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Vol. 9(8), pp. 515-520, 25 February, 2015
DOI: 10.5897/AJMR2014.7260
Article Number: 4A1590450747
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Observations of mycotal species growth on the rainbow trout eggs (*Oncorhynchus mykiss*)

Bazyli Czeczuga*, Ewa Czeczuga- Semeniuk and Adrianna Semeniuk- Grell

Department of General Biology, Medical University, Mickiewicza 2c, 15-222 Bialystok, Poland.

Received 9 November, 2014; Accepted 2 February, 2015

The authors investigated the growth of hydromycobiota on the eggs of rainbow trout (*Oncorhynchus mykiss*) in waters of different trophicity. Of the *O. mykiss* eggs that were investigated, 16.6% were found to be infected by mycotal species. Thirty mycotal species were found on eggs obtained from adult female representatives. *Achlya polyandra*, *A. radiosa*, *Aphanomyces laevis*, *Leptomitus lacteus*, *Saprolegnia ferax* and *Saprolegnia parasitica* belonged to the species that were most commonly encountered. *Aphanomyces frigidophilus*, *Candida albicans* and *Zoopage phanera* were rarely found in salmonid fishes. Amino acid, carbohydrate and urease tests were used, and all analyses of species from the *Achlya*, *Aphanomyces*, *Leptolegnia*, *Pythium* and *Saprolegnia* genera showed that they assimilate glucose and starch. However, they did not assimilate glycine, leucine, lysine, ornithine, and arabinose. Urease was assimilated only by species from the *Leptolegnia*, *Pythium* and *Saprolegnia* genera.

Key words: *Oncorhynchus mykiss*, rainbow trout, eggs, mycotal species, infection, hydrochemistry.

INTRODUCTION

Rainbow trout were classified as part of the *Salmo* genus until 1988 when the use of the generic name *Oncorhynchus* was adopted for all Pacific trout and salmon, to distinguish them as different (Smith and Stearley, 1989). Analyses of mitochondrial DNA showed that the mtDNA of rainbow trout had more similarity to Pacific salmon than to brown trout and Atlantic salmon (Berg and Farris, 1984; Thomas et al., 1986; Gyllensten and Wilson, 1987). This was confirmed through osteological analysis (Smith and Stearley, 1989). Today, all forms of rainbow trout belong to the *Oncorhynchus* genus, as the *O. mykiss* species (derived from the Kamchatkan name "mikizha" or "mykiz") (Ethier and Starnes, 1993).

Both marine and freshwater fish species deliver not only protein and fat, but also biologically active substances that are important for human organisms. Therefore, the consumption of fish is increasing from year to year (Food and Agricultural Organization of the United Nations (FAO), 2012). However, in recent years freshwater fishing has increased much more than fishing in seas and oceans. The growing number of freshwater fish farms may account for this. An important factor to consider in investigating what limits the fish populations in some cultures is the mycotal pathogens that can result in reduced breeding, sometimes affecting from 50% (Hatai and Hoshiai, 1992)

*Corresponding author. E-mail: bazzylio@poczta.onet.pl.

to 75% (Lartzeva, 1986) of the incubated eggs. For example, Chien (1981) has described the mass death of the rainbow trout that occurred off the coast of Taiwan during their reproduction period and which were caused by the *Aphanomyces laevis*. As a result of this, we became interested in the extent to which mycotal species affect the eggs of the rainbow trout in waters of different trophicity.

MATERIALS AND METHODS

Occurrence of rainbow trout

Oncorhynchus mykiss (Walbaum, 1792) (syn. *Salmo gairdneri* Richardson, 1836; *Salmo irideus* Gibbons, 1855) (English name: rainbow trout).

O. mykiss is native to the Pacific Slope, extending from the Kuskokwin River, in Alaska, through Rio Santo Domingo, in Baja, California, to rivers in Mexico, to the upper Mackenzie River (Arctic Basin), in Alberta and British Columbia, Canada, and to the endorheic basins of southern Oregon, U.S.A. This species has also been widely introduced to coastal waters elsewhere in North America and in rest of the world, including South Africa (Page and Burr, 1991; ITIS, 2010).

