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# Dead specimens of fairy shrimp *Streptocephalus dichotomus* (Crustacea) as vectors of mycosis-inducing fungi in fish aquacultures

**B. Czeczuga[1], A. Semeniuk[1], A. Godlewska[1], E. Czeczuga-Semeniuk[1] and C. S. Velu[2]**

[1]Department of General Biology, Medical University, Kilińskiego 1, 15-089 Białystok, Poland.
[2]Department of Zoology, University of Madras, Guindy Campus, Chennai 600 025, India

## ABSTRACT

The authors investigated the occurrence of aquatic zoosporic fungi in the water of three water bodies of varied trophicity. Dead specimens of fairy shrimp *Streptocephalus dichotomus* (Crustacea), fish feed in aquacultures on the Indian subcontinent, were used as a bait. Thirty-five zoosporic fungus species, including 20 known as parasites or necrotrophs of many fish species were identified. They included *Achlya intricata*, *Achlya prolifera*, *Achlya orion*, *Aphanomyces laevis*, *Dictyuchus monosporus*, *Leptolegnia caudata*, *Saprolegnia delica*, *Saprolegnia ferax*, *Saprolegnia monoica and Saprolegnia parasitica*, all known as a frequent cause of mycotic diseases in economically important fish populations. Dead specimens of *Streptocephalus dichotomus* should thus be regarded as vectors of aquatic fungi which induce mycosis in fish.

**KEYWORDS:** fairy shrimp, fungi vectors, mycosis, fish, aquacultures

## 1. INTRODUCTION

Although the number of fish species bred in controlled conditions increases [1, 2], deaths on a large scale of a particular fish population may occur due to bacterial infections [3] and mycotic diseases induced by aquatic parasitic fungi [4, 5]. The latter have most frequently affected eel [6-8], trout [9, 10], salmon [11-13] and acipenserid fish species [14-16]. This phenomenon has also been observed on the Indian subcontinent and in the adjacent regions [17-19].

*Streptocephalus dichotomus* found on the Indian subcontinent was first described by Baird [20]. As this is a relatively common crustacean in that region, it is a feed component of many fish species bred in aquacultures in India and other countries [21, 22].

Aquatic fungi found to grow on 2 other shrimp species utilized as fish feed have been described by few authors, including Shah *et al*. [23] and Monoyama [24].

Therefore, we decided to find out which of the already known fungi responsible for mycotic diseases in fish [25] can grow on the specimens of *Streptocephalus dichotomus* and for which fungi this crustacean is a vector.

## 2. MATERIAL AND METHODS

The study was performed on dried specimens of fairy shrimp (*Streptocephalus dichotomus* Baird) utilized in aquacultures of India as the feed of many species of fish fry and adult specimens, ornamental fish in particular [22]. The water for experiments was collected from three different water bodies; two running (spring Cypisek and river Supraśl) and one stagnant (pond Dojlidy):

- Spring Cypisek, localized in the north part of Białystok: Limnokrenic type, width 0.65 m, depth 0.12 m, discharge 2.4 l/s. The spring is

*Corresponding author: bazzylio@poczta.onet.pl

surrounded by cultivated fields. The bed is covered with sand.

- River Supraśl, right - bank tributary of the middle part of the Narew river flowing through the Knyszyńska Forest: Length 106.6 km. The samples were collected from the site above the municipal swimming pool at the sluice of an arm of the Supraśl river flowing just through the town Supraśl. The sampling site is surrounded by meadows. The bed is muddy.

- Pond Dojlidy, localized near Białystok: Area 34.2 ha, max. depth 2.85 m, its south shores border with coniferous woods and its western part with the town of Białystok. The samples were collected from the western part of this pond, which is used by the inhabitants of the town as a beach.

Nineteen water parameters of the above sampling sites were determined (Table 1) according to the methods recommended by Stirling [26] and Standard Methods for the Examination of Water and Wastewater [27].

For the determination of the presence of aquatic fungal species on the fairy shrimp specimens the following procedure was employed: a certain number of pieces (about 2 mg) was transferred to two samples of water representing each site, in an 1.0 $dm^3$ vessel (all together six vessels) and placed in the laboratory at ambient temperature. A part of pieces from each vessel was observed under a light microscope and the mycelium of aquatic fungi on the pieces of fairy shrimp was recorded. The methods are described in detail by Fuller and Jaworski [28]. The pieces of the fairy shrimp specimens were observed under a microscope every three days. The duration of the experiments was four weeks. Identification of fungi species was based on morphology and biometric data of antheridia and oogonia of the zoosporic fungi. The fungi species were identified using the keys of Johnson [29], Seymour [30], Batko [31], Karling [32], Plaats-Niterink [33], Dick [34] and Pystina [35]. The systematic of fungi was used according to Blackwell *et al*. [36], of straminipilous organisms according to Dick [37] and Johnson *et al*. [38], and of Chytridiomycota according to James *et al*. [39].

