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Trials for the Control of Isopods Infesting Lake Qarun, Egypt, with the Impact of Environmental Stresses on their Parasitism

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ABSTRACT

The Isopod infestation of fish in Lake Qarun has been deemed a catastrophe. Managing crustacean parasites, particularly isopods presents a formidable challenge, and employing chemical or biological remedies seems impractical given the widespread nature of the infestation within a vast water body like Lake Qarun. Hence, the present study aimed to develop new strategic plans for the control of isopods at different salinities and temperatures, using two model fish (*Tilapia zillii* and the European seabass; *Dicentrachus labrax*). The results revealed that *D. labrax* can combat and eat isopod parasites in their territory. At the same time, *T. zillii* was the preferred host for isopods under optimal conditions for infestation (36ppt salinity and 26°C temperature). Unexpectedly, there was a clear association between isopod infestations and salinity levels, with the lowest infestation rates occurring in areas with lower salinity. Physical treatment of infested fish, by lowering salinity or water temperature, proved a futile post-challenge, as the parasite remained firmly attached to its host, even under unfavorable environmental conditions. Conversely, reducing water temperature has a notable adverse effect on the survival of free-swimming isopods, especially at a temperature of 15°C. Conclusively, *D. labrax* is a robust model fish that is difficult to infest with isopods and can combat infestation through unknown defense mechanisms. The fish preyed on isopods, demonstrating their vital role in the biological control strategy for parasites that we proposed in this study.

INTRODUCTION

Pollution can influence the frequency of parasites by impairing the host's immune response, or the parasite possibly will lower the host's endurance to impurities. Parasitism possibly will raise the fish's vulnerability to pollutants, or the impurities may result in either a rise or a decrease in the occurrence of these parasites **(Öktener & Bănăduc, 2023)**. The contaminants may disturb the intermediate hosts as well as the other stages in the parasite life cycle **(Sures *et al.* 2023)**. Lake Qarun is believed to be the most polluted lake of economic importance in Egypt; its water quality parameters were deteriorated and polluted with both sewage and agricultural drain wastes **(Zaghloul *et al.* 2023)**. Hence, lake-polluted water, especially during summer, enhances the infestation rate of parasites like isopods **(Mansour & Sidky, 2002; Fathi & Flower, 2005)**.

Since 2015, the isopod infestation in Lake Qarun has been regarded as a severe crisis. This issue has escalated over time, resulting in significant fish population reduction, major marketing challenges, and a gradual decline in fish stocks. As a result, there have been substantial economic losses and a significant impact on the livelihoods of fishermen in the region **(Mahmoud *et al.* 2016; Helal *et al.* 2018; Khalaf-Allah *et al.* 2019)**. It is difficult to treat and manage crustacean parasites, especially isopods. Furthermore, the usage of chemical therapies makes little sense given how widespread the infestation is in such a huge water body as Lake Qarun.

On the other hand, biological control techniques are one of the hopeful approaches to control such infestations, even though they are difficult to apply **(Khalil *et al.* 2014; Mahmoud *et al.* 2017)**. The use of forceps to manually remove isopods attached to fish could prove beneficial, particularly in the context of ornamental fish. In situations where fish are maintained in aquariums lacking hiding spots like mud; this approach may disrupt the isopods' life cycle (**Noga, 2010)**. Gnathiids, a family of isopod crustaceans, are also preyed upon by cleaner shrimp and cleaner fish, such as the blue-lined cleaner wrasse **(Becker & Grutter, 2004)**.

Certainly, numerous chemical treatments have been experimented to combat cymothoid isopods. Nevertheless, chemical therapies, particularly for addressing the adult stage, typically yield limited success **(Papapanagiotou *et al.*, 2001)**. Cymothoids are sensitive to organophosphates **(Noga, 2010)**. However, the frequent use of these chemicals may potentially harm fish if they are excessively employed. Furthermore, the incorrect application of chemical medications to treat Isopoda-infested fish presents a potential hazard to the well-being of both humans and fish. These compounds may be poisonous, allergic, carcinogenic, and capable of causing resistance in human pathogens **(Buchmann, 2022)**.

