UNRAVELLING AN ALTERNATE LOW COST ECO-FRIENDLY APPROACH FOR TREATING FRESHWATER FISH LICE IN *LABEO ROHITA* (HAMILTON, 1822)

Farhana Hoque*, Ajmal Hussan, Rathindra Nath Mandal, Parthapratim Chakrabarti, Arabinda Das, Subhendu Adhikari and Baidya Nath Paul

ICAR-Central Institute of Freshwater Aquaculture, Regional Research Station- Rahara, Kolkata-700118, West Bengal

*Corresponding author: farhanahoque22@gmail.com*

The infestation of freshwater fish lice belonging to the genus *Argulus* occur repeatedly in freshwater fish farms due to poor management practices. Preventative methods and regular monitoring often fails to control the outbreak, and in turn chemical interventions become necessary. In the present study, the outbreak of freshwater fish lice *Argulus* in Rohu (*Labeo rohita*) has been reported at a farm in Kalyani, West Bengal. A framework was developed for sustainable eco-friendly and cost effective treatment of *Argulus* using salt at 10 g/L for 3 minute, followed by formalin at 40 μL/L for 3 minute, potassium permanganate at 0.5 g/L for 3 minute and turmeric at 5 g/L for 3 minute, respectively *in-vivo*. Mean intensity, abundance and prevalence of the *Argulus* sp. was assessed as the most suitable descriptors to determine the impact of the treatment. The parasite was grouped abundant (85%) in *Labeo rohita* prior to the treatment, whereas, post-treatment, the parasites became rare (5%) in the same fish. Significant reduction in the mean intensity and abundance of *Argulus* sp. was also recorded post-treatment, which implied the efficiency of the treatment process in eradicating *Argulus*. Combined with the multiple bath treatment, mechanical control through vigorous shaking of the affected fish was also found effective and hence may be recommended to the farmers as the suitable control strategy of the freshwater fish lice subjected to further validation in different field condition.

**Keywords:** Parasite, *Argulus*, *Labeo rohita*, control, bath treatment

**INTRODUCTION**

Parasitic infestation is one of the major problems in fish culture causing mass mortalities. In intensive culture, fish are frequently affected by environmental fluctuations and poor management practices, imposing considerable stress, thus, rendering them susceptible to different parasites (Subasinghe, 1997). Apart from the financial losses caused by mortality, parasites may also substantially influence the growth and behaviour of fish (Scholz, 1999; Thilakaratne *et al.*, 2003), consequently reducing production efficiency of the farm, which, in turn, increases cost of production, reducing profit and effects the earning of foreign exchange. *Argulosis* caused by crustacean ectoparasites of the genus *Argulus* is one of the serious problems in fish culture and pose a major threat to freshwater aquaculture industry around the globe. The overall loss has been projected to the magnitude of US$ 615/ha/year due to *Argulosis* (Sahoo *et al.*, 2013). Therefore, top priority needed to be given
for management of this disease to save the aquaculture industry from the vast loss. Several works have been carried out to prevent argulosis (Rahman, 1968; Chandra et al., 2004; Bakshi et al., 2006; Sahoo et al., 2021). For the treatment of this disease, like other ectoparasitic diseases, fish farmers apply various chemical substances such as formalin, hydrogen peroxide, dichlorvos, cypermethrin, levamisole, diflubenzuron, Chloramine-T, deltamethrin, azamethiphos, ivermectin (Powell and Speare, 1996; McAndrew et al., 1998; Pike and Wadsworth, 1999; Toovey and Lyndon, 2000; Hemaprasanth et al., 2012). Padmavathi and Prasad (1998) reported the control measures of argulosis using Nuvan and Ekalux. Despite the use of these chemotherapeutants by farmers, infection by Argulus persist in different fish culture systems. The uses of chemical drugs were restricted due to the bioaccumulation and residual effect in environment as well as fish flesh. Alternative methods to control parasites are needed to be explored. Ahmed (2004) reported the development of environmental friendly methods for treating Argulus in carp brood ponds. Though using medicinal plants for treatment of parasitic diseases in fish are rare but has been practiced (Pandey et al., 2014; Mishra et al., 2017, Hoque et al., 2018). Indigenous and alternative method of treatment is strongly required. This paper reports the outbreak of freshwater fish lice Argulus in Labeo rohita and aims to show its successful low cost alternate treatment strategy using salt, turmeric, formalin and potassium permanganate.