The chemical parameters of the water as well as the fungal flora were investigated by statistical analysis [40].

## 3. RESULTS

Water samples used for the experiment contained differed nutrients. The water from spring Cypisek was the most abundant in nitrogen (all three forms), while the samples from the river Supraśl had the lowest nitrogen content. The highest level of phosphates was observed in the river Supraśl, the lowest in spring Cypisek. The largest amounts of sulphates and chlorides, as water pollution indicators, were noted in spring Cypisek, while their lowest content was found in the river Supraśl (Table1).

Thirty-five aquatic zoosporic fungi were found to grow on dead specimens of *Streptocephalus dichotomus* (Table 2) - 18 in the water of pond Dojlidy, 21 in spring Cypisek and 21 in the river Supraśl. Such species as *Achlya oblongata, Achlya treleaseana, Saprolegnia delica, Saprolegnia ferax and Saprolegnia monoica* developed on this crustacean in the water of the three water bodies. Of the 35 species, 20 are described in literature as fish parasites or necrotrophs. Moreover, the growth of two rare species *Myiophagus ucrainica Pythium indigoferae* was observed on dead *Streptocephalus dichotomus* specimens. The statistical method showed that the factor that had a big influence on the amount of fungal species in all water bodies was the sulphates in water (negative correlation: spring Cypisek- 0.8648, river Supraśl- 0.6821, pond Dojlidy- 0.8124, level of significance 0.04).

## 4. DISCUSSION

It is well known that the water fungi are cosmopolitan organisms which occur in water bodies of Europe, Asia, America and Australia [41]. The fungi types mentioned in the paper were often found in the water on Indian subcontinent. The occurrence of different fungi species depends on occurrence of the substrate in the water, on which the fungi can develop and on the water trophic. So the received data show that dead and dried *Streptocephalus dichotomus*, which are used as a food in aquacultures system, are also a good substrate for the development of fungi which are fish parasites and they can be dangerous for the aquacultures.

In the water of all the water bodies involved in the current study, the respective fungus species appeared

**Table 1.** Chemical composition (in mg $1^{-1}$) of water from different sampling sites.

| Specification | Water from | | |
|---|---|---|---|
| | Cypisek spring | Supraśl river | Dojlidy pond |
| Temperature (°C) | 13.5 | 17.0 | 14.5 |
| pH | 7.45 | 7.83 | 7.58 |
| $O_2$ (mg $L^{-1}$) | 9.0 | 9.4 | 7.0 |
| $BOD_5$ (mg $L^{-1}$) | 1.8 | 2.0 | 5.6 |
| COD (mg $L^{-1}$) | 4.60 | 5.76 | 13.82 |
| $CO_2$ (mg $L^{-1}$) | 11.0 | 6.6 | 11.0 |
| Alkalinity in $CaCO_3$ (mval $L^{-1}$) | 4.9 | 4.7 | 3.6 |
| N-$NH_3$ (mg $L^{-1}$) | 0.100 | 0.310 | 0.530 |
| N-$NO_2$ (mg $L^{-1}$) | 0.010 | 0.006 | 0.008 |
| N-$NO_3$ (mg $L^{-1}$) | 2.000 | 0.050 | 0.070 |
| P-$PO_4$ (mg $L^{-1}$) | 0.800 | 1.600 | 1.300 |
| Sulphates (mg $L^{-1}$) | 56.83 | 16.46 | 48.13 |
| Chlorides (mg $L^{-1}$) | 31.0 | 12.0 | 26.0 |
| Total hardness (mg Ca $L^{-1}$) | 119.52 | 71.28 | 61.20 |
| Total hardness (mg Mg $L^{-1}$) | 18.49 | 15.48 | 13.33 |
| Fe (mg $L^{-1}$) | 0.20 | 0.50 | 1.35 |
| Dry residue (mg $L^{-1}$) | 478 | 183 | 255 |
| Dissolved solids (mg $L^{-1}$) | 316 | 177 | 213 |
| Suspended solids (mg $L^{-1}$) | 162 | 6 | 42 |

