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Occurrence of *Bivagina tai* (Monogenea: Microcotylidae) on the Gills of Cultured Red Sea Bream *Pagrus major*

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Occurrence of a gill monogenean *Bivagina tai* was monitored monthly on a single stock of red sea bream *Pagrus major* artificially spawned and cultured in a net cage. The monogenean was found from the fish stock all the time except for the first few months, significantly in greater numbers on the first gills than on the others.

The infection fluctuated with seasons. There were three peaks of infection during the two-year study period. The O+ fish had the highest level of infection in winter, but the infection was modest in the next winter, suggesting the peak infection resulted from lowered resistance of the small fish to infection at low temperatures. A second and a third peak of infection were formed in May, when the fish was just one and two years from hatching; the rise of water temperature was probably responsible for the peak formation.

From the seasonal changes in the development of *B. tai*, using the clamp number as an indicator, it is deduced that the monogenean had three, possibly four, generations a year. The life-span is estimated as 3–5 months in winter and as 2–3 months in spring through autumn.

Monogeneans generally propagate rapidly on the host fish in warm water seasons. However, a microcotylid *Bivagina tai* is found on the gills of cultured red sea bream *Pagrus major* in much larger numbers in winter than in the other seasons. O+ fish is vulnerable to the attack of the monogenean, while older fish seldom have problem in the monogenean infection even in winter.

The host fish, kept in the same way as they are cultured commercially, was regularly monitored for *B. tai* from fry for two years. This study has primarily been focussed on the seasonal abundance of the monogenean in culture systems, but some of its ecological aspects have also been investigated.

Materials and Methods

The host fish in this study is the same stock as in a previous paper.[1] 20 individuals from the stock in the net pen were randomly sampled once a month. All the gills of fresh samples were either examined under a stereo-microscope for parasites, which were prepared for permanent stained specimens, or fixed directly with 10% formalin for subsequent examination, in which parasites recovered were mounted in glycerin-alcohol. The number of parasites was recorded on each individual fish (a total of 2,561 parasites from 318 infected fish). The number of clamps was counted of all the parasites from 20 fish each month, except for damaged and lost ones during preparation.

Of about 1,100 fish at the start of the experiment, 491 were examined for *B. tai* and 418 remained at the end of it. The rest, about 190 in number, may have been dead or lost of unknown reasons during the study period, though mortalities due to *Bivagina* infection or any other diseases had not been found. Concurrent infection with diplectanid monogeneans *Lamellodiscus* spp. on the gills seemed to produce no or very little effect on *Bivagina* infection.

The fish were 317 g in weight and 25.0 cm in fork length in average at the end of study. Water temperature fluctuated between 10 and 28°C during the sampling period (Fig. 1).

Results

Monthly Fluctuations in the Prevalence of Infection and in the Mean Parasite Number per Fish

The *Bivagina* population among the red sea bream stock fluctuated seasonally (Fig. 1). A shift of the culture site from one place to another in the bay in November 1984 (indicated by a big

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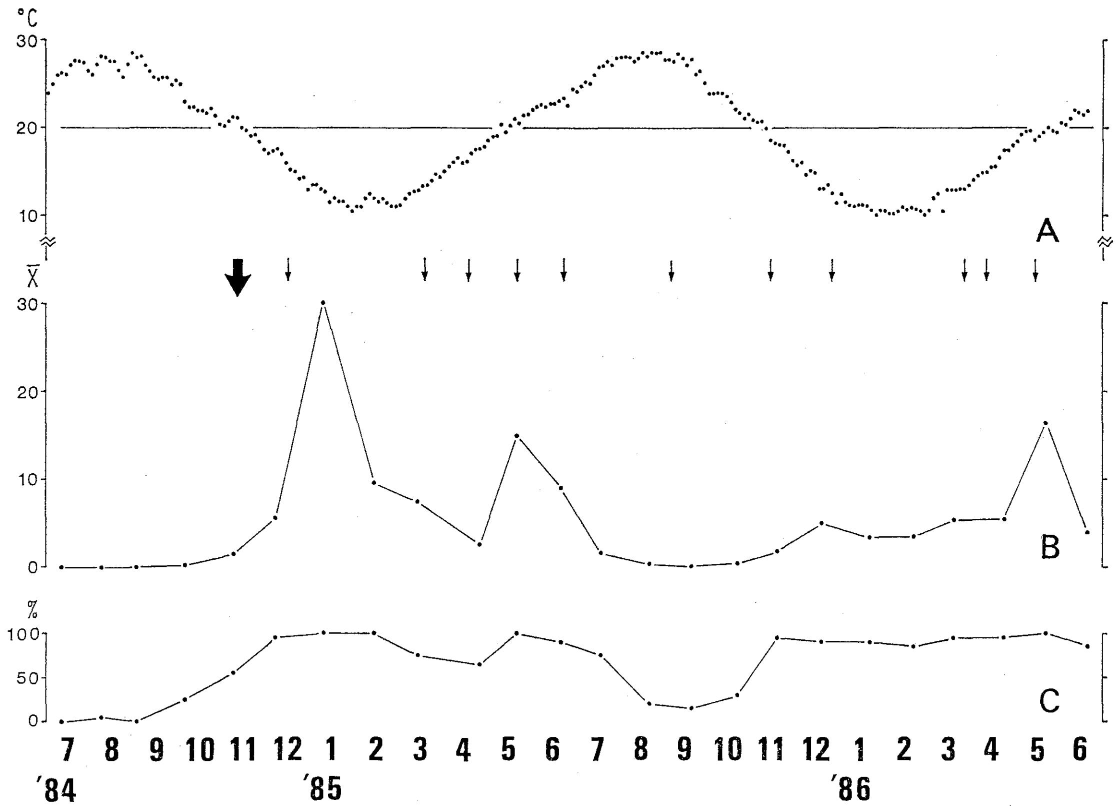

