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Seasonality and the microhabitat of *Microcotyle sebastis* Goto, 1894, a monogenean gill parasite of farmed rockfish, *Sebastes schlegeli* Hilgendorf, 1889

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조피볼락의 아가미에 기생하는 단생흡충 *Microcotyle sebastis* Goto, 1894의 계절별 감염 및 기생 특성

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A total of 353 farmed rockfish, *Sebastes schlegeli* Hilgendorf, 1880 were sampled from 3 localities on the Korean coastline over a 12 month period. Full parasitological examination revealed the polyopisthocotylean monogenean *Microcotyle sebastis* Goto, 1894 to be abundant with infections consistently reaching over 90~100% prevalence throughout the year. A seasonal pattern in parasite abundance on the gills is evident, with the population peaking twice, the largest in winter and again in summer. While the parasitic load on the left and right gills was not dissimilar, parasites within the gill sets were found to favour settlement on the II and III gill arches. A comparison of parasite abundance with host length, revealed that the smaller length fish classes (<17 cm standard body length) had significantly heavier infections than those of larger fish (>17 cm standard body length). This report represents the first record of *Microcotyle sebastis* on farmed rockfish in Korean coastal waters.

Key words : Monogene, Gill parasite, Rockfish, *Sebastes schelegel, Microcotyle sebastis*

Introduction

Korean marine fish culture has developed and expanded quickly since its inception in 1986. Rockfish, *Sebastes schlegeli* Hilgendorf, 1880 culture started in 1988, and to date its production has increased 300 fold and is set to rise further. Fish farms are typically situated close together and the high density of culture conditions is conducive for the propagation of disease agents. The problems most frequently encountered tend to be attributed to bacterial and viral agents (Bang et al., 1991 ; Kim & Lee, 1992). Ecto-parasitic Monogenea however, often cause significant problems in commercial aquaculture systems and have been implicated in causing disease and mortality of fishes (Paperna et al., 1984 ; Das and Pal, 1987 ; Harris et al., 1994 ; Ogawa et al., 1995). The polyopisthocotylean monogenean, *Microcotyle sebastis* Goto, 1894 is the one of the most common gill parasites of rockfish (Thoney, 1986 ; Stanley et al., 1992). Previous study by two of the author (Yoon and Jo, unpublished observation) suggested that heavy infections of parasites combined with high seasonal water temperatures may contribute to observed mass mortalities of fish during high water temperatures. Choi et al. (1996) reported the occurrence of *M. sebastisci* Yamaguti, 1958 on *S. schlegeli* cultured on the south coast of Korea. Their survey represents the only known species of this genus reported in native waters. Our finding of *M. sebastis* Goto, 1894, therefore represents the first record of *Microcotyle sebastis* in Korea. The present study focuses on the population level and microhabitat of *M. sebastis* parasite on its host, *Sebastes schlegeli* collected from 3 sea cage sites throughout one year.

Materials and Methods

Sampling was carried out at 3 different cage cites around Korean coastline (Fig. 1). Fish were first sampled in winter (December in 1995) from cages. These fish were transferred from hatcheries and stocked at the end of summer (August) in 1995. Average standard length of the fish were 5 cm when they were stocked into sea cages, here they enter 2 year growing cycle with random grading as required. Sampling started from December 1995 to November 1996 inclusive. Thirty rockfish (*Sebastes schlegeli*) were collected randomly by hand net at 3 different sea cage localities for each season (winter (Dec.- Feb.), spring (Mar.- May), summer (June- Aug.) and autumn (Sep.- Nov.) (Fig. 1). Collected fish were transported in polythene bags containing oxygenated water to the Pukyong National University, Pusan, Korea. Fish were held in static tanks in seawater (30-33 ppt) brought directly from the collection site and aeration was maintained throughout. All fish were examined within 48 hours following their collection. The standard length of fish was recorded prior to any parasitological examination and sorted into 4 different size length classes (class 1=less than 13.0 cm, class 2=13-15 cm, class 3=15-17 cm, and class 4=greater than 17 cm). Fish were killed by a blow to the cranium and then the nerve cord was severed. The operculum was removed and the gill arches sequentially excised and examined for parasites. The total number of parasites on each hemibranch was recorded for each gill arch before placing the gill arch into a 50 ppm solution of MS 222 (tricaine methane sulfonate). The use of a parasite narcotic such as MS 222 assisted the dislodgement of parasites from the arches. Any parasites still remaining attached to the gill filaments were carefully teased out using a mounted tungsten needle and a paintbrush. Specimens were recounted to confirm the number of each species per gill arch before they were transferred into fixative. The parasites were passed briefly through Berland's fluid to allow relaxation of the tissue before specimens were stored in 10% neutral buffered formalin or in 80% ethanol.

