Comparison of Sampling Methods for Estimating Population Structure of the Freshwater Crab, *Geothelphusa dehaani,* and Infection Levels with Lung Flukes

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Abstract

The relative accuracies of methods for sampling populations of the freshwater crab, *Geothelphusa dehaani,* were compared in respect of the size (carapace width) distribution of the crabs and its effect on estimates of infection levels of metacercariae of lung flukes. Whereas samples collected by a quantitative quadrat method comprised a large number of small individuals, showing clear peaks in width distribution, and a relatively small number of large individuals, those collected by the conventional pick-up method comprised mainly larger individuals. When the two distributions were superimposed and adjusted in relation to the number of individuals in the larger size class of the distribution, significant differences in numbers were observed in the size classes smaller than 16 mm. This result suggested that small crabs tended to be missed in collections by the pick-up method and the proportion of the crabs missing was estimated as 95% of the population. It was shown that the method of sampling can affect estimates of parameters, such as prevalence and intensity, of infection with lung flukes. To standardize methods of estimation and to permit valid comparisons of infection levels in different localities, it is proposed that parameters be quoted only for crabs above a threshold value of size. The threshold is that above which the samples are truly representative of the structure of the parent population.

Key words: *Geothelphusa dehaani, Paragonimus* infection, sampling method, size distribution

Introduction

The freshwater crab, *Geothelphusa dehaani,* is widely distributed throughout the main islands of Japan except in Hokkaido, and is known to serve as the second intermediate host of the lung flukes *Paragonimus westermani* and *P. miyazakii.* Prevalence of lung flukes in the crab populations has been estimated by several authors and values may reach as high as 80% (Ito and Mochizuki, 1975; Hayashi *et al.*, 1977; Shibahara, 1982; Gyoten, 1983). Prevalence can be influenced by many environmental factors, both biotic and abiotic, but, on the other hand,

the sampling method is also believed to have a considerable influence on the estimated values of the infection parameters. In general surveys the crabs are usually collected by a method whereby stones and rocks in and around a stream are moved and crabs in the water or sand are picked up by hand (Kanamori, 1977; Yamaguchi and Takamatsu, 1980; Gyoten, 1983). This method will, for convenience, be referred to as the "pick-up" method in the present study. If a sample taken by this method is truly collected at random, then the estimated infection parameters may accurately reflect the real infection levels in the population. This method, however, has a tendency to be relative in that relatively larger crabs are preferentially sampled. Since crab size is positively correlated with age, it is to be expected that the size distribution in a representative sample will show a large number of small individuals and a small number of large in-

dividuals, as a result of age-dependent mortality. Prevalence values in samples taken by the pick-up method may not therefore necessarily reflect those of the original crab population but only the size composition of the samples. To compare infection levels of a lung fluke in different localities, a sampling method that produces accurate and representative estimates of prevalence and abundance of metacercariae is required.

In the present study, the size structures of crab samples collected by different methods were compared together with estimates of the prevalence and abundance of the fluke infection. Data were further analyzed with special reference to the relative accuracy of the samples and the extent to which they are representative of the original crab population.

Materials and Methods

Sampling sites

Surveys were carried out at two localities in Shizuoka Prefecture with different infection levels with lung flukes; i.e. Dojima, Kawane-cho, and near Tadaki, Mikkabi-cho. Kawane is well known as the locality for the crab that caused the first human case of *P. miyazakii* (Hayashi *et al.*, 1974) and a high level of infection has been maintained there (Kino *et al.*, 1985). Surveys were carried out twice, in June and September 1989. Mikkabi is a small branch stream of the Kawanamiya River, flowing down into Lake Hamana. Preliminary surveys revealed that the crabs in this area were infected with lung flukes at levels as low as 10 to 20%. Sampling was carried out during March, April and November 1989. Environmental conditions in the two localities are similar: surrounded by natural secondary forest, the streams have sandy beds with stones and rocks, the water temperature being as high as approximately 20°C during mid summer and as low as below 5°C in early spring.

Collection of crabs

In order to sample crabs in a manner that reflects the population structure, a quantitative quadrat method was employed. A wire quadrat

1 m^2 was placed in a site where the composition of the bed appeared representative of the whole stream. Every crab within the quadrat was collected, after first removing stones and rocks and then after sieving bed sand to a depth of approximately 20 cm through a wire mesh. The procedure was repeated 2 or 3 times in a survey.