Fig. 1. Water temperature and seasonal fluctuations of *Bivagina tai* infection during the study period. A: water temperature, B: mean number of parasite per fish, C: prevalence of infection. Small and large arrows indicate change of a net cage and shift of a net cage in the bay, respectively.

arrow in Fig. 1) and frequent changes of the net (indicated by small arrows in Fig. 1) due to propagation of fouling organisms on the net seem to have no obvious influences on the infection.

The monogenean was first found on one of the fish examined in August 1984, two months after the transfer from a tank to the net cage. The prevalence of infection and the mean number of parasites per fish increased until January 1985, when the infection was in the highest level; all the fish were infected, with the mean and the highest number of parasites per fish being 29.9 and 99, respectively. Then, the infection level decreased until April. In May 1985, another peak of infection was formed, followed by a sharp decrease in the infection level toward summer. The 1+ fish had a high prevalence of infection (more than 85%) throughout the second winter, but the mean number of parasites stayed as low as less than 5 per fish. With increasing water temperature, a third peak of infection was formed in May 1986, and a rather sharp decrease in the infection level followed next month.

Monthly Fluctuations in the Population Size of B. tai *and in its Composition of Developmental Stages*

In order to know the seasonal changes in the infection in more detail, monthly changes in the composition of the developmental stages of *B. tai* are figured (Fig. 2).

In January 1985, when the infection was most severe, two peaks of frequency, at clamp number 0–4 and around 100, appeared. The presence of many larvae indicated that a large number of oncomiracidia successfully attached to the host fish. As the adult population (clamp number more than 80) disappeared toward April, new oncomiracidial invasions occurred in March–May, and the larvae became matured in May–June. In summer, the parasite population size was so small that no clear peak appeared. The parasite population grew again in winter, but this time it was much smaller than last year. In spring, another wave of oncomiracidial invasion occurred,

