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ORIGINAL PAPER

Myxosporean parasites of *Ceratomyxa merlangi* and *Myxidium gadi* in whiting *Merlangius merlangus*: a comparative epizootiological analysis based on samples from two localities off southern and northern coasts of the Black Sea

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Abstract Totally 690 and 423 whiting *Merlangius merlangus* samples were collected from two localities off Southern (Sinop) and Northern coasts (Balaklava Bay) of the Black Sea, respectively, and examined for myxosporeans in the period from May 2011 to March 2014. *Ceratomyxa merlangi* and *Myxidium gadi* were the only myxosporean parasites identified in the content of gall bladder and their infection indices of prevalence (%) and intensity were calculated for length classes and sex of fish as well as for the seasons at both sampling localities. Overall infection prevalence of *C. merlangi* was 22.6% in Sinop and 27.9% in Balaklava samples while those values of *M. gadi* were 18.4% and 28.6% in Sinop and Balaklava samples, respectively. Both parasite species were also found to be co-existed in whiting samples. This is the first comprehensive epizootiological study yielded comparable data on *C. merlangi* and *M. gadi* infections in whiting in the southern and northern coasts of the Black Sea.

Keywords *Ceratomyxa merlangi* · *Myxidium Gadi* · *Merlangius merlangus* · Comparative analysis · Black Sea

Introduction

Myxozoan are among the microscopic metazoan parasites that infect a wide variety of fish hosts all around the world and there is an increasing interest on their pathogeny, systematics and expansions on geographic areas (Kent et al. 2001; Lom and Dykova 2006; Özer et al. 2014, 2015a, b; Skuratovskaya et al. 2013). The genus *Ceratomyxa*, representing the coelozoic myxozoa, is widely distributed in marine hosts (Eiras 2006) and a total of over 252 nominal *Ceratomyxa* species have been reported globally by MacKenzie and Kalavati (2014) and Özer et al. (2015a, b) reported 13 species in the Black Sea, some of which were also reported from oceans and other seas (Eiras 2006). *Ceratomyxa merlangi* Zaika, 1966 is a parasite of only one fish species, namely whiting *M. merlangus,* in the Black Sea. *Myxidium* is the third most specious myxozoan genus, after *Ceratomyxa*, with 232 nominal species identified from all over the world and a total of 7 *Myxidium* species were reported from marine fish in the Black Sea, all from Ukrainian coasts (Yurakhno 2009; Eiras et al. 2011; Özer et al. 2015a, b). *Myxidium* spp. are typically coelozoic (rarely histozoic) bivalvulidan myxosporeans and they are primarily fish parasites usually infecting the gallbladder of the host (Eiras et al. 2011). Infections with *Myxidium gadi* Georgévitch, 1916 have been reported in a variety of fish including cod (*Gadus morhua*), pollack (*Pollachius pollachius*), saithe (*Pollachius virens*), haddock (*Melanogrammus aeglefinus*), black sole (*Solea solea*), common sole (*Solea vulgaris*), plaice (*Platichthys flesus*) and whiting (*M. merlangus*) in Baltic, Barents, White, Celtic, Adriatic, Black and Azov Seas, Atlantic Ocean (Jayasri and Hoffman 1982; Iskov 1989; MacKenzie and Kalavati 1995; MacKenzie et al. 2005; Özer et al. 2015a, b). Members of both genera, *Myxidium* and *Ceratomyxa,* have been reported to be good indicators of whiting stock structure (Kabata 1963, 1967; MacKenzie and Longshaw 1995).

Whiting, *Merlangius merlangus*, is a widely distributed and commercially important fish species in the eastern North Atlantic Ocean, the northern Mediterranean, western Baltic

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and the Black Sea (ICES 2006) and it has been reported along Romanian, Bulgarian, Turkish, Ukrainian and Caucasus coasts of the Black Sea (Burdak 1960; Svetovidov 1964; Özer et al. 2014). Black sea whiting has been reported to host four myxosporeans parasitizing the gall bladder; *Leptotheca informis* Auerbach, 1910 (only once), *Myxidium incurvatum* Thelohan, 1892 (most probably in fact it was *M. gadi*), *Ceratomyxa merlangi* Zaika, 1966 and *Myxidium gadi* Georgévitch, 1916 (Yurakhno 2008).