Crabs were also collected by the pick-up method around the quadrat site for approximately 1 hr at each sampling. In addition to large crabs, small individuals were also sought for and collected.

A trap method was also employed at Mikkabi in November to compare the efficiency of the method and the resultant size distribution of crabs with results from the other methods. The traps used were small, wire-framed, mesh traps with a mesh size of 5 mm. Fish viscera were put in the trap as bait. Three traps were set in the stream and kept overnight. All crabs trapped and gathered around the traps were taken to the laboratory for examination.

Examination of crabs

After the sex and the maximum carapace width of each crab were determined, the crab viscera were pressed between two glass plates and examined for metacercariae under a dissecting microscope. The remainder of the crab was then minced with a homogenizer at 1200 rpm for approximately 1 min. The homogenate was sieved with water through mesh screens (upper coarse mesh #16 and lower fine mesh #60). Material remaining on the lower screen was washed into a plastic cup and then examined for metacercariae under a dissecting microscope. The total number of metacercariae obtained from the two procedures was taken as the number parasitizing the crab.

Results

Results of the surveys are summarized in Table 1. Data from the surveys at Mikkabi in March and April were combined in a single set, as the number of crabs collected on each occasion was small and the intervals between surveys were short. Although the total numbers of crabs

Table 1 Summary of results of the surveys of *G. dehaani* conducted by different methods.

Method	Month	Location	No. of samples	No. of crabs				Carapace width (mm)			Density
				examined	female	male	unknown	Mean	Max.	Min.	(No./m²)
Quadrat	Mar.–Apr.	Mikkabi	8	223	69	31	123	8.9	27.4	4.1	27.9
	June	Kawane	3	182	37	22	123	7.8	23.4	4.1	60.7
	Sept.	Kawane	2	39	22	17	0	10.5	21.9	5.4	19.5
Pick-up	Mar.–Apr.	Mikkabi	–	119	72	46	1	19.4	28.6	8.4	–
	June	Kawane	–	19	10	9	0	19.8	24.0	12.3	–
	Sept.	Kawane	–	72	36	36	0	19.4	26.4	7.4	–
	Nov.	Mikkabi	–	51	32	19	0	24.0	30.5	14.0	–
Trap	Nov.	Mikkabi	5	94	56	38	0	25.0	30.3	19.7	–

collected varied between samples and there were many small individuals whose sex could not be determined due to lack of sexual morphological differences, the number of females exceeded that of males in all samples except for that obtained by the pick-up method at Kawane in September. However, since the size (mean and range) and the infection rate of females did not differ from those of males, data from the sexes were combined for the following analyses. As is evident in Table 1, mean carapace widths of the crabs sampled by the quadrat method were smaller than in those sampled by the pick-up and the trap methods. This is due mainly to the presence of small individuals in the quadrats with a carapace width of 4 to 5 mm. In the samples taken by the quadrat method, the density of the crabs could be determined as mean number of crabs per unit area. The density varied depending upon place and season, with the highest value observed at Kawane in June.

Since the size:frequency distribution of crabs in every sample taken by the same method showed a similar pattern, even though collected in different seasons, the data from the surveys were combined for each method and then used for analysis. The size:frequency distribution of crab samples taken by the quadrat method showed several peaks in the smaller size classes: each peak probably represents a cohort or sub-population of a different age (Fig. 1a). Small crabs constitute a large part of the sample: the

proportions of crabs with a carapace width of 10 mm or less in 3 samples, for example, were 75.3%, 83.0% and 66.7% (average 77.8%). The samples taken by the pick-up method, by contrast, showed a different pattern of size:frequency distribution with peaks at sizes larger than 20 mm (Fig. 1b). Crabs collected by the trap method showed a similar pattern of size:frequency distribution to those by the pick-up method (Fig. 1c).