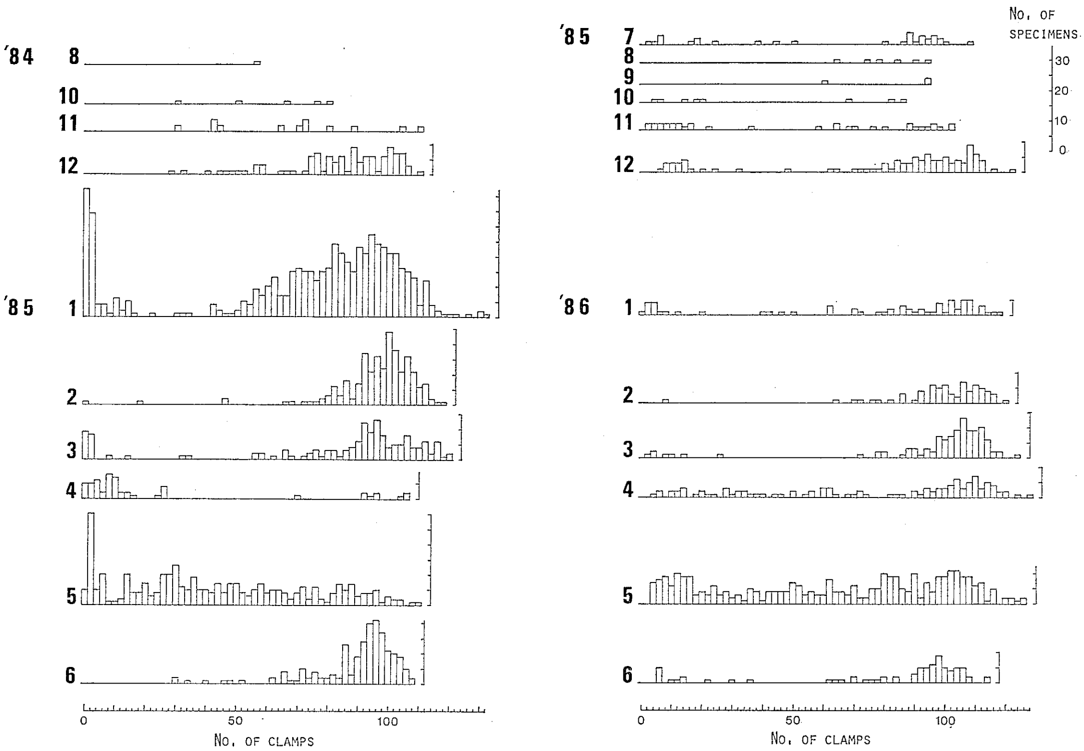

Fig. 2. Monthly changes in the frequency distribution of the clamp number of *Bivagina tai.*

Table 1. Site preference of *Bivagina tai* on the gills of the host

	No. of parasites	No. of parasites on gills I	II	III	IV	χ^2 (3 d.f.)	P
All samples	2270	855 (37.7)	518 (22.8)	449 (19.8)	448 (19.7)	199.87	<0.001
In relation to:							
Fish size; —14.9 cm	185	70 (37.8)	19 (10.3)	52 (28.1)	44 (23.8)	29.08	<0.001
15.0—19.9 cm	1125	448 (39.8)	266 (23.6)	182 (16.2)	229 (20.4)	144.42	<0.001
20.0— cm	960	337 (35.1)	233 (24.3)	215 (22.4)	175 (18.2)	59.62	<0.001
Season*; Dec.–Feb.	954	341 (35.8)	229 (24.0)	194 (20.3)	190 (19.9)	62.60	<0.001
Mar.–May	993	396 (39.9)	219 (22.1)	190 (19.1)	188 (18.9)	119.67	<0.001
Jun.–Aug.	273	96 (35.2)	61 (22.4)	58 (21.2)	58 (21.2)	15.13	<0.01
Infection level;							
Jan. & May 1985, May 1986	1125	423 (37.6)	266 (23.6)	202 (18.0)	234 (20.8)	102.54	<0.001
Feb. & Jun. 1985, Jun. 1986	431	149 (34.6)	100 (23.2)	108 (25.0)	74 (17.2)	26.92	<0.001
Development (Jan. 1985);							
Immature	154	60 (39.0)	25 (16.2)	15 (9.7)	54 (35.1)	37.32	<0.001
Adult	376	145 (38.6)	102 (27.1)	64 (17.0)	65 (17.3)	46.87	<0.001

Parentheses represent percentages.
* Data in Sep-Nov are not listed here because of small sample size (N: 50).

