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Applied Biology

Elixir Appl. Biology 51 (2012) 10901-10903

ISSN: 2229-712X

A Fungal Study on the *Astacus leptodactylus* in Agın Region of the Keban Dam Lake, Turkey

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ARTICLE INFO

Article history:
Received: 25 July 2012;
Received in revised form:
30 September 2012;
Accepted: 5 October 2012;

Keywords
Saprolegnia spp.,
Disease,
Astacus leptodactylus.

ABSTRACT
Fifty female crayfish (46.20 ± 1.37 g live mass) which caught from Agın Region of the Keban Dam Lake (Elazıg, Turkey) in March of 2011were examined in the laboratory to evaluate their health status. The exoskeleton was observed for Saprolegnia spp. Mycological investigations demonstrated the presence of hyphae in the area of the abdomen. *Saprolegnia* spp. was isolated from lesions in infected eggs. Mortalities occurred in fiberglass tanks, after 4 weeks respectively, in the female infected crayfish, with approximately a 38 % cumulative mortality rate (in the last week). In both cases the crayfish were weak, anorexic and lethargic. The main cause of mortality was lesions in infected eggs. Other causes associated with mortality likely can be stressing factors (handling, feeding etc.) in the laboratory.

Introduction

Crayfish, *Astacus leptodactylus* (Eschscholtz, 1823) that the single crayfish species inhabiting Turkish inland aquatic areas is naturally and widely distribute in lakes, ponds and rivers throughout of Turkey. This species is not only important for its economic value but also for its important role playing in inland aquatic food web.

With the growth of crayfish aquaculture worldwide, the occurrence of disease outbreaks is likely to increase. This development will create pressure for investigations on the etiology, pathogenesis, prevention and treatment of crayfish diseases.

Infections in crayfish present as 'burn marks' in the carapace then progress into penetrating ulcers. The risk factors for saprolegniasis in crayfish are likely the same as those recognized in fish, including wounding, stress from water treatments, and poor water quality. Fungal (oomycetes) diseases were classified as fungi are often reported to be the most important pathogens of freshwater crayfish. Oomycetes of the genus *Saprolegnia*, especially *S. parasitica*, are economically important pathogens of fish and fish eggs as crayfish eggs. Infection with these organisms has caused heavy losses in crayfish (Edgerton et al. 2002; Torto-Alalibo et al. 2005). Outbreaks of saprolegniasis have also been reported in wild crayfish and in the eggs (Krugner-Higby et al. 2010). *S. parasitica* infections have been reported in crayfish, both in aquaculture settings and in wild crayfish. Other *Saprolegnia* spp. has been reported in crayfish by culturing the organisms from dead crayfish, but pathogenicity was not determined (Edgerton et al. 2002). Söderhall et. al. (1991) reported that isolated Saprolegnia spp. from *A. leptodactylus*. The researchers reported that they had a low pathogeneses and generally caused melanised lesions by release cellular defense reaction in the cuticle of freshwater crayfish

While some crayfish pathogens and symbionts have been studied in considerable detail, information on most disease agents, disease conditions and symbiont associations are lacking. With the growth of crayfish aquaculture worldwide the occurrence of disease outbreaks is likely to increase. This development will create pressure for investigations on the etiology, pathogenesis, prevention and treatment of crayfish diseases. A stimulus for these types of studies will also come from the need to better understand the environmental consequences of introduction of exotic organisms into aquatic ecosystems. The main purpose of this study was to evaluate the health status of some wild and cultured crayfish populations in Turkey. This base-line study is essential for scientifically monitoring these important crayfish populations and planning for their future.

Material and Methods

Crayfish

Crayfish (*A. leptodactylus*) with brown abdominal lesions were obtained from Agın Region of the Keban Dam Lake (Elazıg, Turkey) in March (Figure 1) in 2011. After capture, all of the live crayfish were rapidly transported to the Laboratory in Aquaculture Faculty of Tunceli University (Turkey) and placed in containers with water from the sampling site. In the laboratory, animals were held in 40-l fiberglass tanks. The total of 50 females (46.20 ± 1.37 g live mass, 26.84 ± 1.83 cm total body length) was stored (10 samples per tank) for four weeks in the laboratory at a temperature of 14±1 °C. An artificial photoperiod of 12D-12L was maintained. All crayfish were provided with air-bricks as shelters to reduce injuries caused by cannibalistic attacks during molting and were fed twice weekly on a formulated diet. Each day, crayfish were examined for the occurrence of morbidity and mortality. Water in tank was replaced every week.