It is assumed that the difference in results between the methods was due to non-random, size-relative sampling by the pick-up and trap methods, such that smaller crabs in the population were missing from the samples, and that the distribution revealed by the quadrat method more correctly represented the original population structure. On the basis of these assumptions, the size distribution patterns in samples taken by the quadrat and pick-up methods were compared. If randomness is satisfied in respect of collecting larger crabs, the two distributions should have a similar pattern in the larger size classes at the tails of the frequency distributions, despite actual differences in numbers of crabs. If the size:frequency distribution of crabs taken by the pick-up method can be adjusted to that shown by the quadrat method, the magnitude of the portion of the population missed by the pick-up method can be determined. The procedure for adjustment and calculated values are summarized in Table 2. After crabs had been divided into size classes
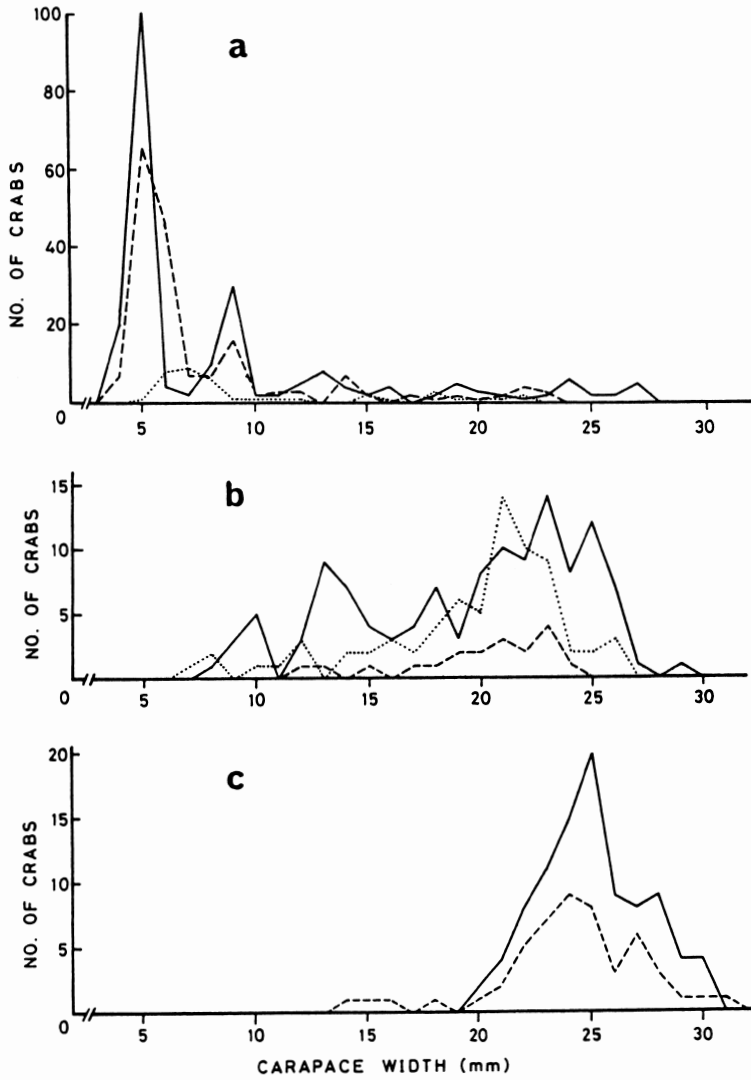
Fig. 1   Size distributions of crabs collected by the quadrat method (a) and the pick-up method (b). The solid line represents the sample at Mikkabi, the broken line at Kawane in June and the dotted line at Kawane in September. Size distributions of crabs at Mikkabi in November (c) collected by the trap method (solid line) and the pick-up method (broken line) are also shown.

of 2 mm intervals, the cumulative number of crabs, starting from the largest size class, and the ratios of cumulative numbers between the two methods were calculated for every size class. A correction factor, 0.32, which was determined in the range of the ratios so as to make the total value of X^2 smallest, and the crab number in each class for the pick-up method was then

multiplied by this value. The statistical difference (D) was calculated for each class using the equation

$$D_i = (E_i - O_i)^2 / E_i,$$

where E and O are, respectively, the expected value obtained by the quadrat method and the observed value obtained by the pick-up method. The X^2 values were then calculated as the

Table 2 Adjustment and the comparison of size distributions of *G. dehaani* collected by two different methods.

| C.W.*	Quadrat		Pick-up		Ratio	Recalculated No.	D	ΣD	d.f.	Significant level
(mm)	$N^{(1)}$	$\Sigma N^{(2)}$	$N^{(3)}$	$\Sigma N^{(4)}$	(2)/(4)	$(3) \times 0.32$	$((5)-(1))^2/(1)$	(X^2)		(P)
26≤	7	7	12	12	0.583	3.84	1.43	1.43		
24−25	8	15	25	37	0.405	8.00	0.00	1.43	1	−
22−23	12	27	48	85	0.318	15.36	0.94	2.37	2	−
20−21	10	37	42	127	0.291	13.44	1.18	3.55	3	−
18−19	14	51	23	150	0.340	7.36	3.15	6.70	4	−
16−17	8	59	13	163	0.362	4.16	1.84	8.54	5	−
14−15	17	76	16	179	0.425	5.12	8.30	16.85	6	<0.01
12−13	17	93	17	196	0.474	5.44	7.86	24.71	7	<0.01
10−11	11	104	7	203	0.512	2.24	6.98	31.68	8	<0.01
8− 9	70	174	6	209	0.833	1.92	66.21	97.89	9	<0.01
6− 7	76	250	1	210	1.190	0.32	75.36	173.26	10	<0.01
4− 5	194	444	0	210	2.114	0.00	194.00	367.26	11	<0.01