Information available about myxosporean parasites in the Black Sea have been largely faunistic and, thus, the objective of the present study is to investigate the comparative ecology and host-parasite interrelationship of myxosporean parasites in whiting, *Merlangius merlangus* collected from Turkish and Crimean Black Sea coasts for the first time.

Materials and methods

Details of whiting sampling, parasitological investigations and identifications were presented by Özer et al. (2015a, b). Briefly, totally 690 and 423 fish specimens from Turkish (Sinop) (N 42° 05′ 68″ E 35° 10′ 55″) and Crimean (Balaklava Bay) (N 44° 49′ 54″ E 33° 59′ 48″) samples, respectively, were examined for parasites using conventional methods. Myxosporeans were determined by screening smears of muscles, gills, gall bladder, urinary bladder, kidney and intestinal serous membrane of fish using a light microscope at ×400 and ×1000 magnifications under oil immersion. Parasite species were identified using Olympus microscope (BX53) equipped with a digital camera (DP50) and hand drawing attachment in Turkey and Sunny microscope (CE, T3.15A) in Crimea. Infection prevalence (%) values were determined according to Bush et al. (1997). Sterne's exact 95% confidence limits were calculated for prevalence using Quantitative Parasitology 3·0 software (QP3·0) (Reiczigel and Rózsa 2005). Difference in prevalence values between two fish populations from two sampling localities and between two fish sexes were determined by the exact unconditional test that maintains the prescribed type I error rate, which has a higher statistical power in a small sample size ($N < 100$). Differences in prevalence values between sampling seasons, age and length categories were determined using the Fisher's exact test. All the statistical tests were performed at the significance level of 5%. The intensity of infection of myxozoans was semiquantitatively evaluated following a scale from + to ++++++, based on the number of myxosporean parasites per microscopic field at ×300, as described by Alvarez-Pellitero et al. (1995). This protocol was modified for ×200 magnification to intensity ranges: (+) 1–5; (++) 6–10; (+++) 11–25; (++++) 26–50; (+++++) 51–100; (++++++) > 100.

Results

Ceratomyxa merlangi Zaika, 1966 and *Myxidium gadi* Georgévitch, 1916 were the only myxosporean parasites identified in the content of gall bladder of whiting *Merlangius merlangus* collected from both localities of the Black Sea. Of the 690 and 423 fish specimens examined in Sinop and Balaklava, infection prevalence values and intensity levels were determined for each parasite species and their co-existences (Table 1). Prevalence of infection values of individual parasite species in Sinop samples were lower than those in Balaklava Bay samples except for both co-existing parasites. Each parasite species at both sampling localities were observed in hundreds and thousands immature and mature individuals of spores and their intensities were ranked as ++++++ per microscopic field while intensity rank was similar but slightly lower for co-existing levels of both parasite species at both sampling localities (Table 1).

The distribution of *C. merlangi* and *M. gadi* with respect to season

Seasonal prevalence values of *C. merlangi*, *M. gadi* and their co-existences in *M. merlangus* at both sampling localities are presented in Table 2. Infection prevalence values for both parasite species were very similar without any statistically significant difference between seasons ($P > 0.05$), however co-existing *C. merlangi* and *M. gadi* in Sinop samples had significant differences in the prevalence values between seasons ($P < 0.05$) (Table 3). On the other hand, in Balaklava samples, the situation was reversed that there were statistically significant differences in the prevalence values between seasons for each *C. merlangi* and *M. gadi* infections only ($P < 0.05$) (Table 3). When the prevalence values of each parasite species and their co-existence in each season are compared in two sampling localities, statistically significant differences were found for individual parasite species infections between autumn and winter ($P < 0.05$), while their co-existing prevalence values had a significant difference only in spring ($P < 0.05$) (Table 4). Prevalence values of each *M. gadi* and *C. merlangi* species and their co-existence were very low in Balaklava samples in summer while myxosporeans were absent in Sinop samples during the same season (Table 2).