Fig. 1. Farmed rockfish, *Sebastes schlegeli* Hilgendorf 1880 sample sites.

The terms used throughout this study are consistent with those defined in Margolis et al. (1982) - "Abundance : total number of individuals of a particular parasite species in a sample of hosts divided by the total number of individuals of the examined hosts ; Prevalence : number of individuals of a host species infected with a particular parasite species divided by the total number of host examined. Intensity : range of individuals of a particular parasite species in each infected host in a sample., and Mean intensity : total number of individuals of a particular parasite species in a sample of a host species divided by the number of infected individuals of the host species in a sample".

The seasonality of the infection, the parasite numbers in each microhabitat on the gills and the relationship between the host size and the parasite population were analysed statistically. Data were not normally distributed and therefore, the data for each season and each gill arch were analysed using the non-parametric statistics tests Dunn's and Tukey.

Results

The polyopisthocotylean monogenean was identified as *M. sebastis* Goto, 1894 using keys of Bonham and Guberlet (1937), Yamaguti (1963) and Beverley-Burton (1984). The key features identifying this as *M. sebastis* were 24~42 testes, 25~29 clamps arranged in two symmetrical lines, and sclerite morphology in accordance with -the drawings presented in Beverly-Burton (1984).

The prevalence of infection was high throughout the sampling period with a 100% infection during both the winter and summer seasons. The mean intensity and abundance data is presented in Table 1. It showed differences in the parasite population at different times of the year. The mean intensity (41.9± 30.3) and abundance (41.9) of parasites peaked in winter, and were significantly higher than those of the other seasons ($P<0.05$). Parasite numbers were observed to peak again in summer when the mean intensity and abundance of the parasite was 23.8± 18.3 and 23.8 respectively. Summer infection figures were significantly higher than the figures obtained for autumn and winter ($P<0.05$) but were not significantly different for the fish sampled in spring.

The parasite load was heavier in fish in the smaller length classes. The mean intensity of the parasite in the host size class I, II and III were 26.3± 19.8, 26.1± 20.5 and 27.3± 30.0 respectively. The mean intensity of classes I~III were similar and significantly higher than that of size class IV (12.5± 8.9) ($P<0.05$). Summary statistics often obscure real effects or trends in the data, but by using the median among others, it can be seen from Table 2 that the parasitic load was indeed lighter on fish exceeding 17.0 cm long. Parasite numbers on fish exceeding 17 cm in length were significantly different ($P<0.05$) from those on the other size classes of fish.

The data was further analysed separating the fish into the 4 size classes. Table 3 shows the parasite burden for each size class for each season. There were no significant differences among the size classes I,

Table 1. The seasonality of the gill parasite, *Microcotyle sebastis* on the gills of farmed rockfish, *Sebastes schlegeli*

Season	Host (n)	Total parasite number	Intensity	Median	Mean intensity	Abundance	Prevalence (%)
Winter	85	3561	3~156	34.0	41.9± 30.3	41.9	100.0
Spring	90	1484	0~ 67	14.5	18.1± 12.2	16.9	93.2
Summer	90	2138	1~ 88	19.0	23.8± 18.3	23.8	100.0
Autumn	90	1142	0~ 68	9.0	13.9± 11.9	12.7	91.1

Winter=Dec.~ Feb., Spring=Mar.~ May, Summer=June~ Aug., Autumn=Sep.~Nov.

Table 2. The population distribution of the gill parasite, *Microcotyle sebastis* on different size classes of farmed rockfish, *Sebastes schlegeli*

Season	Host (n)	Total parasite number	Intensity	Median	Mean intensity	Abundance	Prevalence (%)
Class I	124	3161	0~ 88	22.0	23.3± 19.8	25.5	96.8
Class II	95	2375	0~120	18.0	26.1± 20.5	25.0	95.8
Class III	84	2213	0~156	16.5	27.3± 30.0	26.3	96.4
Class IV	50	576	0~ 40	8.5	12.5± 8.9	11.5	92.0

Table 3. The parasite burden of the gill parasite, *Microcotyle sebastis* for each size class and for each season