MATERIALS AND METHODS

Ethics statement

All experimental procedures fulfilled the ethical guidelines including adherence to the legal requirements of India.

Study site

The outbreak was observed in May, 2018 in a cemented pond measuring 44.86x24.15x1.5 m³ stocked with Labeo rohita@ 4000 nos. ha⁻¹ along with other carps (Catla catla @ 2000 nos. ha⁻³, Cirrhinus mrigala @ 1500 nos. ha⁻¹, Cyprinus carpio @ 1000 nos. ha⁻³, Hypophthalmichthys molitrix @ 250 nos. ha⁻¹ and Ctenopharyngodon idella @ 250 nos. ha⁻³) situated at Kalyani Field station (22°57’47” N and 88°26’38” E) of Regional Research Station of the institute located at West Bengal, India.
Fig. 1. Location of the farm and the Argulus infested pond

Sample

*Labeo rohita* weighing 645 ± 143g were found infested with crustacean parasites *Argulus* sp. Collected fishes were clinically examined for the determination of abnormalities on the external body surface as described by Lucky (1977), and the clinical signs of the infected fishes were noted.

Analysis of parasitological indices

Randomly 60 fish from the infected stock was chosen for determination of parasitic dominance, prevalence, mean intensity and abundance to assess parasitological indices as explained by Roohi *et al.* (2014) and Oscar *et al.* (2015) before and after treatment.

Parasitic frequency index (%) = (No. of infected fish/Total no. of fish examined) x 100

The Parasitic Frequency Index (PFI) was calculated by taking the percentage of fish (hosts) infected by a certain parasite species compared to the total number of fish (hosts) assessed in a specific region under observation. The PFI was classified into rare (0.10 – 9.90%), occasional (10.00–29.90%), common (30.00 – 69.90%) and abundant (70.00-100.00%) as explained by Paperna (1996).

Mean intensity = No. of collected parasites/ No. of infested fish

Abundance = No. of parasites/ No. of fish examined

Bath treatment of Argulus infested fish

Though the treatment was done in all fish of the pond, but the study was conducted on *Labeo rohita* only. A total of 120 individuals of *L. rohita* infested with *Argulus* sp. were collected from the pond for treatment. Four FRP tanks of 400 L capacity containing 300 L of seasoned rain water was taken. The infected fish were divided into three batches comprising 40 fish in each batch for ease of treatment. Fish were shaken vigorously and briefly (20 to 30
s) with a hand net in a separate FRP tank filled with rain water. For each batch of fish, four consecutive bath treatment was given viz. salt bath at 10 g/L for 3 minute, followed by formalin bath at 40 μL/L for 3 minute, potassium permanganate bath at 0.5 g/L for 3 minute and turmeric bath at 5 g/L for 3 minute, respectively and were subsequently moved to an empty pond. Treatment was done for three consecutive days.

![Diagram of bath treatments](image)

**Analysis of physico-chemical parameters of water**

Physico-chemical parameters of water like pH, dissolved oxygen, alkalinity, temperature and ammonia were measured. Dissolved oxygen, alkalinity and ammonia were determined as per the standard procedures described in APHA (2007) and expressed in mg/L. Water temperature and pH were measured using digital thermometer and pH meter (Labman Multiparameter water quality meter LMMP-30, India), respectively.

**Statistical analysis**

The results are presented as the mean ± standard deviation (SD). The data were analyzed by one-way analysis of variance (ANOVA) and Tukey’s comparison of means (P<0.05). All statistical analyses were performed using SPSS 16.0 for Windows software (SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Clinical signs**

*Labeo rohita* (n=60) infested with *Argulus* sp. were examined. Affected fish showed haemorrhages in the body surface with small sized wound. The crustacean parasites are visible on fish by naked eye. The fish were found to rub their body on the pond embankments and swim restlessly.