The distribution of *C. merlangi* and *M. gadi* with respect to the length classes of the host fish

Of the three different fish length classes studied (Table 2), consistent gradual increases in infection prevalence values were observed from the smallest length class up to the largest length class of fish infected with either individual or co-

Table 1 Infection prevalence (%) and intensity levels of myxosporean parasite species identified in whiting, *M. merlangus*, collected from Sinop ($n = 690$) and Balaklava Bay ($n = 423$) coasts of the Black Sea, CI, 95% confidence intervals based on Stern's exact score limit given in parentheses. Both infection indices indicate the host fish to be infected only one species, either *C. merlangi* or *M. gadi*, and mixed infections by both parasite species at the same investigated host

Parasite species	Infection Intensity		Infection Prevalence (%) (CI)	
	Sinop	Balaklava	Balaklava	Balaklava
Ceratomyxa merlangi	+++++	+++++	22.6 (19.6–25.9)	27.9 (23.7–32.3)
Myxidium gadi	+++++	+++++	18.6 (15.7–21.6)	28.6 (24.4–33.0)
C. merlangi + *M. gadi*	++++	++++	13.9 (11.5–16.7)	8.7 (6.3–11.9)

existing myxosporeans at both sampling localities (Table 2). Between length classes in Sinop samples, statistically significant differences were determined in the prevalence values for each *C. merlangi* and *M. gadi* parasites and their co-existence (Table 3). On the other hand, only *C. merlangi* had statistically significant difference in the prevalence values between length classes of fish in Balaklava samples (Table 3). When the differences between the sampling localities in terms of the infection prevalence values of each length classes were compared, statistically significant differences were determined for *M. gadi* only in the smallest length class and the co-existing *C. merlangi* and *M. gadi* infections in the middle length class ($P < 0.05$) (Table 4).

The distribution of *C. merlangi* and *M. gadi* in host fish of different sexes

When the infection prevalence values of individual parasite species *C. merlangi*, *M. gadi* and their co-existing infection in both sex of fish in sampling localities were compared, statistically significant differences were determined only in *M. gadi* in Sinop samples and *C. merlangi* in Balaklava samples ($P < 0.05$) (Table 3). On the other hand, when the differences in the infection prevalence values of each sex of fish are compared, there were statistically significant differences between Sinop and Balaklava samples in terms of the prevalence values of *C. merlangi, M. gadi* and their co-existence in female fish while only *M. gadi* showed significant difference in male fish in these sampling localities ($P < 0.05$) (Table 4).

Discussion

Myxosporean parasites are among the most diverse parasitic groups of fish all around the world and a total of 69 species have so far been identified from fishes in the Black Sea (Özer et al. 2015a, b). Members of the genera *Ceratomyxa* and *Myxidium* have a world-wide distribution and parasitise a wide range of fish species, usually infecting the gall bladder of their hosts (Eiras 2006; Eiras et al. 2011). Significant pathology associated with gall bladder myxosporeans in general, but with particular reference to those infecting gadids, was reported by Kabata (1967) and Feist and Bucke (1992) as a result of atrophy, hypertrophy and increased thickness of the gall bladder wall and loss of contractility of the bladder, with the likely final outcome of a heavy infection causing complete functional elimination of the bladder. Skuratovskaya et al. (2013) reported significant decreases of spleenosomatic index and increases of hepatosomatic index in whiting *M. merlangus* with average and high intensity of infection with *Ceratomyxa merlangi* and *Myxidium gadi* along with a nematode *Hysterothylacium aduncum*. *Ceratomyxa merlangi* and *M. gadi* have been reported from the gall bladder of whiting in the Black Sea (Zaika 1966; Karataev and Iskov 1984; Yurakhno 1994, 1997, 2004, 2008, 2009; Skuratovskaya et al. 2010, 2011, 2013; Özer et al. 2015a, b). In the present study, individual infection prevalence values of each parasite species in Balaklava samples were higher than those in Sinop samples and the differences were statistically significant. However, the case was reversed in co-infection prevalence values of both parasite species (see Table 1). Seasonal infection prevalence ranges of each parasite species also indicated higher values in Balaklava samples than those in Sinop samples (see Table 2). Previously reported infection prevalence values were between 7% and 47% for *C. merlangi* and 30% and 63% for *M. gadi* in the northern coasts of the Black Sea including where this study was conducted by Yurakhno (1994, 1997, 2008). Our results in Balaklava samples suited well in the ranges mentioned above, however, there are no previous reports for both parasite species in the southern coasts of the Black Sea would enable us to make comparison in the infection prevalence values in this region. This study provided the first data on the co-infection of both parasite species at both localities in the Black Sea.