Nonetheless, certain studies have indicated that active ingredients derived from medicinal plants can be more efficacious than those present in other commercially produced products. *In vitro* experiments were conducted to assess the effectiveness of these natural compounds, including extracts from neem leaves (*Azadirachta indica*), chamomile flowers (*Matricaria chamomilla*), and Shih (*Artemisia herba-alba*), in combating isopod parasites. These herbal-based compounds demonstrated their efficiency when compared to deltamethrin bath, a chemical drug renowned for its effectiveness against parasitic isopods **(Shaheen *et al.*, 2017)**. Looking at it from a different scientific perspective, experiments involving hypo-salination as a physical treatment approach led to a noticeable decrease in both morbidity and mortality rates in the infested *Argyrosomus regius* (Lute fish) **(Fadel *et al.*, 2020)**

In this context, our treatment trials utilized Lake Qarun as a case study due to the intensive spreading of isopods affecting numerous economically significant fish species, leading to substantial financial losses amounting to over 70% of the lake's harvest **(Mehanna, 2020)**. This initial pilot study was designed with the objective of formulating novel strategic approaches for managing isopod populations under varying salinity and temperature conditions. The study shed light on the potential uses of physical approaches for combating isopod infestation as an environmentally friendly and sustainable remedy, aligning with conservation efforts and reducing the ecological footprint associated with chemical interventions. It also proposed the initial use of *D. labrax* as a potential biological means to control isopods although additional research was deemed necessary in this regard.

MATERIALS AND METHODS

Collection and preservation of samples

Two fish species, 150 *T. zillii* (22± 5g and 12± 2cm) and 30 *D. labrax* (18± 5g and 13± 2cm) were collected from Lake Qarun and Wadi El-Rayan Lakes, respectively. The collected fish were caught alive during the summer season (a year season of isopod infestation) and transferred in aerated polyethylene bags to the Shakshouk research station of the National Institute of Oceanography and Fisheries (NIOF), the Fayoum Governorate. Moreover, free-swimming isopods, 300 individuals, were collected from Lake Qarun and immediately transferred in water fill-plastic bags to the research station, where isopods adaptation was held in Lake Qarun water for 12h to validate their vitality and activity. The collected isopods were classified and identified previously as different haplotypes of *Livoneca redmanii* (order: Isopoda, family: Cymothoidae) **(Saied *et al.*, 2024)**.

Isopod-host fish experimental trials

Three experimental trials were performed using the collected isopods against different host species, *T. zillii*, and *D. labrax*. The first two trials were performed in closed circular fiberglass tanks, each of 1000L holding capacity, while the physical treatment trial was performed using small glass aquaria of 120L. All experimental units were supplied with an ample amount of sand-filtered brackish water (35ppt) obtained from Lake Qarun. Continuous aeration was ensured using an electric air pump to maintain the dissolved oxygen levels around 6.5± 0.2mg/ L, whereas water pH and temperature were adopted at 7.1± 0.1 and 26± 1°C, respectively. The fish underwent a one-week acclimatization period in the experimental tanks before the start of the trials. Throughout the trials, the experimental fish received a daily diet consisting of a commercial feed with 30% protein content (obtained from Elmorshidy Company, Egypt), provided at a rate equivalent to 3% of their body weight.

Test of isopod virulence (infestation ability) on fish species at optimized salinity and water temperature

In order to indicate the rate of infestation of *L. redmanii* among the tested fish species, 30 apparently healthy *T. zillii* and 30 apparently healthy individuals of *D. labrax* were divided into three replicates (10 individuals each), free-swimming isopods (60 individuals) were evenly distributed in each tank, maintaining an intensity ratio of ~2 isopods/ fish. The temperature and water salinity in each tank were optimized for isopod infestations at 26± 1°C and 35ppt, respectively. Fish were daily observed for infestation by isopods according to

Athanassopoulou *et al*. (2001). Additionally, any characteristic clinical signs and gross macroscopic lesions were observed.