Microscobical techniques

In macroscopic investigation, the samples were isolated from brown abdominal patches of *A. leptodactylus* stained with methylene blue. The affected crayfish were observed under an optic microscope 100x and 400x.

Microscopically identified Saprolegnia spp. was transferred to sterilized Petri plates containing distilled water. The affected

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crayfish were observed under an optic microscope 100x and 400x every day starting from the third day of the culture. The identification of fungi and fungus like organisms involved such morphological features as shape and size of hyphae, shape of sporangium and spores. Isolated fungi and fungus-like organisms were identified according to classical mycological methods (Diéguez-Uribeondo et al., 1994; Unestam 1973).

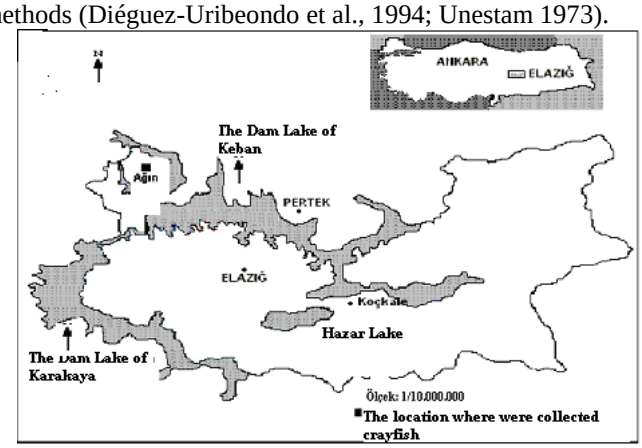


Figure 1. Geographic location of the study

Results

Disease outbreaks occurred in the all tanks but especially intensively 3th and 4th weeks respectively after being brought into the laboratory. 50 crayfish were caught from Agın Region of the Keban Dam Lake (Elazıg, Turkey) in 2011 showed white cotton-like patches distributed predominantly over the anterior part of the body. The main cause of mortality was lesions in infected eggs. Mortality rates were showed %6 in the first week, % 8 in the second week, % 28 in the third week and % 38 in the last week.

The moribund crayfish were collected and underwent mycological examinations. Microscopic investigation demonstrated the presence of hyphae in the area of the abdomen, specially infected eggs (Figure 1, 2). Isolation on culture medium, microscopic examination of morphological characteristics enabled identification. Colonies were initially cottony white but progressively developed a characteristic rose violet pigmentation. Identification results revealed that all isolates mycelium samples belonged to the genus Saprolegnia. Saprolegnia spp. caused melanized lesions in the cuticle of freshwater crayfish. Saprolegnia spp. invades dead eggs in the egg masses of freshwater crayfish. Saprolegniales fungus was shown to infect eggs.

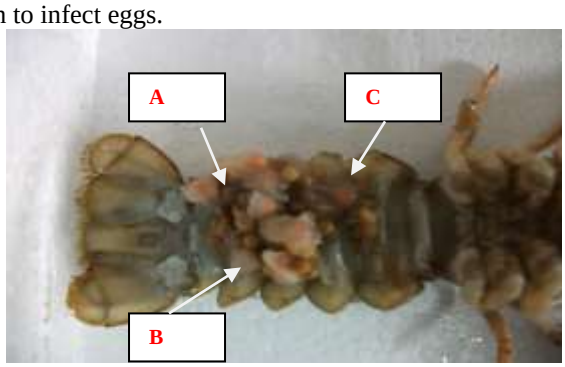
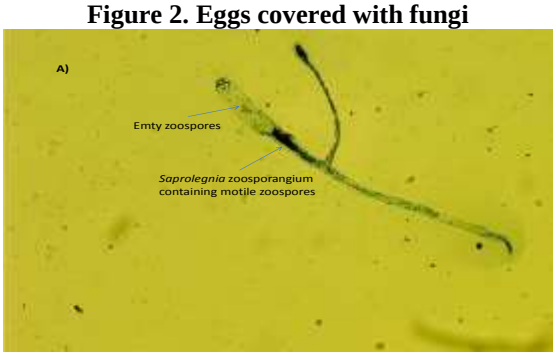
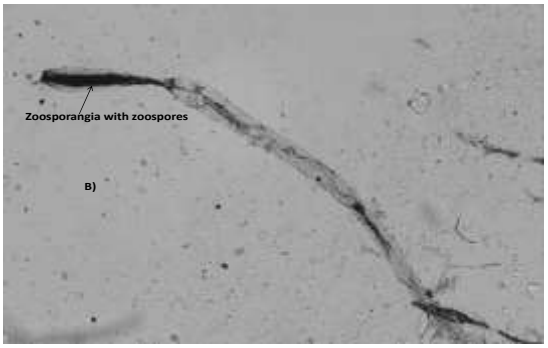