Myxosporean parasites of freshwater fishes comprise two-stage life cycles, with a myxosporean stage in fish and an actinosporean stage in invertebrate alternate hosts. However, in marine myxosporeans of fishes, both alternate life cycles with two hosts (Køie et al. 2007, 2013) including two *Ceratomyxa* species involving polychaetes as alternate hosts (Køie et al. 2004, 2008) and a direct fish-to-fish transmission

Table 2 Levels of infection of *Ceratomyxa merlangi, Myxidium gadi* and co-existing *C. merlangi + M. gadi* in whiting, *M. merlangus,* from Sinop and Balaklava Bay of the Black Sea during 2011–2014; N = number of fish examined, CI – 95% confidence intervals based on Stern's exact score limit given in parentheses, SE - Standard Error

Season/fish length/sex	N	Fish Length ± SE	*Ceratomyxa merlangi* Prevalence (%) CI	*Myxidium gadi* Prevalence (%) CI	*C. merlangi + M. gadi* Prevalence (%) CI
Sinop Samples					
Seasons					
Summer (June–August) (20.4–26.1 °C)	26	15.5 ± 0.2	0	0	0
Autumn (September–November) (15.2–21.4 °C)	222	15.5 ± 0.2	21.2 (16.1–26.9)	16.2 (11.8–21.7)	10.4 (6.9–15.0)
Winter (December–February) (8.3–13.1 °C)	222	15.2 ± 0.1	23.4 (18.2–29.4)	17.6 (13.0–23.1)	14.0 (9.8–19.1)
Spring (March–May) (9.5–17.2 °C)	220	15.0 ± 0.1	25.9 (20.4–32.2)	23.6 (18.3–29.7)	18.6 (13.8–24.2)
Fish Length Classes					
<15 cm	323	-	18.9 (14.8–23.5)	14.6 (11.0–18.8)	10.5 (7.5–14.3)
15–18 cm	326	-	25.2 (20.6–30.1)	23.3 (18.9–28.2)	17.2 (13.3–21.7)
>18 cm	41	-	34.1 (20.5–50.0)	26.8 (15.3–42.6)	14.6 (6.5–29.0)
Fish Sex					
Male	229	14.5 ± 0.2	23.1 (18.0–29.0)	13.1 (9.1–18.0)	10.5 (6.9–15.2)
Female	461	15.6 ± 0.1	22.3 (18.7–26.4)	21.3 (17.6–25.2)	15.6 (12.5–19.2)
Balaklava Bay Samples					
Seasons					
Summer (June–August) (21.7–25.2 °C)	40	15.1 ± 0.2	10.0 (3.4–23.4)	10.0 (3.4–23.4)	2.5 (0.1–13.3)
Autumn (September–November) (13.9–21.6 °C)	97	18.6 ± 0.3	33.0 (24.1–43.2)	35.1 (26.1–45.3)	12.4 (7.0–20.5)
Winter (December–February) (8.2–11.0 °C)	80	17.0 ± 0.2	33.8 (24.1–44.9)	40.0 (29.7–51.2)	12.5 (6.6–21.7)
Spring (March–May) (8.4–16.4 °C)	206	15.7 ± 0.2	26.7 (21.0–33.2)	24.8 (19.1–31.2)	6.3 (3.5–10.6)
Fish Length Classes					
<15 cm	117	-	16.2 (10.5–24.2)	29.9 (22.1–38.8)	6.0 (2.8–11.8)
15–18 cm	181	-	27.6 (21.5–34.7)	27.6 (21.5–34.7)	8.8 (5.4–14.0)
>18 cm	125	-	39.2 (30.7–47.9)	28.8 (21.4–37.5)	12.0 (7.0–19.1)
Fish Sex					
Male	69	14.8 ± 0.2	14.7 (7.8–25.5)	39.9 (20.6–42.8)	5.8 (2.0–14.2)
Female	354	16.9 ± 0.2	30.4 (25.7–35.4)	28.2 (23.6–33.0)	9.3 (6.6–12.8)

