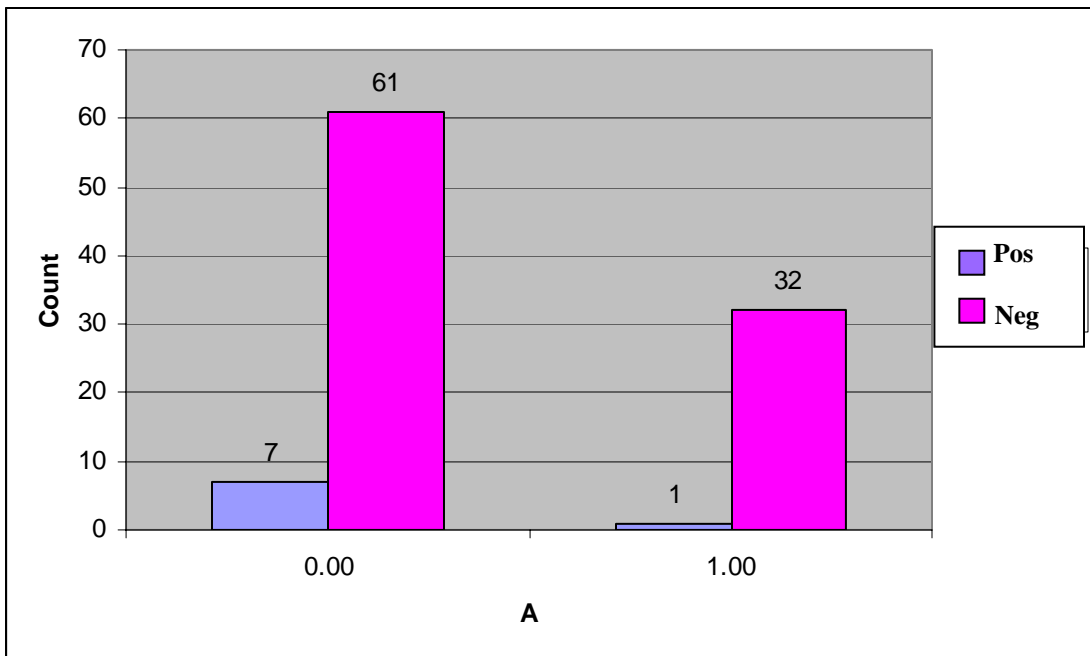


Fig 5: Multi analysis between height of sea level and parasitism level

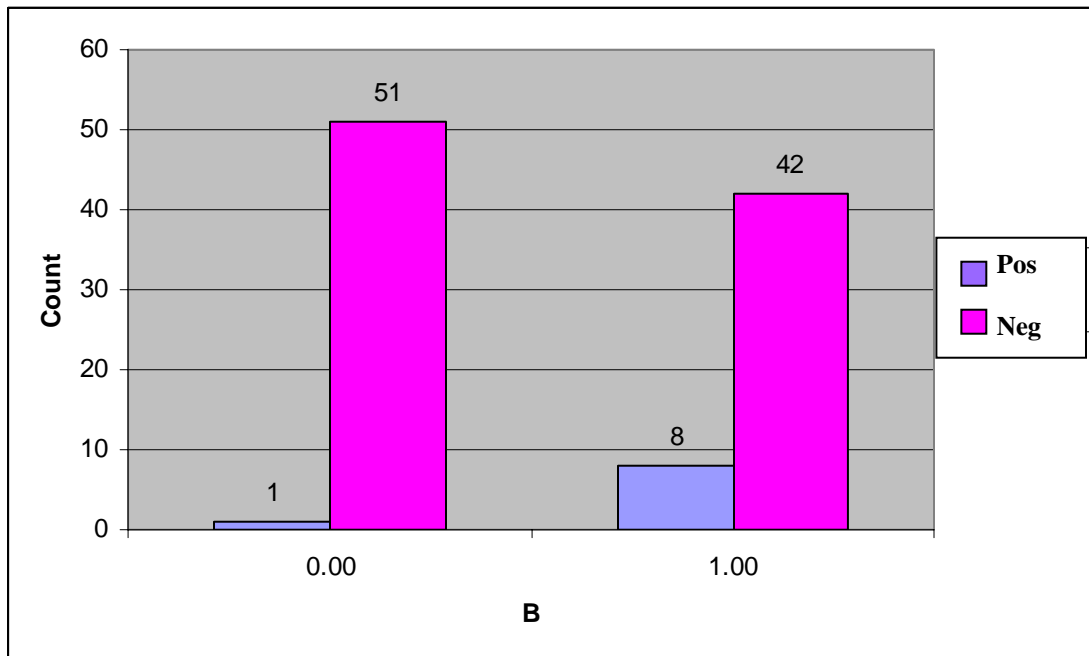


Fig 6: Multi analysis between low height, temperature, and transferred baby fish (outside - inside)