Season	Size class	Host (n)	Total parastic number	Intensity	Mean intensity	Prevalence (%)
Winter	Size I	50	1659	3~85	33.2± 21.1	100
	Size II	27	1161	1~2120	18.1± 12.2	100
	Size III	8	741	17~156	92.6± 47.6	100
	Size IV	0	0	0	0	0
Spring	Size I	56	888	0~67	15.8± 12.1	94.5
	Size II	29	561	0~51	19.3± 14.2	93.1
	Size III	1	34	34	34	100
	Size IV	2	18	0~18	9.0± 12.7	50
Summer	Size I	13	520	6~88	40.0± 249	100
	Size II	29	505	1~49	17.4± 12.5	100
	Size III	40	988	1~80	24.7± 17.7	100
	Size IV	8	125	5~30	15.6± 9.0	100
Autumn	Size I	5	71	0~35	14.2± 15.2	80
	Size II	9	154	0~68	17.1± 20.8	77.8
	Size III	36	484	1~57	13.4± 12.1	91.2
	Size IV	40	433	0~40	10.8± 9.1	92.5

II and III, even though the size class III had more parasites than those of size classes I and II in winter and spring. This can be explained by a small sampling size in winter and spring. Statistical analysis however, showed a significant difference in the parasite level for size class IV.

The mean intensity and prevalence of the parasite on the left and right gill arches were found not to be significantly different ($P>0.05$). A breakdown of infection data, as presented in Table 4, reveals that the parasitic load on gill arches as II and III were significantly higher than that on gill arch I ($P<0.05$). The mean intensity and abundance of gill arch IV was significantly lower than those of the other gill arches. The parasite mean intensity on the gill arches, as shown in Fig. 2, was found to be independent of seasonality.

Discussion

In the present study, population numbers were found to peak twice over the sampling period, once in winter and again in summer. This is markedly different to the pattern of prevalence and abundance of *M. sebastisci* reported by Choi et al. (1996). From Choi et al.s survey, covering spring (April) to autumn (Oct.), the prevalence of *M. sebastisci* was highest in the months of April - May (c. 70%) and lowest in the summer months of June and July (c. 0%). Ogawa (1988) found that *Bivagina tai* on the gills of red sea bream, *Pagrus major* peaked three times over a two year study period peaking in both spring seasons and the intermediate

Table 4. Microhabitat of the gill parasite *Microcotyle sebastis* on the farmed rockfish, *Sebastes schlegeli* (all seasons combined) (n=353)

Gill arch	Total parasite number	Intensity	Mean intensity	Abundance	Prevalence (%)	Sub total
Left I	1014	023	4.53.7	2.9	63.7	
Left II	1267	024	4.73.9	3.6	77.1	
Left III	1426	029	5.44.6	4.0	75.1	
Left IV	332	012	2.72.2	0.9	34.6	4039
Right I	1045	020	4.63.5	3.0	64.9	
Right II	1430	024	5.44.0	4.1	75.4	
Right III	1512	027	5.34.3	4.3	80.5	
Right IV	368	018	3.02.7	1.0	35.1	4355

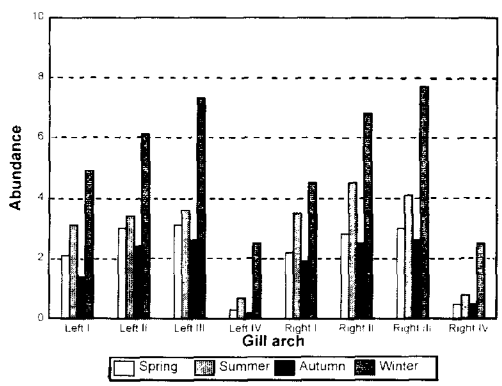

Fig.2. The seasonal changes in abundance of the gill parasite *Microcotyle sebastis* Goto, 1894 on the gills of the farmed rockfish *Sebastis schlegeli* Hilgendorf, 1880.