have been proven (Diamant 1997; Redondo et al. 2002). Køie et al. (2007) reported a two-host life cycle for a Gadimyxid myxosporean species in two gadid fish species, namely *Gadus morhua* L. and *Arctogadus glacialis* (Peters). However, there is no report indicating how the infection processes of *C. merlangi* and *M. gadi* are completed. Based on above mentioned reports on the life cycle strategies of marine myxosporean parasites, and the infection levels of both parasite species found in whiting at two distinct localities of the Black Sea, we can assume that both parasite species may have the same type of life cycle strategy, either alternate or direct fish-to-fish transmission. On the other hand, we should keep in mind that both myxosporean species may undergo actinosporean development within invertebrate hosts as was speculated by Køie et al. (2004) for marine myxosporean parasites which have fish-to-fish transmission. The sabellid polychaetes are known to act as invertebrate hosts to three members of the 'marine clade', *C. shasta, C. auerbachi,* and *Parvicapsula minibicornis* (Bartholomew et al. 1997, 2006; Køie et al. 2013), and it is likely that members of this cosmopolitan filter-feeding family, which is common in the Black Sea (Kurt Şahin and Çınar 2012), play an important role as

Table 3 Comparative statistical differences in prevalence of *Ceratomyxa merlangi, Myxidium gadi* and co-existing *C. merlangi + M. gadi* in Sinop and Balaklava Bay samples relative to season, fish size and sex, *level of significance with $p < 0.05$

Parasite Fish samples	Variable	*C. merlangi* Prevalence (%)	*M. gadi* Prevalence (%)	*C. merlangi + M. gadi* Prevalence (%)
Sinop	Seasons	0.508	0.112	0.045*
	Length classes	0.031*	0.008*	0.044*
	Sex	0.847	0.009*	0.079
Balaklava Bay	Seasons	0.028*	0.002*	0.085
	Length classes	0.000*	0.907	0.280
	Sex	0.008*	0.662	0.485

Table 4 Comparative statistical differences in prevalence of *Ceratomyxa merlangi, Myxidium gadi* and co-existing *C. merlangi* + *M. gadi* between Sinop and Balaklava Bay samples relative to season, fish size and sex; * level of significance given as $p < 0.05$

Variable	*C. merlangi* Prevalence (%)	*M. gadi* Prevalence (%)	*C. merlangi* + *M. gadi* Prevalence (%)
Seasons			
Spring	0.912	0.821	0.000*
Summer	-	-	-
Autumn	0.034*	0.000*	0.697
Winter	0.077*	0.000*	0.850
Length classes			
<15 cm	0.578	0.000*	0.193
15–18 cm	0.598	0.286	0.011*
>18 cm	0.585	1.000	0.787
Sex			
Female	0.010*	0.026*	0.008*
Male	0.176	0.002*	0.347

invertebrate hosts for both *C. merlangi* and *M. gadi* myxosporeans. Moreover, despite similar water temperature values in summer at both sampling localities, no myxosporean species were detected in Sinop samples as a possible result of the alternate host be different species in these places and this would be related to the absence of the alternate host in summer months probably because of the raise in water temperature.

Environmental factors, mainly temperature, influence the myxosporean life cycle, and that specific physical, chemical and biological conditions are necessary for the successful development of parasites (Rocha et al. 2011). In the present study, infection prevalence values of *C. merlangi* and *M. gadi* in the bile showed marked seasonality throughout the year, summer being the least preferred season, as was previously reported in the Black Sea by Yurakhno (1994, 1997). Rocha et al. (2011) reported a temperature dependent developmental stages and infection prevalence of *Triangulamyxa psittaca* from the Amazon River. They also reported that the infection prevalence values are higher when the temperature is higher. A time period from 4 to 25 days has been reported for the longevity of actinospores, depending on temperature and species by Markiw (1992), Yokoyama et al. (1993) and Xiao and Desser (2000). Several authors reported decreases in life-span of actinosporeans with increasing temperature (Yokoyama et al. 1993; Özer and Wootten 2002). We can assume that null infection value in summer in Sinop samples and a very low value in Balaklava samples in the same season could be resulted from lower infection period, if the life cycle models of *C. merlangi* and *M. gadi* involve two alternate stages.