## Discussion

*L. intestinalis* infection has a wide distribution in different areas in Iran. Many researchers have studied the most aspects of this parasite, such as the life cycle, new hosts and effects of parasite on fish host (as plerocercoid–intermediate host). Karimi *et al.* have determined new infection area in Sattarkhan Dam located in East Azerbaijan Province (7, 8). Studies in Ekbatan Dam have shown that the maximum parasite / fish weight ratio is higher than 17.7% and mean value was determined as 7/4% (9, 10).

The present study has been carried out in one of the most prevalent parasitic sites for *L. intestinalis* cestode infection in fishery regions of Chalous with different seasonal-regional conditions. The sampling was carried out in 6 ponds (A-F). These areas had been grouped into different classes based on mean annual temperature, the highest possible temperature, altitude from sea level, age of accessible fish from reproductive fish (egg-stage), transferring or preparing from inside and kind of pond (sand or cement). The first group had mechanized cul-

ture-ponds (cemented) with mean annual temperature < 11 °C. In data analysis, there was no significant difference between the age (length of fish) and parasite (5 cm equal to 1 month age, 10-15 cm to 4 months, more than 25 cm to 9 months). So it is clear that parasite could also be seen in every age of cultured fish and if fish is infected in early stages of its life, (phytoplankton feeding–stage), it will continue for the rest of the fish life, although it should be noted that there was no identical age in fish transferred to ponds. Hence, it is worth mentioning that if all fish studied from first day of life for clearly defining parasitic infection was followed, it does not seem that such effects exist except for less growth and small size fish if they are infected with parasite in comparison with the uninfected ones (Fig. 1).

In evaluation of the effect of season on the rate of parasitism, sampling was done in cold and warm seasons, but in group 1, the temperature was already low. Other factors like intermediate host rate (cyclops) (Fig. 2, 3) and first host persistence may change with the temperature variable (11, 12), but in this study none of these

factors existed. Although the highest rate of parasitism was in the warmest month of year (July-in F region), in the lowest altitude from sea level, almost all the time, had the highest prevalence of parasites.

One of the key elements about the existence of more parasites in sandy ponds is the capability of residence of cyclops as an intermediate host, although if fish were transferred from outside, this factor would be negligible for cyclops residence. Regarding to all suitable factors in one region, the highest prevalence of parasite will be observable (such as F region with negative altitude from sea level, mean temperature > 11 °C, transfer of baby-fish from outside and sandy pond) and all have proved statistically significant. One of the limiting factors in determining the parasite rate was the impracticality of diagnosis of pro-plerocercoid's microscopic shape and size (abnormal and unpublished shape). For establishing the existence of parasite in this stage of life, determination of pro-plerocercoid stage is necessary. The effect of altitude is in direct relation with mean temperature because this area already had lower temperature. In fish spending long winter, low weight is one of the susceptible factors for infection. The studies showed that the parasite induces secretion of growth factor-like hormone in fish that can improve more length in infected fish (13-15). This parasite is valuable for human health with respect to economical view. It could be harmful in considerable amount of fish and it reduces protein content in human food. According to studies (16, 17) the parasite could secrete sex related hormones that affect fish and infertilize it, so by affecting on consumers, sex hormone balance is altered (and probably reproductive ability as well). Therefore, it will be important to evaluate and establish the rate of parasitism. It is notable to state that some contraceptive drugs have previously been prepared from net parasite body content in Czech Republic (18). In conclusion, this parasitic has great flexibility in selecting its intermediate hosts so that can itself be its own final host (based on parasite's

life cycle) (19, 20). It is expected that wide range of organisms adapt themselves to parasite life cycle (21, 22) and regarding to these characteristics of parasite, large-scale study and specie's molecular determining in this field is strongly recommended.

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