In the Eastern Pacific, *O. mykiss* occurs on the Kamchatkan Peninsula and has been recorded on the Commander Islands (east of Kamchatka) and sporadically in the far south of the Okhotsk Sea where the mouth of the Amur River flows along the mainland (ITIS, 2010).

This species inhabits cold headwaters, creeks, small-to-large rivers and lakes (usually not stocked in water that reaches summer temperatures above 25°C) and ponds with very low oxygen concentrations. It feeds on a variety of aquatic invertebrates and on small fishes. *O. mykiss* is cultured in many countries (including some in the south of Africa) and is often hatched and stocked in rivers and lakes, especially to attract recreational fish species (Frimodt, 1995; ITIS, 2010). With the exception of the common carp, the rainbow trout is one of the oldest species that is being cultured (Gall and Crandell, 1992). According to Wales (1939), the first transfer of eggs took place from McCloud River (northern California) to a hatchery in Caledonia, New York, in 1874. Rainbow trout eggs were also shipped from North America to Japan in 1877 and to England in 1885. The European rainbow trout farming industry began in Denmark (Laird and Needham, 1988; Gall and Crandell, 1992).

Characteristics of water bodies

Three water bodies located in the north-eastern region of Poland (53°07'N, 23°10'E to 53°13'N, 23°20'E) were chosen for this study: Pond Fosa: an area of 2.5 hectares (ha); maximum depth of 1.75 m; breeding site of wild ducks; a culture of crucian carp intended for anglers; Pond Komosa: an area of 12.1 ha; maximum depth of 2.25 m; surrounded by coniferous trees of the dense Knyszynska Forest; River Suprasl: a length of 106.6 kilometers (km); a right-bank tributary of the middle part of the Narew River, flowing through the Knyszynska Forest.

Nineteen (19) parameters of those water samples were measured (Table 1) according to generally accepted methods (APHA, 2005).

Isolating and identifying mycotal species

Eggs were collected (after fertilization) at the end of April from the hatchery at the Gawrych Ruda Farm. 150 - 450 eggs for each of the water bodie were investigated.

Table 1. Chemical and physical properties of water in particular water bodies (in mg l^{-1}).

Specification	River	Pond	
	Supraśl	Fosa	Komosa
Temperature (°C)	17.0	18.0	17.4
pH	7.8	7.1	7.6
DO	11.2	6.4	12.8
BOD_5	2.8	12.8	7.4
Oxidability(COD)	6.60	20.2	13.20
CO_2	6.6	22.4	8.3
Alkalinity in $CaCO_3$ (mval l^{-1})	4.5	5.8	3.9
$N\text{-}NH_3$	0.142	0.864	0.161
$N\text{-}NO_2$	0.006	0.114	0.009
$N\text{-}NO_3$	0.014	0.552	0.034
$P\text{-}PO_4$	0.158	3.598	0.255
Sulphates (SO_4)	32.38	85.1	42.75
Chlorides (Cl)	17.12	79.3	23.51
Total hardness in Ca	73.42	24.2	68.40
Total hardness in Mg	11.58	20.6	28.81
Fe	0.48	1.06	1.54
Dry residue	197.0	429.0	375.0
Dissolved solids	179.0	370.0	312.0
Suspended solids	18.0	59.0	63.0

Water samples from specific water bodies 800 ml each were placed into 1000-ml vessels and 50 eggs were transferred to each vessel in accordance with the general principles of culture (Watanabe, 2000). The vessels were stored at temperature of 7± 0.5°C, with access to daylight that resembled natural conditions and following the recommended instructions (Seymour and Fuller, 1987). The pH of the water was analysed separately for every vessel (Peterson and Brindge, 1994). The water analysis and experiments were done in three parallel repetitions. Eggs were taken for each vessel, and the eggs that were covered with fungal mycelia were observed every 3 - 4 days under a light-microscope. The presence of any morphological structures, such as zoospores, antheridia and oogonia, belonging to aquatic fungi were recorded. Fungal species were identified using the keys of Johnson et al. (2005), Pystina (1998) and Petrini and Petrini (2013). The systematics of straminipiles species according to Dick (2001) were used in this experiment. The experiments were carried out for one month, and the results were then tested for significance using ANOVA and evaluated by the Scheffe test (Winer, 1997).