Test of isopod infestation behavior on *Tilapia zillii* at different water salinities

A total of 120 apparently healthy *T. zillii* were randomly distributed into four groups (*n*= 30), each contributing three replicates (*n*= 10/ replicate). Group 1 was maintained at the normal salinity level of 35ppt without isopod infestation, serving as a negative control group (NC). The positive control group corresponds to the first tank used in the virulence trial mentioned above. The water salinity in the tanks of the remaining groups (G2-G4) gradually decreased by about ~3ppt every 24h using a water bath of sterile, dechlorinated fresh water until the intended salinity reached 25ppt (G2), 15ppt (G3), and 8ppt (G4). Free-swimming *L. redmanii* were evenly introduced into the fish tanks with an intensity ratio of ~2 isopods/ fish, except in the negative control group tank. The tested fish were observed daily for infestations. The prevalence and mortality rates were calculated.

Physical treatment of infested fish species

The remaining survival-infested fishes, *D. labrax* (nine individuals) and *T. zillii* (six individuals), were reallocated into two small glass aquaria of 120L holding capacity for further physical treatment trials:

- **Treatment by reducing water salinity**

The water salinity was gradually reduced by 2 degrees every hour to 8ppt by adding fresh sterile dechlorinated water, and the results were reported.

- **Treatment by lowering water temperature**

The water temperature was gradually decreased from 26 to 10°C using ice bags. The temperature was monitored daily using a multiparameter device, and the results were reported.

Effect of water temperature on isopod viability

An experiment was conducted to assess the impact of reducing water temperature on the viability and behavior of 10 active free-swimming isopods. This trial was performed in small glass aquaria, and the outcomes were carefully observed and documented.

RESULTS

Prevalence and mortality rate of isopod infestation

The prevalence and mortality rates of *Livoneca redmanii* infestation among different fish species following the challenge with active free-swimming cymothoid isopods at 26°C and 35ppt salinity were recorded (Table 1). As shown, *L. redmanii* successfully infested most of the tested *T. zillii* fish, displaying varying prevalence rates ranging from 80 to 100%, particularly within the first day of the challenge. Remarkably, the infestation prevalence notably decreased to 30% in the case of *D. labrax* by the second day of the challenge toward the end of the trial. Moreover, mortality rates were variable among different species, with the highest rate recorded in *T. zillii* (80%), while it was the lowest among *D. labrax* (10%).

Table 1. Results of virulence test among different fish species experimentally infested with free live swimming isopods at 26°C and 35ppt salinity for 7 days

Fish species	**Infested fish**			**Total**	**Dead fish (Mortality)**					**Total**	**Recovered fish**			**Total**	**Remained healthy fish**	**Remained infested fish**
	Day 1	**Day 2**	**Day 3**		**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5-7**		**Day 1**	**Day 2**	**Day 3-7**			
T.zillii	**30**	**ND**	**ND**	**30/30 (100%)**	**9**	**7**	**7**	**1**	**ND**	**24/30 (80%)**	**ND**	**ND**	**ND**	**ND**	**6 (20%)**	**ND**
D. labrax	**18**	**6**	**ND**	**24/30 (80%)**	**2**	**1**	**ND**	**ND**	**ND**	**3/30 (10%)**	**ND**	**5**	**7**	**12 (40%)**	**9/30 (30%)**	**18/30 (60%)**

ND: Not detected, Infestation, Recovery and mortality rates are calculated based on the number of fish / group (*n* = 30) divided into three replicates (10 fish/ replicate).