*: Carapace width
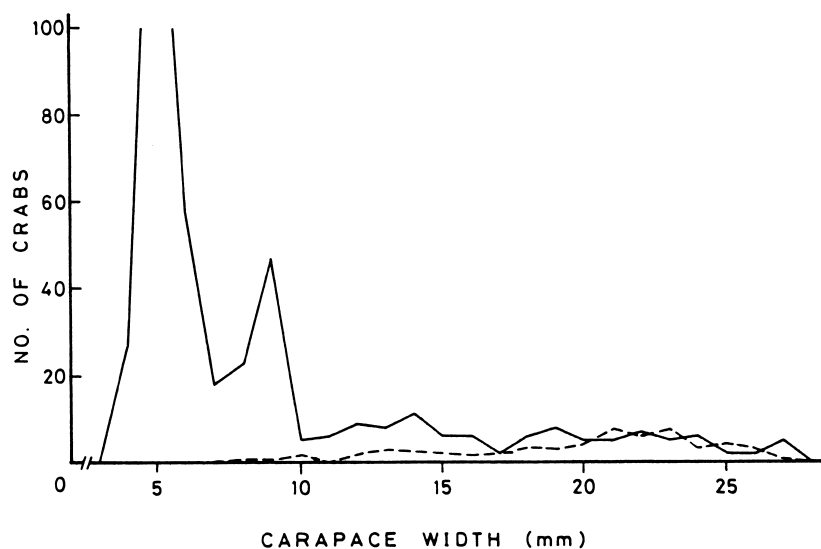
Fig. 2 Adjusted size distributions of crabs collected by the quadrat method (solid line) and the pick-up method (broken line).

cumulative sums of D starting at the largest class and assuming that no significant difference could be revealed by a X^2 test, since fundamental distribution pattern shown by the pick-up method should be identical to that of the quadrat method. Significant differences were observed for the classes of 14–15 mm and smaller, implying that it was the crabs smaller than 16mm in size that were missing from the pick-up samples. The missing individuals constituted 95% of the total when compared with the quadrat method (Fig. 2). A similar result was obtained when the size classes were further divided into 1 mm intervals.

Each of the grouped samples was divided into 2 classes with respect to crab size around a threshold of 15 mm and the infection levels of

Table 3 Comparison of infection levels of *G. dehaani* with lung flukes in different size classes and in relation to three sampling methods.

Date	Location	Method	No. of crabs			Infection rate (%)			Mean No. of mc		
			≥16mm	<16mm	Total	≥16mm	<16mm	Total	≥16mm	<16mm	Total
Mar.–Apr.	Mikkabi	Quadrat	34	189	223	14.7 NS	0.5	2.7	0.21 NS	0.01	0.04
		Pick-up	87	32	119	31.0	0.0	22.7**	0.40	0.00	0.29**
June	Kawane	Quadrat	16	166	182	93.8 NS	13.3	20.3	3.00 NS	0.25	0.49
		Pick-up	16	3	19	93.8	66.7	89.5**	2.19	1.33	1.84**
Sept.	Kawane	Quadrat	9	30	39	66.7 NS	20.0	30.8	1.89 NS	0.20	0.59
		Pick-up	60	12	72	88.3	41.7	80.8**	3.58	0.67	3.10**
Nov.	Mikkabi	Trap	94	0	94	37.2 NS	—	37.2 NS	0.65 NS	—	0.65 NS
		Pick-up	49	2	51	26.5	0.0	25.4	0.84	0.00	0.80 NS

NS: Not significant
** : $P < 0.01$

crabs in each class were then compared in samples taken by different methods (Table 3). When the total numbers, i.e., crabs of all sizes, were compared, prevalence and abundance of infection (expressed as the mean number of metacercariae per crab) differed significantly between methods ($P < 0.01$). Among crabs larger than 15mm, however, there were no significant differences in either prevalence or intensity ($P > 0.05$). All crabs collected by the trap method were larger than 15 mm and there were no significant differences in infection levels of crabs obtained by this and by the pick-up method.