successively on dead specimens of *Streptocephalus dichotomus* in a certain order. During the first days of the experiment, the species known in literature as animal saprophytes, either parasites or necrophils of fish and other aquatic animals were identified. They included four species of the genus *Achlya* (*Achlya klebsiana, Achlya oblongata, Achlya oligacantha, Achlya prolifera), Aphanomyces laevis, Apodachlya brachynema, Dictyuchus monosporus, Leptolegnia caudata, Myiophagus ucrainica, Phlyctochytrium aureliae),* and four species of the genus *Saprolegnia* (*Saprolegnia ferax, Saprolegnia hypogyna, Saprolegnia monoica* and *Saprolegnia parasitica*). Some of them have been frequently encountered on fragments of dead specimens of various aquatic crustacean species [42-44] and aquatic insects [45]. After 10-14 days, other fungus species began to appear, which in literature are mainly referred to as plant saprophytes, fish parasites or necrophils.

A similar phenomenon was observed in a study of aquatic fungi growing on various keratinine-containing substrates [46-48]. Keratinophil group species are the first to appear both on hair and feathers, and then follow plant saprophytes with substrate decomposition.

Some of these species have caused great losses in fish pond aquacultures in Island. *Achlya intricata* is a known parasite of fish bred in ponds in Island [49], while *Achlya prolifera* is responsible for death of the whole *Tor tor* eggs in ponds - hatcheries in India, causing its total extinction [50]. Other species of the genus *Achlya*, e.g., *Achlya orion*, also occasionally induce substantial losses both in fish fry and grown-up populations [51].

**Table 2.** Zoosporic fungi found on the fairy shrimp, *Streptocephalus dichotomus*.

| Taxa | Spring Cypisek | River Supraśl | Pond Dojlidy |
|---|---|---|---|
| Fungi | | | |
| Chytridiomycota | | | |
| Chytridiales | | | |
| 1. *Phlyctochytrium aureliae* Ajello | | x | |
| Straminipila | | | |
| Hyphochytriomycetes | | | |
| Hyphochytriales | | | |
| 2. *Myiophagus ucrainica* (Wize) Sparrow | x | | |
| Peronosporomycetes (Oomycetes) | | | |
| Leptomitales | | | |
| 3. *Apodachlya brachynema* (Hildebr.) Pringsh. | x | | |
| Pythiales | | | |
| 4. *Pythium helicandrum* Drechsler | x | | |
| 5. *Pythium indigoferae* E. J. Butler | | | x |
| 6. *Pythium intermedium* de Bary | x | | |
| Saprolegniales | | | |
| 7. **Achlya americana* Humphrey | x | x | |
| 8. *Achlya androgyna* (W. Archer) T. W. Johnson et R. L. Seym. | | x | x |
| 9. *Achlya colorata* Pringsh. | | x | |
| 10. *Achlya debaryana* Humphrey | | x | |
| 11. **Achlya diffusa* J. V. Harv. ex T. W. Johnson | | | x |
| 12. **Achlya intricata* Beneke | x | | |
| 13. **Achla klebsiana* Pieters | | x | |
| 14. *Achlya oblongata* de Bary | x | x | x |
| 15. *Achlya oligocantha* de Bary | x | | |
| 16. **Achlya orion* Coker et Couch | | x | x |
| 17. **Achlya prolifera* C. G. Nees | x | | x |
| 18. **Achlya radiosa* Maurizio | x | | |
| 19. *Achlya rodrigueziana* F. T. Wolf | | x | |
| 20. *Achlya treleaseana* (Humphrey) Kauffman | x | x | x |
| 21. **Aphanomyces laevis* de Bary | x | x | |
| 22. **Dictyuchus monosporus* Leitg. | x | x | |

Table 2 continued..

| | | | |
|---|---|---|---|
| 23. **Isoachlya monilifera* (de Bary) Kauffman | x | x | |
| 24. **Leptolegnia caudata* de Bary | | | x |
| 25. **Protoachlya paradoxa* (Coker) Coker | | x | x |
| 26. *Saprolegnia anisospora* de Bary | | x | x |
| 27. **Saprolegnia delica* Coker | x | x | x |
| 28. **Saprolegnia ferax* (Gruith.) Thur. | x | x | x |
| 29. *Saprolegnia glomerata* (Tiesenh.) A. Lund | | x | x |
| 30. **Saprolegnia hypogyna* (Pringsh.) de Bary | x | | x |
| 31. **Saprolegnia monoica* Pringsh. | x | x | x |
| 32. **Saprolegnia parasitica* Coker | x | x | |
| 33. **Saprolegnia torulosa* de Bary | | x | x |
| 34. *Saprolegnia uliginosa* Johannes | x | | x |
| 35. **Thraustotheca clavata* (de Bary) Humphrey | x | | x |
| **Total number** | **21** | **21** | **18** |

*Species known in literature as parasites or necrotrophs.