Specifically, isopodosis in *T. zillii* occurred just 30min after introducing the fish into the isopod tank, with a prevalence rate of 100% during the first day of challenge. Initially, the isopods attached to the fish's external body surface and subsequently moved forward to settle in the fish's buccal cavity and inside the branchial cavity on the gills (Fig. 1). The initial response to external infestation provokes irritation in the fish, leading to rapid and distressed movements as they attempt to dislodge the firmly attached parasite. Most infested fish displayed common signs associated with ectoparasitism, such as sluggish movement, fin erosion, skin darkness, hemorrhages, and detached scales (Fig. 1). In terms of mortality rate, *T. zillii* displayed daily mortalities, lasting up to 4 days after infestation and reached 80%.

In comparison, the onset of isopodosis in *D. labrax* was slightly delayed by about 2h after introducing fish to the isopods. Infested fish exhibited an aggressive escape response, and the infestation rate reached 80% by the end of the second day and dramatically decreased to 30% by the end of the trial, as most of the infested fish (40%) succeeded in managing their infestation and could get rid of the parasite. Isopods were found attached to the external body surface of the fish, particularly on the body trunk and around the tail, pectoral, and dorsal fins. Clinical investigation revealed skin ulcers and erosions with detached scales (Fig. 2). No infestations were observed in the branchial cavity, and ~20% of *D. labrax* showed resistance to isopod infestation by the end of the experiment.

In terms of mortality rate, only 10% of the infested fish died during the first two days of the challenge, and no further deaths were documented after that. The infested fish exhibited signs of stress, including vigorous shaking of the tail fin and body trunk.

Concerning the density of isopod infestation, experimentally infested fish, regardless of the species, could be invaded by one, two, or sometimes three *L. redmanii* isopods. Overall, following the experimental infestation of *D. labrax* with isopods, the number of free-living isopods was notably decreased at the end of the experiment, while those infested *T. zillii* remained stable and active. Moreover, the postmortem inspection of the experimentally infested *D. labrax* revealed the presence of the isopod inside the fish gut (Fig. 3).