**Parasitological indices**

*Argulus* sp. was isolated from moribund fish. Among the total number of fish species (n=60) studied, 368 *Argulus* individuals were isolated from 56 rohu samples, whereas only 11 individuals of *Argulus* were collected from 3 fish samples post treatment.

Prior to treatment, the mean intensity and abundance of *Argulus* sp. were recorded as 6.57 and 6.13, respectively, whereas post-treatment, the same were reported as 0.19 and 0.18, (Fig.1).
Interestingly, the prevalence of *Argulus* sp. as determined by the parasitic frequency index (PFI) was 93.33% before treatment, but reduced to 5.00% post-treatment (Fig. 2).

![Graph showing parasitological indices and prevalence of Argulus](image)

**Fig.1.** Parasitological indices of *Argulus* spp. affecting *Labeo rohita* pre and post treatment  
**Fig.2.** Prevalence of *Argulus* spp. in *Labeo rohita*

**Analysis of physico-chemical parameters of water**

Temperature, dissolved oxygen (DO), pH, ammonia, alkalinity and hardness of the pond water were recorded during Argulus infection. Significant increase in ammonia, alkalinity and hardness were observed in pond water during the infection, which may have directly affected the fish health making them more susceptible to Argulus during the period of study as mentioned in Table 1.

**Table 1. Water quality parameters during Argulus infestation in *Labeo rohita***

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.80 ±0.35</td>
</tr>
<tr>
<td>Water temperature (°C)</td>
<td>34.50 ±0.50</td>
</tr>
<tr>
<td>Dissolved oxygen (ppm)</td>
<td>5.83±0.50</td>
</tr>
<tr>
<td>Ammonia (ppm)</td>
<td>0.05 ±0.02</td>
</tr>
<tr>
<td>Alkalinity (ppm)</td>
<td>235.00±7.83</td>
</tr>
<tr>
<td>Hardness (ppm)</td>
<td>270.00 ±6.37</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Intensification in aquaculture has invariably given rise to favourable environment for proliferation of parasites. High abundance of parasites jeopardises the production, and in turn, causes economic loss. *Argulus* are some of the most prevalent parasites affecting fish culture. As soon as *Argulus* is identified, suitable management and treatment is necessary considering their capability for rapid escalation of infection. There are currently no FDA-approved drugs for the treatment and control of *Argulus*. The need of this hour is to
recommend cost-effective and eco-friendly control methods to the farmers (Hakalahti-Sirén et al., 2008). Apart from applying a single set of chemical for parasite treatment, creating and implementing an eco-friendly process involving a series of treatment would be a step towards sustainable and profitable fish production. In view of this situation the present study was undertaken. Though prevention is the fundamental to reduce the likelihood of a parasite becoming rampant, yet when the bridge of prevention breaks down, the ‘process of control’ plays the central role. The most effective treatment against argulus was achieved by using organophosphates (Noaman et al., 2010; Wafer et al., 2015), but use of these chemicals as pesticide in fish is restricted. Other treatments of Argulus infestations include the use of common chemicals such as salt, formaldehyde, potassium permanganate and formalin (Noaman et al., 2010). Abiotic environmental conditions have a significant impact on freshwater fish lice populations. In the present study, during Argulus infestation in Labeo rohita, significant increase in ammonia, alkalinity and hardness were observed in the pond water as compared to the unaffected ponds of the farm, which may have directly affected the fish health making them more susceptible to Argulus. Additionally, numerous studies have emphasised the significance of physico-chemical properties of water include oxygen content, pH etc. for the survival of Argulus populations (Pettersen et al., 2006; Mikheev et al., 2007). An additional (though not separate) step in the application of a suitable management or control process of any infection is the monitoring of parasite infection levels. Mean intensity, abundance and prevalence of the Argulus sp. was assessed as the most suitable descriptors to determine the impact of the treatment. Consequently, the parasitic frequency index (PFI), the parasite was grouped abundant (85%) in L. rohita prior to any treatment, whereas, post-treatment, the parasites became rare (5%) in the same fish. Significant reduction in the mean intensity and abundance of Argulus sp. was also recorded post-treatment. This implies the affectivity of the treatment process. Vigorous, brief shaking of the affected fish along with consecutive bath treatment with salt, formalin, potassium permanganate and turmeric at the mentioned doses, for three consecutive days may be recommended to the farmers as the suitable control strategy of the freshwater fish lice subjected to further validation in different field condition. Hakalahti-Sirén et al. (2008) also opined the suitability of a combination of chemical, mechanical and biological treatment in eradicating A. coregoni. In the present study, apart from the bath treatment, the mechanical control process by using transportable artificial egg laying trays can also be recommended for removal of parasite eggs as observed by Hakalahti et al. (2004). Similarly, Fenton et al. (2006) also suggested a model, which revealed that Argulus infection level generally relies on the quantity of eggs in the egg-bank and destroying eggs with all available methods would greatly reduce the size of the parasite population. Hence, it has been recommended to drain and dry the egg laying sites of Argulus sp. (Bauer, 1962) along with application of lime (Chen 1933, Hoffman 1980) has been also suggested as a useful technique for controlling eggs of this parasite.