*Dictyuchus monosporus* and *Leptolegnia caudata* have been described as fish pathogens causing enormous losses in the eggs of white salmon in the delta of the river Volga in Russia [52]. *Aphanomyces laevis* is responsible for death of substantial amounts of acipenserid eggs in hatcheries in the lower course of the Volga [16]. The same fungus species are also encountered on other fish species in the river Volga [53]. Dangerous pathogens of the eggs of various fish species in hatcheries [54, 55] and of fish fry and grown-up specimens in ponds and other water reservoir types include some of the *Saprolegnia* species [56], particularly *Saprolegnia delica, Saprolegnia ferax*, *Saprolegnia monoica* and first of all *Saprolegnia parasitica*. *Saprolegnia delica* is a known pathogen of tropical regions [57] and is also encountered on other fish species inhabiting waters in the continental climate zone [53, 58-60]. However, the most common pathogens in aquacultures of various fish species, causing the greatest losses, are *Saprolegnia ferax* and *Saprolegnia parasitica*. *Saprolegnia ferax* kills the eggs of sterlet [14], carp [61] and of other fish species bred on the Indian subcontinent [62]. We have frequently found this pathogen on the eggs of European fish species, both of the salmonid and cyprinid families [59, 63, 64]. *Saprolegnia parasitica* has been described as a pathogen of the eggs of various fish species [54, 63] and of fish fry in breeding farms of Pacific salmon where it causes death of almost the whole population [12, 13, 65, 66]. It is also responsible for considerable losses in fish populations in lakes [67].

There has been a long search for effective measures that would prevent or delimit mycotic infections of eggs in hatcheries as well as of fish fry and grown-up specimens in aquacultures [4, 68]. As shown in the current study, dead specimens of *Streptocephalus dichotomus* used as feed for fish fry in aquacultures can be the source of infection in aquacultures in fish farms of southern Asia, particularly on the Indian subcontinent. Zoosporic fungi growing on this crustacean do not lose their vitality even when not in aquatic conditions [69], which has been confirmed in the climatic conditions of India [70, 71]. Hyphae found in land conditions in dry air were still vital when transported to aquatic conditions after a year. The uneaten dead specimens of *Streptocephalus dichotomus* can

serve as a substrate for the growth of many fungus species, fish parasites or necrotrophs. Thus, not only should antimycotic agents be used against fungi in fish specimens but also fungus growth on the crustaceans they consume should be prevented.

From the mycological point of view, worth noting is the finding of two fungus species only occasionally encountered in waters - *Myiophagus ucrainica* growing on fairy shrimp specimens in the water of spring Cypisek and *Pythium indigoferae* in pond Dojlidy. These water bodies differ in hydrochemical parameters. Compared to the other two reservoirs, the spring water contained the highest amounts of $N\text{-}NO_3$, sulphates, chlorides, Ca, Mg, dry residue, dissolved and suspended solids, and showed the lowest content of $N\text{-}NH_3$, $P\text{-}PO_4$, Fe, and the lowest $BOD_5$ and oxidability. The water in pond Dojlidy was the most abundant in $N\text{-}NO_3$, Fe, had the highest $BOD_5$ index and oxidability, and the lowest levels of oxygen, Ca, Mg and alkalinity.

*Myiophagus ucrainica* has been known as a parasite of insect pests of cultivated plants. It was first described by Wize [72] in the Ukraine as a parasite of the insect *Cleonus punctiventris*, a beet pest, and later as a parasite of other land insects in Great Britain [73], in Bermuda [32], and in Florida [74, 75]. Moreover, worth noting is the already reported occurrence of *Myiophagus ucrainica* on aquatic insect species [45] and planktonic crustacean species [44].

*Pythium indigoferae* described in India by Butler [76] as epiphytic on the leaves of *Indigofera arrecta* Hockst., and Royal Botanic garden, Calcutta [77]. This species was found also in soil in China [78]. Sideris [79] transferred this species to *Nematosporangium* genus. It can also grow on certain species of submerged aquatic plants in lakes of Poland [80].

The statistical analysis showed that sulphates influenced the amount of species growing on the specimens of *Streptocephalus dichotomus.* As it is known [81], sulphates is pollution indicator for some water bodies. The amount of sulphates increases with the eutrophication of the water bodies. The effect of the sulphates on the number of zoosporic fungi growing on specimens of dead crustaceaus was observed earlier [43, 44, 47, 82].

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