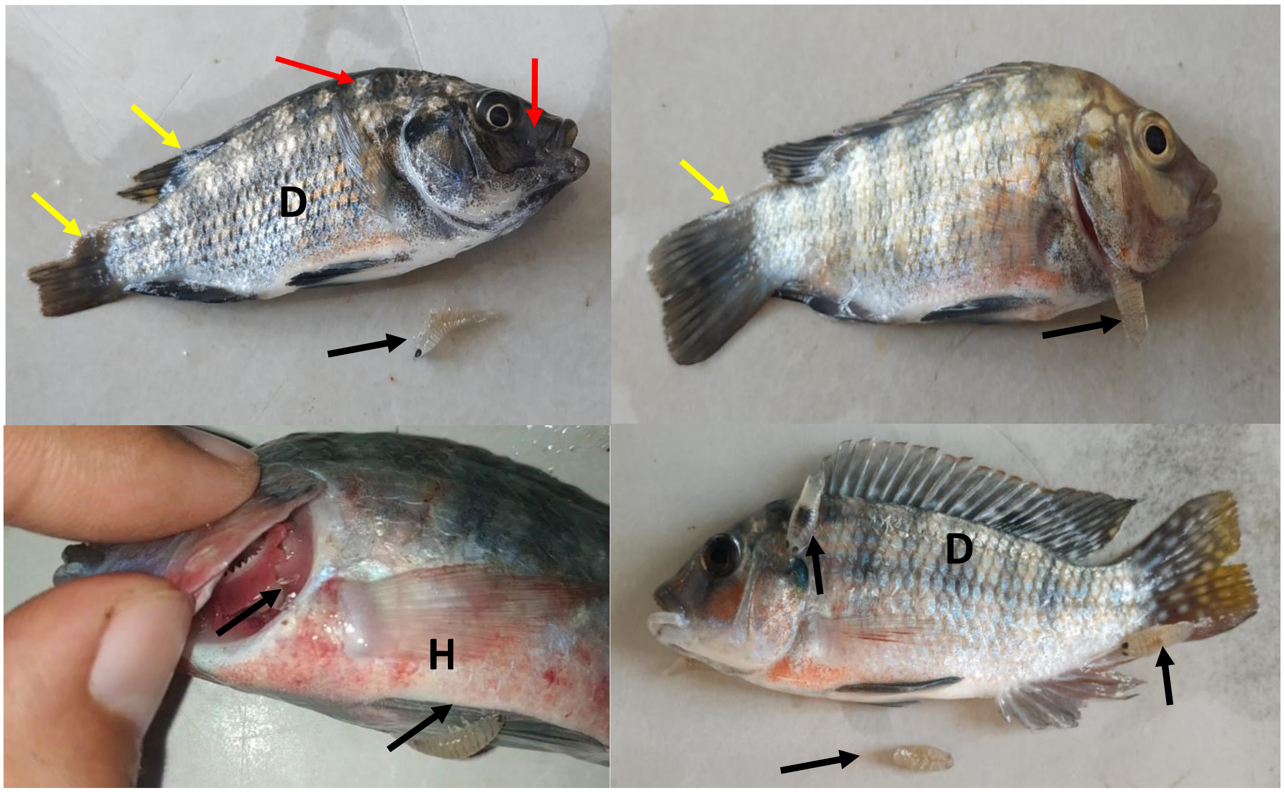

Fig. 1. Experimentally infested *T. zillii* with isopods showed fins erosion (yellow arrows), skin darkness (red arrows), hemorrhages (H), and detached scales (D). Isopods were found on the external body surface and subsequently migrated forward to settle inside both the buccal and branchial cavities (black arrows)

Experimental infestation at different water salinities

The results of the experimental infestation of *T. zillii* with free-living *L. redmanii* at different salinities for seven days were recorded and tabulated (Table 2). As noted, 35ppt water salinity was optimal for isopod infestation, where the highest prevalence and mortality rates were documented as 100 and 80%, respectively (Table 1). Extensively, fish in group 2, reared at a salinity of 25ppt, exhibited an 80% isopod infestation rate and a 40% mortality rate by the end of the experiment. The gradual decline in salinity levels led to a notable reduction in both prevalence and mortality rates. At a salinity level of 15ppt, the overall prevalence and mortality rates were 50 and 10%, respectively. However, at a salinity of 8ppt, only 10% of the fish were infested, and no mortalities were recorded.

Fig. 2. Experimentally infested *D. labrax* with isopods showed fins erosion (yellow arrows) and skin ulcers (red arrows). Isopods were found on the external body surface, particularly on the body trunk and around the tail, pectoral, and dorsal fins (black arrows)

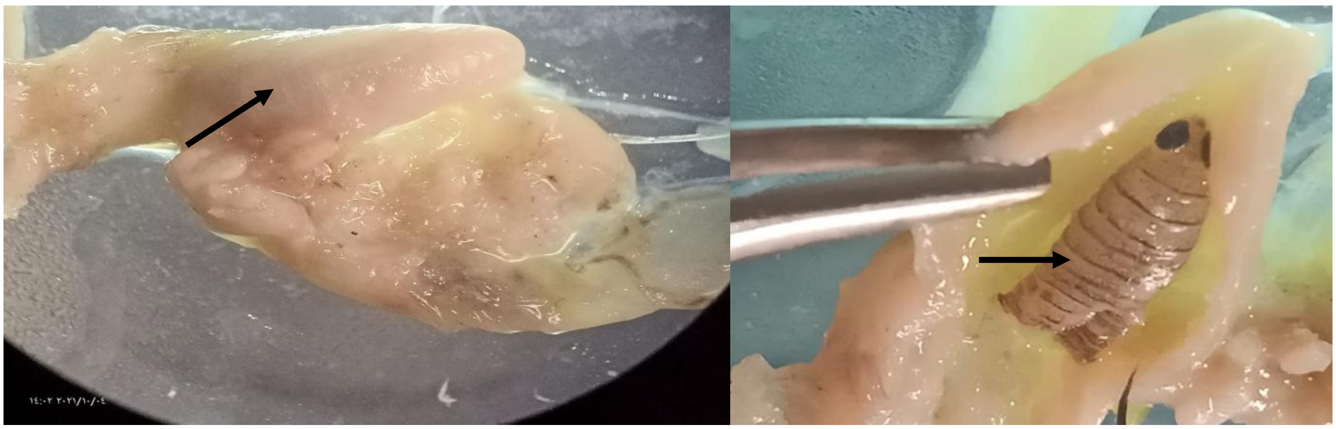

Fig. 3. Postmortem examination of the experimentally infested *D. labrax* showing the presence of *Livoneca redmanii* inside the fish gut