Determination of the amino acid, carbohydrate and urease assimilation tests

Amino acid, carbohydrate and urease tests were performed on the *Achlya*, *Aphanomyces*, *Leptolegnia*, *Pythium* and *Saprolegnia* genera, based on Yuasa and Hatai (1996). For the carbohydrate utilization test, Yeast Nitrogen Base agar (Difco) was the medium used for the cultures of the fungal isolates. GY agar (Difco) was used for the urease test. The basal medium used in the amino acid assimilation test was the same as that used for the carbohydrate assimilation test. Bromo thymol blue and phenol red that was added to the yeast nitrogen-based broth and the GY broth, respectively, were used as indicators. These methods are described in detail in

our previous paper (Czeczuga et al., 2011b).

RESULTS

Table 1 shows the hydrochemical parameters of the water that was used in the experiment. The water from Pond Fosa was the most eutrophic. It showed heightened oxidability and alkalinity and higher levels of CO_2, N (NO_3), phosphates and other parameters. The lowest indices of the parameters that have been mentioned, as well as the lowest amounts of chlorides and iron, were found in water from Pond Komosa and River Supraśl.

Thirty mycotal species, including twenty four (24) belonging to the *Saprolegniales*, two to the Zoopagales, two to the Pythiales and one each to the Leptomitales, and Saccharomycetales, were found to be growing on the eggs of the *O. mykiss* (Table 2). The highest number of species was growing in water samples from Pond Fosa (the most eutropic), while the lowest number occurred in water from River Supraśl and Pond Komosa (the lowest trophicity). It is worth making a special note that *Aphanomyces frigidophilus*, *Candida albicans* and *Zoopage phanera* have rarely been found in fish living in Polish waters. Table 3 shows the assimilation tests for species from the *Achlya*, *Aphanomyces*, *Leptolegnia*, *Pythium* and *Saprolegnia* genera. All analysed species from the *Achlya*, *Aphanomyces*, *Leptolegnia*, *Pythium* and *Saprolegnia* genera showed that they assimilated glucose and starch. However, they did not assimilate glycine, leucine, lysine, ornithine or arabinose. The urease was assimilated by species from the genera of *Leptolegnia*, *Pythium* and *Saprolegnia* only.

DISCUSSION

The present study has proven that the growth of aquatic mycotic species on the eggs of rainbow trout depends on the chemical characteristic of the water bodies from which the water samples are obtained for the experiment. Chemical analyses of the water samples that were collected enabled water differentiation with respect to the content of chemical compounds. Water from Pond Fosa contained more biogenic compounds, mainly phosphorus. This confirms once again our earlier assumptions (Czeczuga and Woronowicz, 1993) that the degree of infection of the fish eggs in hatcheries depends largely on the state of cleanliness and trophicity of the water that supplies the hatchery.

As shown in Table 2, the most commonly encountered species included *Saprolegnia parasitica*, *S. ferax*, *Achlya polyandra*, *Laptomitus lacteus* and *Aphanomyces laevis*. All these species belong to the group of opportunistic sapro- and necrotrophic pathogens (Bruno and Wood, 1999). *S. parasitica*, which has been described as a pathogen in the eggs of various fish species (Hatai et al., 1990) and in the fish fry in Pacific salmon breeding farms, causes the death of almost the entire population (Neitzel et al., 2004; van West, 2006). It is also responsible for considerable losses in fish populations in lakes. *S. ferax* kills the eggs of sterlet (Lartzeva, 1986) and cyprinids (Czeczuga and Muszynska, 1999). *A. polyandra* was observed quite frequently on the eggs of four lampreys that were examined (Czeczuga, 1997) and on Atlantic salmon (Czeczuga et al., 2011a), and *L. lacteus* infects many fish species in different water bodies. *Aphanomyces frigidophilus* was described as occurring on the eggs of the Japanese char *Salvelinus leucomaenis* (Kitancharoen and Hatai, 1997) and, for the first time in Europe, on the eggs of *Coregonus lavaretus* (Czeczuga et al., 2004). *Aphanomyces frigidophilus* also grows on some species from the *Salmo* genus (Czeczuga et al., 2011a), including sturgeonid fishes (Czeczuga et al., 2012b), Chinook salmon (Czeczuga et al., 2012a) and African catfish (Czeczuga et al., 2013), and on the alevins of the Nile tilapia (Czeczuga et al., 2014b), and on the eggs of *Stenodus* species (Czeczuga et al., 2014a).