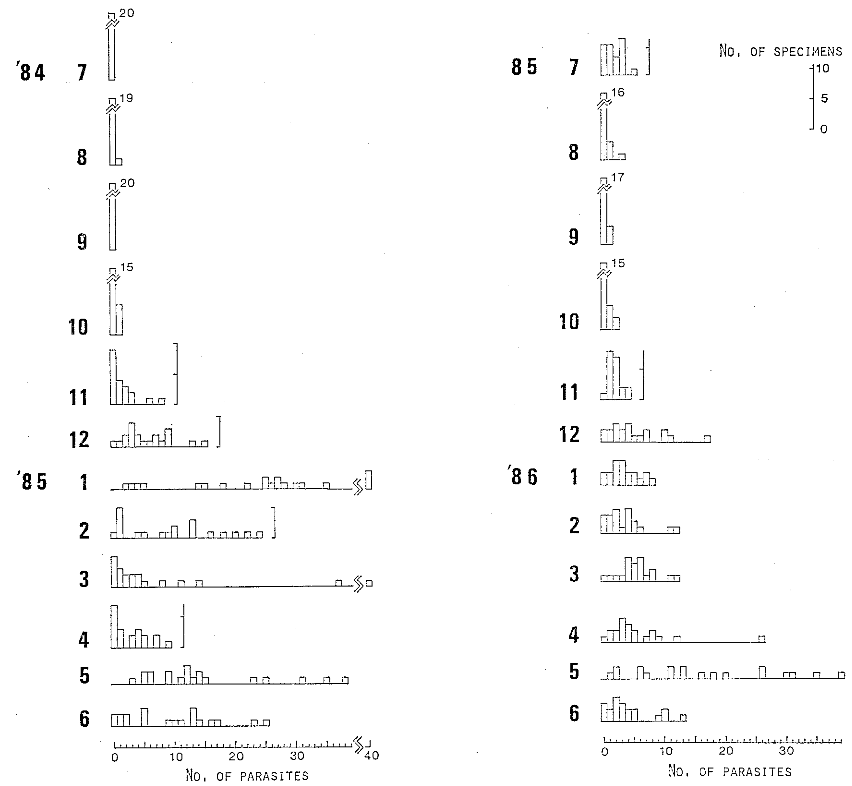

Fig. 3. Monthly changes in the frequency distribution of the parasite number of 20 fish.

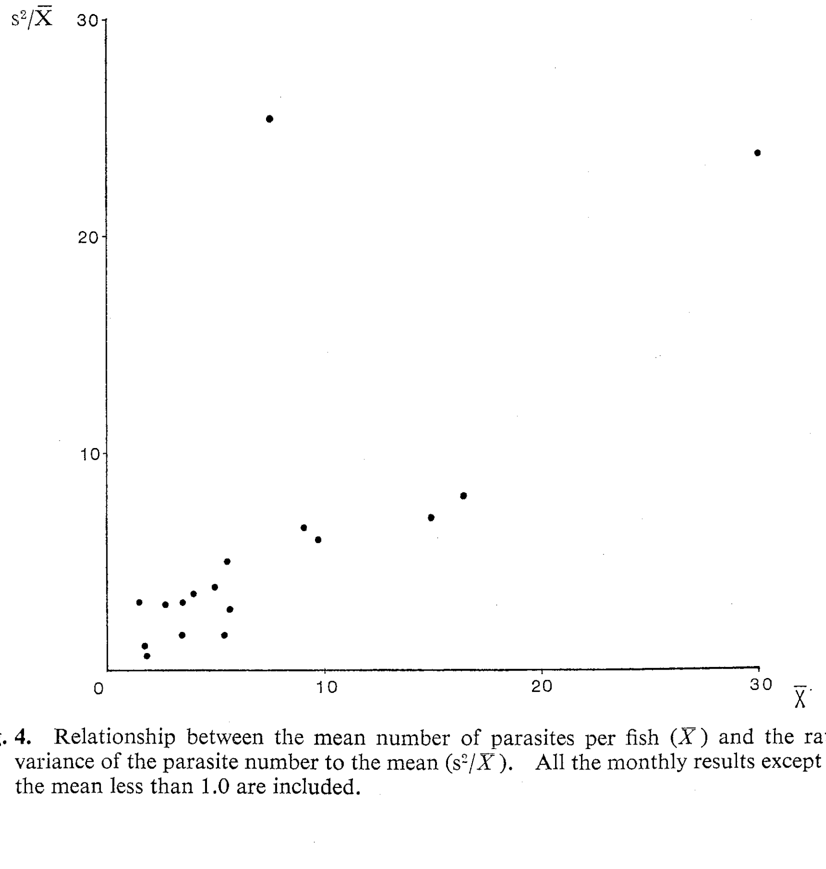

Fig. 4. Relationship between the mean number of parasites per fish ($\bar{X}$) and the ratio of the variance of the parasite number to the mean ($s^2/\bar{X}$). All the monthly results except ones with the mean less than 1.0 are included.

resulting in forming a small peak of the adult population in May–June.

Site Preference of B. tai *on the Gills of the Host*

During the study period, out of 379 fish, in which the infection site on the gills was investigated, 285 (75.2%) were infected with *B. tai* and a total of 2,270 parasites were recorded of their infection sites, except ones whose infection sites were not determined. Parasites were found in almost equal numbers from both sides of the gills; of 2,270, 1,162 were collected from the right gills and 1,108 from left, with no significant difference by means of the χ^2 test ($P>0.1$). Results of analyses in the site preference on the four gills (right and left gills combined) are listed on Table 1. Based on all the specimens, they were not randomly distributed over the four gills ($P<0.001$), and the first gill is the most preferable site of attachment. There seemed no apparent change in the site preference in relation to host size and seasons. A comparison is made of the preference between the three peak months (January and May 1985 and May 1986 combined) and their next months (February and June 1985 and June 1986 combined). In the former, many immature parasites were included (stage I and II[1]), while, in the latter, most of the parasites collected were adults (see Fig. 2). Greater number of parasites were recorded on the third gill and smaller number on the fourth in the next months. In January 1985, when the highest peak of infection was formed, parasites recovered were divided into immature forms and adults. Immature parasites were mostly collected from the first and the fourth gills. In adults, the proportion of the recovery from the fourth gill decreased, but that from the first gill stayed almost the same. Apparently, these results suggest that the site preference change according to the development of the monogenean.