Lom and Dykova (1992) reported more susceptibility to myxosporean infections in younger fish and more accumulation with age. Hallett et al. (1997) explained this observation with longer exposure to infections rather than differences in their susceptibility. A significant relationship was observed in the present study where the larger size groups of whiting had gradually increasing infection prevalence than the smaller size group. This suggests either that fish have acquired more parasites with age or that the parasites have proliferated with time within their host as was recommended by Hallett et al. (1997) due to positively correlated prevalence of *Kudoa ciliatae* infections and length of Indo-Pacific whiting *Sillago maculate* in their study.

Physiological, morphological and behavioral differences between females and males could operate to create a slight but consistent sexual bias in infection levels (Poulin 1996). However, such consistent bias is not apparent in myxosporean infections of fish; studies on the effects of host sex on myxosporean infections are quite rare and the data available do not show any preference to either of host fish sex, except myxosporean species specifically infecting gonads (Alvarez-Pellitero and Sitja-Bobadilla 1993; Özer 2003; Azevedo et al. 2011). Özer (2003) reported similar prevalence values of individual *Sphaerospora elegans* and *Myxobilatus gasterostei* infections at both sexes of *Gasterosteus aculeatus* in the Black Sea. Azevedo et al. (2011) also reported relatively similar infection prevalence values of *Myxidium volitans* in female (38.5%) and male (28.0%) *Dactylopterus volitans* in the Atlantic coasts of Brazil. In our study, prevalence values of individual parasite species did not show any consistently significant differences between sex of host fishes, on the other hand, co-existing prevalence values of both parasites had no significant differences between sexes of host fish at both sampling localities. Along with above mentioned findings in the present study, only few cases showing males were more sensitive than females to these parasites at both sampling localities which revealed that this sex difference could be irrelevant for our parasite species (Poulin 1996).

Myxosporean parasites are among the well-suited indicators of fish stock discriminations (Kabata 1963, 1967; Khan and Tuck 1995; Larsen et al. 1997; Cantatore et al. 2016). Kabata (1963, 1967) used *Leptotheca, Sphaeromyxa, Ceratomyxa* and *Myxidium* to identify different stocks of their whiting hosts based on variations in the geographical distributions of the parasites as they are site-specific and concluded that whiting occupied the northern and southern North Sea were two separate stocks, based largely on the dominant prevalence of *Ceratomyxa arcuata* in the north and *Myxidium sphaericum* in the south. Khan and Tuck (1995) reported *Myxidium gadi* as a good indicator of stock discrimination of Atlantic cod, *Gadus morhua,* on the continental shelf off Newfoundland, Canada. Cantatore et al. (2016) also used two myxosporean species, namely *Kudoa rosenbuschi* and *Fabespora* sp., for the discrimination of northern and southern stocks of the Argentina hake *Merluccius hubbsi* in the Southern Atlantic. Here in the present study, the differences in the infection values of each parasite species and their co-existences at both sampling localities in the northern and southern coasts of the Black Sea were statistically significant possibly and this possibly indicates two different whiting stocks and the differences in infection prevalence values may have resulted from the difference in fish stocks.

Whiting feed on benthic organisms including worms and small crustaceans and, since many freshwater myxosporean species have been found to have alternate life cycle stages in oligochaetes and marine myxosporeans with direct fish-to-fish transmission or alternate life cycle involving marine polychaetes, the different levels of *C. merlangi* and *M. gadi* infections in whiting may be related to their proximity to either infected fish hosts or invertebrate hosts. Knowledge of the epidemiology of myxozoan parasites is important in elucidating and understanding the life cycle of myxozoan parasites, either for two host life cycle or fish-to-fish direct life cycle. Thus, conducting such long term studies with comparable data is so valuable to make contribution to our current knowledge about their infection processes. Further studies should also be performed in order to elucidate the actual life cycle strategies of both myxosporean parasite species.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and institutional guidelines for the care and use of animals were followed.

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