Figure 1. A) Fertilized egg fully infected with fungi B) Infected egg changed color (from brown to orange) C) Infected egg died (orange)

Figure 2. Eggs covered with fungi





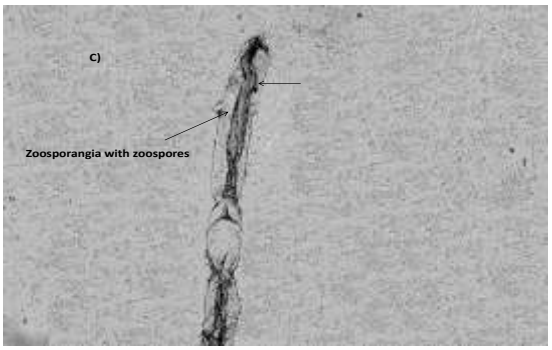


Figure 3. Saprolegnia zoosporangium A) containing motile zoospores (X100) B) empty zoosporangia (x100) C) with zoospores (x400)

Discussion

Saprolegnia spp. as pathogens and egg parasites are of major economic concern since they are responsible for devastating infections on fish and crayfish in aquaculture and farms (Melendre et al., 2006; Van West 2006).

Saprolegnia spp. are water mould, now classified in the kingdom Protoctista, phylum Heterokonta, class Oomycotea (Bruno and Wood, 1999), and include species which are responsible for significant infections involving both living and dead fish. The identification of these species, by cultural and microscopic methods, is based on obtaining the sexual structures *in vitro* (oogonia, antheridia and oospores). However, one

problem facing the diagnostic mycologist when culture conditions fail to stimulate the production of sexual structures, making accurate identification of the species becomes very difficult (Pickering and Willoughby, 1982).

In crayfish *Saprolegnia parasitica* has been recently recognised as an important pathogen, causing up to 60% mortalities. Various ubiquitous fungi have been assumed to play a role in the density regulation of crayfish populations (Bower & McGladdery 2005) and also Söderhäll et al. (1991) speculate about mortality in crayfish caused by *Saprolegnia*. As pointed out by Söderhäll et al. (1991), although *S. parasitica* causes a severe problem in fish, it does not appear to be an important parasite for crayfish. However, during intensive culture of crayfish, *Saprolegnia* spp. may cause some mortality, especially in females with eggs; furthermore Dieguez-Uribeondo et al. (1994), in a challenge test with zoospores, infected healthy (non-injured) *Astacus astacus*, *Pacifastacus leniusculus* and *Procambarus clarkii* inducing 20% mortality. Death could result in a few of these crayfish under certain conditions either from the fungal infection or from invasion of the crayfish by other pathogens.

In our study, our strain was isolated from the melanized areas of the exoskeleton and from the eggs of crayfish and induced 38 % mortality rate. The main cause of mortality was lesions in infected eggs. Different mortality rates can probably be severe of pathogenic activity but their pathogenic role, primary or secondary, is not clear. The appearance of higher levels of mortality can be due to stressing factors (handling, feeding etc.) in environmental conditions of the laboratory. It can be considered as an anomaly. Therefore, particular care must be given to crayfish culture to prevent environmental stressors causing disease outbreaks.

Due to lack of the formation and observation in the characteristic of secondary cysts and the molecular studies we were couldn't to identify the species. Therefore, further studies on the pathogenic potential of *Saprolegnia* spp. in crayfish are needed studies which are involved histopathology and bacteriology techniques

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