The relationships between size of crabs, grouped into 2 mm intervals, and prevalence (Fig. 3a) and abundance of infection (Fig. 3b) showed a tendency for values to increase with increasing size. Prevalence in small crabs and absolute values of abundance were higher in Kawane, reflecting the high infection level overall.

Discussion

Previous investigations on the population structure and life history of *Geothelphusa dehaani* have been mainly focussed on its juvenile stages (Minei, 1976; Kanamori, 1977; Yamaguchi and Takamatsu, 1980; Hara, 1983; 1984). According to Minei (1976), eggs hatch in late summer and juvenile crabs with an average size of approximately 4 mm leave the mother crab a week or more after hatching. They molt once or more during the rest of the year and then overwinter without molting. Growth recommences in spring accompanied by several molts in a year, depending on food supply and/or water temperature. Young crabs mature at the age of 4 years old on average and the females lay eggs in summer. The life span of this species has been estimated to be 7 to 8 years. This developmental process produces a series of peaks in size distribution in a population. As a result of synchronized annual reproduction, every year class creates a discrete peak in the size:frequency distribution of young crabs (Kanamori, 1977; Hara, 1983). In the samples taken by the quadrat method, one or two peaks were seen at sizes smaller than 10 mm in different samples (Fig. 1a). These peaks presumably represent crabs aged 0 and 1 year, and the differences between samples represent the seasonal growth of juvenile crabs. The peaks gradually become obscured, with a lower frequency and wider range in older crabs due to age-dependent mortality and variation in the number of molts. On the whole, the distribution of any population exhibits a pattern of decline in numbers with increasing individual size. Although previously the quadrat method has
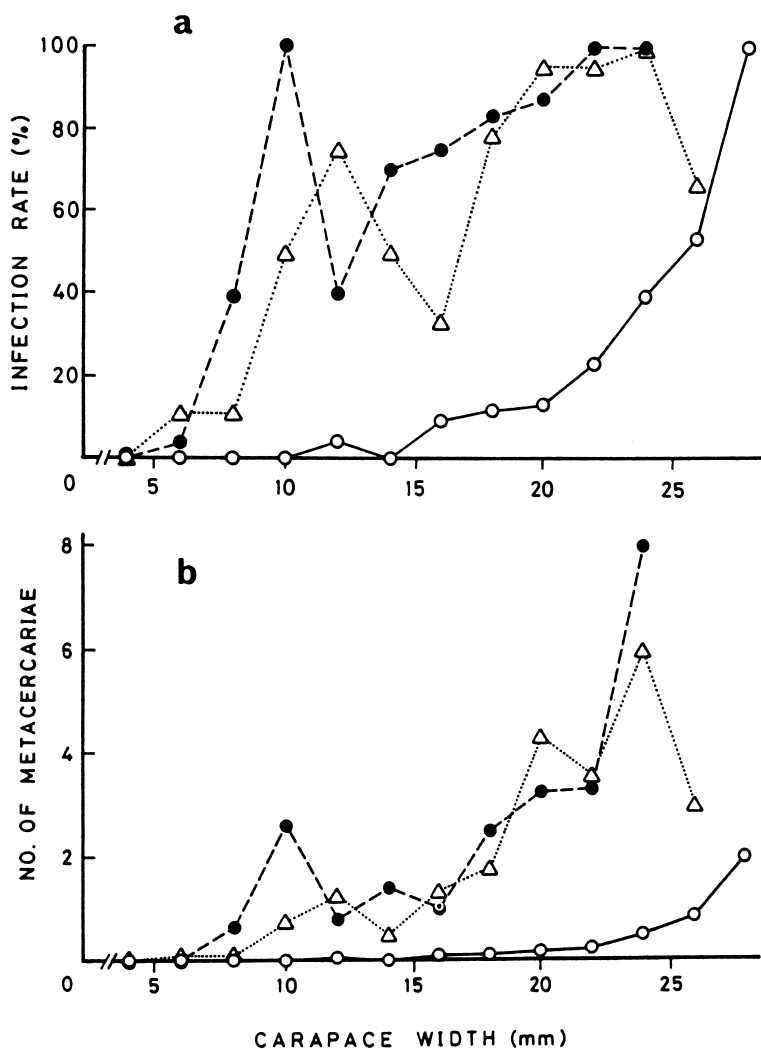
Fig. 3   Relationships between the size of crabs and prevalence with lung fluke (a) and mean number of metacercariae of lung fluke per crab (b). Solid line represents the sample at Mikkabi, broken line at Kawane in June and dotted line at Kawane in September.

mainly been used to estimate the density of various plants and animals in ecological surveys, it has already proved to be a satisfactory method of sampling *G. dehaani* (Hara, 1983; 1984), and the results of the present samples indicate the further suitability of the method for the study of population structure in crabs.