Table 2. Prevalence and mortality rates among *Tilapia zillii* experimentally infested with free live swimming isopods at different water salinities

Group number	Salinity level (ppt)	Day of challenge								Total	
		Day 1		Day 2		Day 3		Day 4-7			
		P	M	P	M	P	M	P	M	P	M
Group1 (NC)	35	--	--	--	--	--	--	--	--	ND	ND
Group2	25	15	6	9	3	--	3	--	--	24/30 (80%)	12/30 (40%)
Group3	15	9	--	3	3	3	--	--	--	15/30 (50%)	3/30 (10%)
Group4	8	--	--	--	--	--	--	--	--	3/30 (10%)	ND

NC, Negative control, P: Prevalence, M: Mortality (--), Not recorded. Morbidity and mortality rates are calculated based on the number of fish/ group (n = 30) divided into three replicates (10 fish/replicate).

Recovery rate

T. zillii didn't show any recovery from the isopod's infestation. At the same time, *D. labrax* was notably able to recover from infestation, as on the third day of infestation, 40% of infested fish were recovered without any external interference.

Physical treatment of experimentally infested fish

It was noted that *L. redmanii* continued to cling to the infested host fish, *T. zilli* and *D. labrax*, at various salinity levels ranging from 30 to 15ppt. However, at 10ppt, only one isopod changed its position from the body trunk to the caudal fin but remained attached. At a salinity level of 8ppt, one isopod detached from its host and attempted to reinfest it but was unsuccessful. Conversely, treatment involving a decrease in water temperature did not yield a promising effect on isopod detachment. All isopods remained firmly attached to their hosts and appeared more stable, even at lower temperatures ranging from 15 to 10°C.

Effect of water temperature on isopod viability

It was observed that free-living isopods showed different obvious reactions after a gradual decrease in water temperature. The isopods displayed heightened activity at a water temperature of 26°C. Nevertheless, their activity steadily declined as the temperature decreased, ultimately leading to complete inactivity at 15°C. At this point, they were observed lying motionless on the tank's floor, resembling a state of near paralysis.

DISCUSSION

The isopod infestation in Lake Qarun is a severe and worsening crisis, causing a significant decline in the fish population **(Kua *et al*. 2022)**. Managing crustacean parasites, especially isopods, is challenging. Chemical treatments are impractical due to the widespread infestation. Biological control methods show promise but come with their challenges. Hence, this study aimed to explore methods for controlling infestations caused by the different haplotypes of *livoneca redmanii* identified from Lake Qarun based on nuclear *18S rDNA* and mitochondrial *COI* **(Saied *et al.*, 2024)**. This approach involved assessing the virulence of the isopod at the optimal water salinity on *T. zillii* and *D. labrax*, evaluating the predation capability of *D. labrax* as a biological control method, testing the effectiveness of physical treatments, such as lowering water salinity and temperature and examining the impact of reduced water temperature on isopod viability.

The results revealed that under the normal water conditions of 26°C and 35ppt salinity, free-living isopods exhibited highly virulent behavior against fish hosts. The prevalence rate reached 100% on the first day post-challenge, and the mortality rate was 80% for *T. zillii*. This result aligns with earlier findings from **Fadel *et al*. (2020)**, who reported a 100% infestation rate of isopods against *A. regius* at 33 and 25ppt salinity levels, with a mortality rate of 73.33%. Ideal water conditions are crucial for free-swimming parasites to find host fish, offering them shelter, a source of nourishment, and the right environment for growth and reproduction. **(Rhode, 2005)**.