Distribution of B. tai *among the Host Population*

Monthly frequency distributions of the number of parasites on the hosts exhibit a wide variability in the number of parasites on individual hosts, especially in months when the mean number of parasites per fish ($\bar{X}$) was more than five (Fig. 3). For example, in January 1985, all the fish were infected with 2–99 monogeneans per fish ($\bar{X}=29.9$), showing an overdispersed distribution among the host population. This tendency is more clearly demonstrated in Fig. 4. There seems to be a positive correlation between the mean parasite number and the ratio of the variance of the parasite number to the mean.

Discussion

Several types of site preference of monogeneans on the gills of host have been reported. Of these, some polyopisthocotyleans are recovered in greater numbers from the first gill than from the other: *Diclidophora merlangi* on *Merlangius merlangius*,[2] *Discocotyle sagittata* on *Salmo trutta*,[3] *Pseudothoracocotyla indica* on *Scomberomorus commensoni*[4] and *Kuhnia scombri* on *Scomber scombrus*[5] (all these data were based on more than 100 parasite specimens). In the present study, *B. tai* showed this type of preference under any circumstances (Table 1). The proportion of the number of parasites appears to change as *B. tai* develops. This may be because of a disproportionate rate, among the four gills, of probable elimination of parasites by the host (see the last paragraph about the host's elimination of parasites).

The *Bivagina* population among the present bream stock showed an overdispersed distribution, which was especially clear when the infection level was high (Fig. 4). No further analysis has been made here, because of many unestimated factors in the culture condition.

During the two-year observation, there were three peaks of infection. The first winter, when the highest peak occurred, was very different from the second in terms of the infection level. A comparative examination was made in February 1986 of ten 0+ breams from a different stock in a neighbouring net cage; all the fish were infected with *B. tai*, with the number of parasites per fish ranging from 2 to 52 and a mean of 22.8. This was much higher than that of the present stock at that time (1+; 60% infected with a mean of 3.5), but similar to that of the present stock of last year (0+). It is, thus, likely that the infection in winter is generally intensive among 0+ fish but modest among 1+ fish. The egg hatching will take as long as about three weeks at that time of year (10–13°C),[1] but the eggs were laid in great numbers in a field observation made in January.[6] The intensive infection of 0+ fish in winter was probably due to lowered resistance to oncomiracidial invasions at the low temperature period. The other two peaks both occurred in May, when the water temperature was increasing to about 20°C. The increase of temperature must be an essential factor to facilitate the growth of *Bivagina* population; at least it contributed to a shorter incubation time of the egg and to a shorter maturation time on the host. The infection level drastically decreased in summer. The optimal temperature for propagation appears to be 20°C and below. A higher temperature is probably unfavorable for the propagation of the monogenean because of the lowered hatching rate at 30°C.[1] Thus, it seems obvious that vulnerability of the host and water temperature are major factors determining the level of infection. It will be concluded that the winter propagation of *B. tai* among 0+ fish was related to the host's vulnerability and the spring propagations to water temperature.

From the seasonal changes in the developmental stages of *B. tai* (Fig. 2), it will be deduced that there are three (possibly four) parasite generations a year: a winter (December-March), spring (March–June) and summer-autumn generation. In summer–autumn, there may be multiple generations because of higher temperatures, though it has not been made clear. The respective life span will be estimated as 3–5 months for the winter generation and as 2–3 months for the spring and summer–autumn generation. Several species of polyopisthocotyleans, to which *Bivagina tai* belongs, have been studied on the life span (*Diplozoon paradoxum*,[7] *Mazocraes alosae*,[7] *Polystoma integremum*[7] and *Discocotyle sagittata*[8]), and it has been widely accepted that they generally have a life span of more than a year. The relative shorter life span in *B. tai* may apparently be resulted from the elimination of the monogenean by the host, as was suggested in the monopisthocotylean *Tetraonchus* infection of cultured *Oncorhynchus masou*.[9] Then, perhaps the life-span will become longer, unless the elimination by the host works. The *Bivagina* infection is very different in many points between culture systems and natural environments. For example, a number of *Bivagina* eggs were found entangled in the meshes of culture nets,[6] but the eggs will be dispersed into a wide area in natural wateıs. No data have been available on the *Bivagina* infection of feral breams, and it cannot exclude the possibility that the rather quick turnover of generations is observed only among cultured fish populations.

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