While the overall size structure of the crab population appeared to be accurately represented in the samples taken by the quadrat method, other biotic factors can affect or even distort the representative nature of samples. Differences in densities at Kawane between June and September may reflect a change in behavior of juvenile crabs after growing season (Minei, 1976). Distribution patterns of crabs related to changes in habitat selection may also affect the age composition of a sample and so its representative nature. The habitat of juvenile crabs is restricted to water, whereas adult crabs tend to leave the water but

return there again during the reproductive season. Adult crabs often construct and inhabit burrows at some distance from the water (Yamaguchi and Takamatsu, 1980), and since sampling was undertaken only in the stream itself, the number of large, adult crabs may have been underestimated. The sex ratio in favor of females in the samples may also be explained by different behavioral patterns of males related to differential habitat selection. However, since crabs are distributed within the stream in a uniform or slightly aggregated pattern (Hara, 1984), the samples were believed to be representative of the crab population within the stream itself.

When sampling with the pick-up method, the smallest size of crabs collected will vary from person to person in relation to their experience and skill and will also may be influenced by the population density of the crabs: at low density there is a great likelihood of collecting many more small crabs than at high density. If a lung fluke does not parasitize all age classes of the crab equally or randomly, such biases in samples could significantly affect and distort estimates of parasite prevalence. Thus age (size) dependent infection rates are an important factor to be considered in sample estimates of population infection levels.

A size-dependent increase in infection levels has frequently been reported for *P. miyazakii* infection in *G. dehaani* (Ito and Mochizuki, 1975; Hayashi *et al.*, 1977; Gyoten, 1983), for *P. kellicotti* in crayfish (Stromberg *et al.*, 1978), and for *P. westermani* in *Eriocheir japonicus* (Suzuki, 1958; Nakagawa, 1960) and in *G. dehaani* (Shibahara, 1982). In general, changes in parasite abundance with host size are more evident than changes in prevalence. In the present study, the tendency for infection levels to increase with host size was evident in both localities (Fig. 3). This is primarily due to the cumulative mode of parasite acquisition by the crab host: parasites are long-lived and can be infected throughout their life span. Samples consisting only of larger, and hence older, individuals should thus exhibit higher infection rates than those of the natural population from which they were drawn. The proportion of the population of flukes in the smaller crabs, missing from the pick-up samples, will affect the prevalence estimates but may be less important in estimates of total number of metacercariae. If sampling is conducted merely in order to collect a number of metacercariae for experimental use, for example, the pick-up method would have the advantage since it requires less effort and has a high efficiency. The trap method was also shown to be efficient for the collection of large crabs: its tendency to select larger crabs than by the pick-up method is due probably to a wider range of foraging activity by the large crabs. It is, however, pertinent to note that even small crabs, less than 10 mm, were often infected (Fig. 3a). Thus, when overall prevalence is high, any size-dependent change in infection rate may become obscured.

In order to compare accurately the abundance and/or prevalence of flukes in time and/or space, a quantitative method of sampling such as the quadrat method is clearly preferable. However, such a method is very demanding of time and effort and so can not always be employed in a rapid, general survey. A solution to this problem would be to determine, and then standardize upon, an optimum threshold of crab size: estimation and comparison of infection levels would only be carried out on crabs above this size. The present results suggest that for *G. dehaani* a size of 15 mm in carapace width would be an appropriate threshold. This value could, however, be considered to be too small when using the trap method, since no individuals smaller than 19 mm were collected (Fig. 1c). This suggests that crabs between 15 and 20 mm were under represented in the samples, and implies that a higher threshold value should be preferred. Although a higher threshold would decrease the number of crabs available for analysis, and so estimates of infection level would become less reliable, in one respect, the validity of the sample would undoubtedly improve, in other respects. Thus, on the premise that a number of crabs can be collected, a threshold value of 20 mm, as Gyoten (1983) adopted, might be preferable as it permit the use of any of the sampling methods employed in this study.

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