The immune response of rainbow trout to *Aphanomyces invadans* has also been examined (Thompson et al., 1999), and according to Khan et al. (1998) and Oidtmann et al. (2008), the rainbow trout is moderately susceptible to *Aphanomyces invadans* through intramuscular infection.

Rainbow trout is one of the main species that is bred on a large scale in fish farms, not only in Europe (Backiel, 1964; Goryczko, 2000) but also on other continents (MacCrimmon, 1972), including Africa (FAO, 2012). Therefore, from the 1930s to the present, mycosis has caused huge losses in the populations of this species and has been studied intensely. Members of this species can be infected by *A. laevis*, *L. lacteus*, *Saprolegnia delica, S. ferax, S. monoica* and *S. parasitica* (Tiffney, 1939a, b; Scott and O'Bier, 1962; Scott, 1964; Chien, 1981; Noland-Tintigner, 1970; Hatai et al., 1990). In addition, *A. laevis*, *Aphanomyces* sp., *L. lacteus*, *S. delica*, *S. monoica*, *S. parasitica* and *S. asterophora* have been observed in rainbow trout eggs (Scott and O'Bier, 1962; Scott, 1964; Florinskaja, 1971; Czeczuga and Woronowicz, 1993). Czeczuga and Muszynska (1996) also revealed the presence of such straminipiles as *Achlya polyandra* and *Achlya radiosa*. Experimental infection with *Saprolegnia* spp. in the eggs of rainbow trout has been investigated by Kitancharoen and Hatai (1996), Kitancharoen et al. (1997), Fregeneda-Grandes et al. (2001), Hussein et al. (2001) and Hussein and Hatai (2002).

It is also worth noting that the fungi *Zoopage phanera* that was found on eggs that were examined from water in the Biala River have been described as predacious fungus-catching soil amoebae (Drechsler, 1935). The growth of *Z. phanera* in fish has been reported on peled eggs (Czeczuga and Woronowicz, 1993). *Candida albicans* yeast has also been seen very rarely as a fish parasite, although it was found on eggs from water samples taken from Pond Komosa. The growth of yeast-like fungi has now been found on coregonid and salmonid fry. Bauer et al. (1973) reported yeast infections on salmon

Table 2. Fungi and straminipiles from the reservoirs analysed (number of investigated eggs – 450, infected – 75, 16.6%).

Taxa	Pond Fosa	Pond Komosa	River Suprasl
Fungi			
Ascomycota			
Saccharomycetales			
1.*Candida albicans* (Robin) Berrk.		x	
Zygomycota			
Zoopagales			
2. *Zoopage phanera* Dreschsler			x
3. *Zoopagus insidians* Sommerst	x	x	x
Straminipila			
Peronosporomycota			
Leptomitales			
4. *Leptomitus lacteus* (Roth) Agardh			
Pythiales			
5. *Pythium artotrogus* de Bary	x		
6. *P. ultimum* Trow	x	x	
Saprolegniales			
7. *Achlya americana* Humphrey			x
8. *A. bisexualis* Coker et Couch		x	
9. *A. colorata* Pringsh	x		
10. *A. hypogyna* Coker et Pemb	x		
11. *A. klebsiana* Pieters		x	
12. *A. orion* Coker et Couch	x		
13. *A. polyandra* Hildebr			x
14. *A. radiosa* Maurizio			x
15. *A. treleaseana* (Humphr.) Kauf.		x	x
16. *Aphanomyces frigidophilus* Kitauch. et Hatai		x	
17. *Ap. laevis* de Bary	x	x	x
18. *Ap. stellatus* de Bary	x		x
19. *Dictyuchus anomalus* Nagai	x		
20. *D. monosporus* Leitgeb		x	
21. *D. sterilis* Coker	x		
22. *Isoachlya monilifera* (de Bary) Kauf.	x		
23. *Leptolegnia caudata* de Bary	x		x
24. *Protoachlya polyandra* (Lindst.) Apinis	x		
25. *Saprolegnia anisospora* de Bary	x		
26. *S. ferax* (Gruith) Thurnet	x	x	x
27. *S. hypogyna* (Pringsh.) de Bary		x	
28. *S. mixta* de Bary		x	
29. *S. parasitica* Coker	x	x	x
30. *Scoliolegnia asterophora* (de Bary) M. W. Dick	x		
Total species (the different letters indicate difference of statistical significance (≤ 0.05)	17a	13b	11b
Number of infected eggs	48a	15b	12b
	10.6%	3.3%	2.7%