In contrast to previous findings, the mortality rate among *D. labrax* was remarkably low, standing at 10%. This can be attributed to the natural behavior of this fish species, known for its aggressiveness and territorial tendencies. When confronted with a genuine threat, they typically respond by either rapidly retreating or assuming a distinct defensive posture. *D. labrax* displays a flashing behavior in which it turns on one side and seems to rub that side against the substrate. This behavior is believed to serve the purpose of either disturbing small crustacean food items buried in a sandy bottom or eliminating ectoparasites **(Pickett & Pawson, 1994)**.

Herein, infestations in *T. zillii* developed quickly with the parasite reaching the opercular cavity. In contrast, in *D. labrax*, infestations took longer to develop, and the parasite couldn't reach the opercular cavity. This difference may be linked to the passive and vulnerable nature of *T. zillii* **(Eccles, 1992; Costa-Pierce, 2003)**, making it a preferred target for isopods, which is consistent with the high infestation rates observed in the previous studies **(Abdel-Latif, 2016; Mahmoud *et al.*, 2017)**. Nonetheless, *D. labrax* fish were observed preying on free-swimming isopods in their tanks, confirming their predatory nature. Younger fish have a higher preference for invertebrates compared to older fish. European sea bass exhibit opportunistic and aggressive predatory behavior. They employ a variety of tactics to locate and capture prey, including ascending toward the water surface and launching attacks from below at a steep angle. Their diet adapts to the seasonal abundance of prey species in a given location **(Oikonomidou *et al*., 2019)**.

In this study, lowering water salinity has proven to be an effective prophylactic measure against isopod infestations. The morbidity and mortality rates significantly decreased from 100 and 80% at 35ppt to 10% prevalence rates with no mortalities at 8ppt. Our findings agree with those recorded by **Fadel *et al*. (2020)**, where the prevalence rates decreased to 63.33% at 15ppt and 40% at 8ppt. On the other hand, attempting physical treatment of infested fish by reducing salinity or lowering water temperature proved ineffective post-challenge, since the parasite remained firmly attached to its host even under unfavorable environmental conditions. Several studies have shown that, in the wake of environmental changes like temperature increases or eutrophication events, parasites frequently gain an advantage over their hosts **(Mitchell *et al*., 2005; Johnson *et al*., 2010; MacNab & Barber, 2012; Scharsack *et al*., 2016)**.

Conversely, reducing water temperature impairs the survival of free-swimming isopods, especially at 15°C, causing complete paralysis and sinking to the tank bottom. This may account for the lower infestation rates during the winter season. These findings are consistent with those reported by **Davidson *et al*. (2013)**, who noted a reduced activity of crustacean isopods when exposed to lower temperatures.

CONCLUSION

Physical treatment of infested fish proved futile post-challenge since the parasite remained firmly attached to its host even under unfavorable environmental conditions. Lowering the water salinity and temperature affected the survival of free-swimming isopods and could serve as a prophylactic measure. *T. zillii* is a preferable host for parasitization by the isopod in Lake Qarun, and it actually acts as a shelter host needed for isopod growth and reproduction. *D. labrax*, a robust model fish, exhibited resistance to isopod infestation, potentially using undisclosed defense mechanisms. These fish also actively preyed on isopods, highlighting their pivotal role in the proposed biological control strategy for parasites. This study is one of the few to investigate the direct relationship between isopods and host fish under varying environmental conditions. The results offer hope for finding effective solutions to combat isopod infestations in large water bodies through physical or biological methods, providing an alternative to chemicals with adverse effects on the fisheries sector and public health.

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ETHICAL APPROVAL FOR EXPERIMENTAL ANIMAL CARE

Fish handling and all the experiments were approved by the NIOF Committee for Ethical Care of Marine Organisms and Experimental Animals (NIOF- IACUC) (2023/ No: NIOF-AQ6-F-23-R-044).

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