winter season. He suggested that the winter peak resulted from a lowered resistance by the host to infection at low water temperatures, whilst higher temperatures contributed to a shorter incubation time of the egg and a shorter maturation time of the parasite on the host. Nie and Kennedy (1991) found that the seasonal dynamics of *Pseudodactylogyrus anguillae* on the European eel *Anguilla anguilla* was closely linked with water temperature. The manner in which water temperature affects parasite population dynamics has been investigated by a number of researchers (Gelnar, 1987 ; Scott and Nokes, 1984 ; Kamiso and Olsen, 1986). Briefly, their results concluded that firstly, water temperature directly stimulates parasite development and reproduction, and secondly, it indirectly changes the immunological resistance of the host. In the present study, the parasite burden was highest in the winter and lowest in autumn suggest a combined effect of a lowered resistance of the host and an increased longevity of the oncomiracidia and adult parasites. The second, though slightly smaller, peak in summer may be explained by an increased rate of egg production, rapid developmental time of the egg and increased activity of the oncomiracidia. Even though the settlement of the oncomiracidia may be considerably less than populations settling in the winter season, the rapid rate of egg production and the sheer number of oncomiracidia being released, results in an increase in the number of parasites successfully finding the host. Further study is required to determine the optimum temperature range of this parasite and the range of temperatures that induce changes in the immunological response of the host.

Gorbunova (1936) distinguished three groups of parasites infecting a host : a) parasites independent of the age of their host ; b) parasites decreasing in abundance with the age of their host. and c) parasites increasing in abundance with the age of their host. Indeed, Winch (1983) found that the gill parasite *Atrispinum labracis* on the sea bass *Dicentrarchus labrax* did not begin to infect fish until they were at least 3 years of age. Fischer and Kelso (1990) also found that the prevalence and mean intensity of the parasite, *Posthodiplostomum minimum* from the bluegill, *Lepomis macrochirus* and the largemouth bass, *Micropterus salmoides* increased with increasing host length. The result of this study however, clearly indicates that there is a decrease in parasite intensity with the age/length of the host. The precise reason why smaller fish were more heavily infested by parasites is not clear and requires elucidation. This phenomenon may indicate a developing immunity correlated with the age of the host . Alternatively, there may be a difference in the behaviour of small fish (*i.e.* schooling, which may increase the chances of parasite transmission) and larger fish (*i.e.* solitary or territorial), or in the ability of the parasite to maintain its attachment on its host. The efficiency of attachment being greater on smaller fish, where the parasite is able to span the distance between individual gill filaments (*i.e.* morphological restrictions). Further, it may be more difficult for parasites on larger fish to obtain a blood meal. Further insight into the relationship between this particular species of *Microcotyle* and its host are required with parallel studies on wild fish.

In the present study, there was no difference in the parasite numbers on the right and left gills. It was, however, found that *M. sebastis* occurred more on gill arches II and III than on gill arches I and IV. Wiles (1968) found that *Diplozoon paradoxum* on *Abramis brama* favoured gill arches I and II. Dzika and Szymanski (1989) found that *D. auriculatus* preferred the first gill arch with lowest numbers of worms on the third gill arch. Further, Koskivaara et al. (1992) found that the abundance of *Dactylogyrus* species on the gill arches of the roach, *Rutilus rutilus* were highest on the third gill arch and lowest on the first gill arch. Site specificity of the parasite is often presumed to result from active site selection by the parasite (Anderson et al., 1993). Very little however, has been done previously to determine whether the rate of gas exchange is equal over all the gill arches and/or whether the arrangement of the gills in some species are such that more of the respiratory current passes over some gill arches more than others. Paling (1968) attempted to estimate the different volumes of water flowing over the four pairs of gills in the brown trout *Salmo trutta.* His experiments led him to conclude that the majority of the water current was over the second and third gills, a reduced proportion over the first pair of gills and the smallest proportion across the posterior most or the fourth pair of gills. Similarly, Wootten (1974) stated that the greatest volume of water passed over the second gill and the third gill arches in the ruffe, *Gymnocephalus cernua.* This observation mirrored the distribution of *Dactylogyrus amphibothrium* on the gills, where the majority attached to the second and third gills. This distribution of the parasite coincides with the greater proportion of water passing across these gills and it is apparent that *Dactylogyrus amphibothrium* did not actively avoid these gill arches where the flow of water is greatest. His results however, markedly contrast those of Paling (1969) who found that *Discocotyle sagittata* actively avoided strong water currents flowing though the two middle sited gills but this parasite species was found in their largest numbers on the first gill arch. These findings confirmed Suydam's (1971) suggestion that the direction of the ventilating current may influence the position of monogeneans on the gills. Hence, the predomination of *M. sebastis* on the II and III gill arches of the rockfish may merely reflect the greater surface area available for the parasite to attach to on these gills or the greater volume of water passing over them.

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