Though in the present study complete control (100% removal) of Argulus was not reported when treated with potassium permanganate, turmeric, salt or formalin in-vivo but
still the effect of these compounds can’t be ignored. Bandilla et al. (2006) opined that 100% removal of parasites are not needed either for high yields nor appropriate for integrated parasite management approach. Hakalahti-Sirén et al. (2008) also opined that fish can withstand a certain level of lice infestation without significant consequences on vigor. The economic edge for freshwater fish lice infestations has yet to be discovered, and it is anticipated to vary among several types of culture systems. Instead of waiting for the damage to manifest itself, eco-friendly and profitable corrective actions should supplement preventative measures after the pre-determined action threshold has been reached.

The anti-parasitic effects of turmeric as observed in the present study, may be due to the presence of the active ingredient of turmeric i.e. curcumin, which have received a significant amount of attention in recent decades (Shahiduzzaman et al., 2009). Curcumin has been shown to have antiparasitic effects both in-vitro and in-vivo. The biomolecular and cellular processes in parasite eradication are not completely understood but it may be due to the pharmacological and antioxidant activities (Surh, 2002; Aggarwal et al., 2003; Choi et al., 2006; Shen and Ji, 2007; Goel et al., 2008, Subash et al., 2012). Formalin because of its electrophilic nature, it can react with proteins and with DNA and RNA to inactivate microorganisms mostly through reactions with amino acids and nucleic acids, mediating the killing of parasites (Fox et al., 1985; Kiernan, 2000; Lu et al., 2010) as reported in the present study. On the other hand, treating Argulus with salt is considered as the most common and inexpensive technique. The mortality of Argulus sp. during in-vitro study may be due to the osmoregulatory failure in salt solution. Moreover, in host, salt solution provides osmoregulatory stabilization, mucus production and hence provides enhance control to parasite in fish. The use of sodium chloride (NaCl) in farm is safe for water environment as it is inherent therein. Dewi (2018) reported that the highest sodium chloride concentration (9 ppm) give the lowest prevalence (37.33%) of Argulus sp. in Oreochromis niloticus, which corroborate to the present study. Contrarily, potassium permanganate is a strong oxidising agent that reacts with any material that it comes into contact as observed in the present case. But the primary issue for treated fish is the oxidative activity of potassium permanganate and, hence, to prevent fish death, the treatment dose must be carefully chosen. Water pH, temperature and exposure time of the treatment also play a significant role in this regard (Markings and Bills, 1975).

In this study, we present a framework for sustainable eco-friendly and cost effective process to manage Argulus. Using different strategies and rotational treatments is preferred over a single costly chemical treatment as it mitigates the development of parasite tolerance and resistance towards therapeutics as reported by Hakalahti-Sirén et al. (2008). However, for further validation of this process, investigation and field trials are necessary to identify elements influencing the growth potential in different geographical region of fish louse populations.
ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, India for providing necessary facilities during this study.

REFERENCES


Shahiduzzaman, M., V. Dyachenko, R. E. Khalafalla, A. Y. Desouky and A. Daugschies,