fry, while Nagornaya et al. (1996) observed the growth of several species of the genus *Candida* on the eggs of rainbow trout. *C. albicans* growth was observed on the eggs of *Coregonus albula* in a hatchery (Czeczuga and Woronowicz, 1993), and species of fungi belonging to *Candida*, *Rhodotorula* and *Torulopsis* have been isolated

Table 3. Amino acid, carbohydrate and urease assimilation by straminipiles isolated from eggs of *Oncorhynchus mykiss*.

Species of genus	Amino acid	Carbohydrate	Urease
Achlya	Asp, Glu, Arg, Ala	Fru, Glu, Man, Raf, Suc, Mal, Lac, Mel, Cel, Tre, Sta, Dex, Rha, Gly	-
Aphanomyces	Glu, Ala, Cys, Phe	Glu, Sta	-
Leptolegnia	Asp, Glu,	Fru, Glu, Man, Mal, Mel, Cel, Tre, Sta, Dex, Gly	+
Pythium	Ala, His, Orn, Phe, Cys	Fru, Glu, Man, Gal, Raf, Suc, Mal, Lac, Mel, Cel, Tre, Sta, Dex, Rha, Gly, Sal	+

in fish from the African continent (Refai et al., 2010).

Finally, the amino-acid, carbohydrate and urease assimilation by straminipiles on the eggs of rainbow trout in the water bodies that have been mentioned differ from the assimilation by straminipiles in the waters of Japan (Yuasa and Hatai, 1996; Kitancharoen and Hatai, 1998). Perhaps it is related to huge biological variety of straminipiles species.

Conclusion

Examination of the growth of fungi and straminipiles organisms on the eggs of rainbow trout (*O. mykiss* Walbaum), in three trophically different water bodies was performed. Thirty species of mycotal organisms, developing and growing on the eggs of rainbow trout (24 belonging to the Saprolegniales, 2 to the *Pythiales*, 2 to the *Zoopagales*, 1 to the *Leptomitales* and 1 to the *Saccharomycetales*), were found. The greatest number of mycotal organisms was found in water from the most eutrophic Pond Fosa (17 species) and the lowest was identified in water from the less eutrophic River Supraśl (11) and Pond Komosa (13). Also, the greatest number of infected was found in water from Pond Fosa (48 eggs – 10.6% out of 450 investigated) and the lowest in water from River Supraśl (12 – 2.7%) and Pond Komosa (15 – 3.3%). *Achlya* and *Saprolegnia* were the most prevalent genera. The most commonly encountered species were: *Z. insidians*, *A. laevis*, *S.ferax* and *S. parasitica*. *C. albicans*, *Z. phanera* and *A. frigidophilus* were rarely found.

Species of *Achlya*, *Aphanomyces*, *Leptolegnia*, *Pythium* and *Saprolegnia* genera did not assimilate methionine, lysine, ornithine, leucine and glycine. All species of *Achlya*, *Aphanomyces*, *Leptolegnia*, *Pythium* and *Saprolegnia* genera assimilated glucose and starch, but did not assimilate arabinose. Urease was only assimilated by species from the *Leptolegnia*, *Pythium* and *Saprolegnia* genera.

The investigations showed that different trophicity of respective water bodies increases the prevalence of mycotal infections of the eggs of the rainbow trout.

Conflict of interests

The authors did not declare any conflict of interest.

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