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THERSITINA GASTEROSTEI (PAGENSTECHER, 1861) (Copepoda : Ergasilidae) infecting the stickleback *Pungitius pungitius* L. at Chalk Marshes, Gravesend, Kent.

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SUMMARY. 10-spined sticklebacks, *Pungitius pungitius*, taken from a drainage dyke at Chalk Marshes, Kent, between October 1979 and July 1980 were infested with adult females of the parasitic copepod *Thersitina gasterostei*. Relative density of the overwintering parasite population fell during the winter months to a minimum in April ; however during this winter period the proportion of ovigerous females rose, reaching 100 % in April. Young infective adults were first seen in May, and the peak of infection by the first summer generation occurred in June. The parasite was found to be overdispersed within the host population : in 8 of 10 monthly samples the negative binomial distribution was a good model for the observed data. Relative density was related to host length in only 3 of the 10 samples. Evidence is presented to suggest that infected fish are more likely than non-infected fish to recruit young parasite.

Key-words : Parasitic Copepoda. Fish. Ergasilidae. Gasterosteidae. Host-Parasite Relationships.

Infection de l'épinoche *Pungitius pungitius* L. à Chalk Marshes, Gravesend, Kent par *Thersitina gasterostei* (Pagenstecher, 1861) (Copepoda : Ergasilidae)

RÉSUMÉ. Des épinoches à dix épines, *Pungitius pungitius*, prises d'un fossé à Chalk Marshes, Kent, d'octobre 1979 à juillet 1980 étaient infestées par des adultes femelles du Copépode parasite *Thersitina gasterostei*. La densité relative de la population des parasites pendant les mois d'hiver est tombée au minimum en avril ; cependant pendant cette période la proportion des femelles ovigères a augmenté et est arrivée à 100 % en avril. Des jeunes adultes infectées ont été vues d'abord en mai, et la densité maximale d'infection par la première génération d'été est arrivée en juin. Le parasite était sur-dispersé chez la population des hôtes : dans 8 des 10 échantillons mensuels la répartition binominale négative était un bon modèle pour les données obtenues. La densité relative avait un rapport avec la longueur de l'hôte dans seulement 3 des 10 échantillons. Ceci suggère de toute évidence que des poissons infectés ont plus de chance que des poissons non parasités de contracter des jeunes parasites.

Mots-clés : Copédodes parasites. Poisson. Ergasilidae. Gasterostei. Relations hôte-parasite.

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Introduction

Thersitina gasterostei is a parasitic copepod belonging to the family Ergasilidae ; the adult female being parasitic on the inner surface of the opercula of the sticklebacks *Gasterosteus aculeatus*, *G. wheatlandi* and *Pungitius pungitius* in brackish water (Wootten, 1976 ; Kabata, 1979). Little is known of the population biology of *T. gasterostei*, although aspects of the population biology of other ergasilid species have been studied (Tedla & Fernando, 1970 ; Cloutman & Becker, 1977 ; Rawson, 1977 ; Bortone *et al.*, 1978).

This account describes a population of *T. gasterostei* infecting *P. pungitius* in a drainage dyke at Chalk Marshes, Gravesend, Kent (southeast England). Seasonal variation in prevalence and mean density (as defined by Margolis *et al.*, 1982) ; age structure ; and relationships between levels of infection and host sex, and host length are described for the period October 1979 to July 1980. In addition the dispersion pattern of the parasite population is analysed and an attempt is made to elucidate the mechanisms causing the observed distributions.

Materials and Methods

Chalk Marshes are an area of reclaimed marshland lying on the eastern edge of Gravesend, Kent (Ordnance Survey Sheet 178, Grid Reference 677736), now used as mixed agricultural land. Drainage is achieved by a system of dykes. During a survey of the parasites of the sticklebacks of Chalk Marshes carried out in late summer and early autumn 1979 *T. gasterostei* was found in only one of the dykes surveyed. Since this dyke was easily accessible it presented a good opportunity to study a population of *T. gasterostei* infesting *P. pungitius* over a period of time. The dyke is 4-5 m. in width and 1.5 m. in depth. Bottom substrate consists of rotting organic matter overlying anoxic black mud. The predominant rooted vegetation is *Myriophyllum* sp., *Lema* sp. is also abundant. Salinity measured on a single occasion was 1 .9 $^{0}/_{00}$ NaCl (estimated from a conductance of 3130 μsiemens).

P. pungitius were taken from the drainage dyke at Gravesend at monthly intervals from October 1979 to July 1980. During June and through September 1980 the sampling site became covered by a thick layer of the green alga *Enteromorpha intestinalis*, resulting in deoxygenation of the dyke. The stickleback population almost completely disappeared (through death or emigration). In August the total number of *P. pungitius* caught numbered only 10 ; in September no sticklebacks were caught ; in October only 13 fish were captured. These samples were all statistically inadequate and are therefore not recorded here. Samples were obtained by hand netting from the bank along the length of the dyke. At least 29 fish were sampled on each occasion.

The fish were killed by pithing within 3 days of return to the laboratory, having been held in aquaria in a cold room at a temperature of 8-10 °C. Left and right opercula were removed from the fish and examined for the presence of *T. gasterostei*. The numbers of parasites, and their stages of maturity were recorded, together with the host sex and length.

Individual parasites were assigned to one of 4 stages of maturity as follows. (This system is based on that of Walkey *et al.*, 1970).

I : Immature adult female : cephalothorax longer than broad.
II : Mature adult female : cephalothorax as broad as long ; distinctly swollen.
III : Ovigerous female : possessing paired egg-sacs.
IV : Ovigerous female with fully ripe (greenish-blue) eggs.

For each monthly sample the following were calculated :

1) Prevalence of *T. gasterostei* (% of hosts infected) ; 2) Relative density $(\bar{x})$ of infection per host (including all zero counts) ; 3) Age (maturity) structure of the parasite population ; 4) Spearman Rank Correlation of parasite numbers on host length ; 5) The frequency distribution of the parasite population.

Additionally, in an attempt to clarify the factors generating the observed distributions of *T. gasterostei* the method of Iwao (1970) involving regression of mean crowding $(\overset{*}{x})$, (Lloyd, 1967) on mean density $(\bar{x})$ was applied. This technique was usefully employed by Boxshall (1974b) to analyse the dispersion pattern of the parasitic copepod *Lepeophtheirus pectoralis*. The method was fully explained by Boxshall but it is briefly redescribed below :

Mean crowding $(\overset{*}{x})$, the mean number per individual of other individuals in the same sampling unit, is estimated (when the population can be described by the negative binomial distribution) by :

$$\overset{*}{x} = \bar{x} + (\bar{x}/k),$$

where $\bar{x}$ is the sample mean and k is the exponent of the negative binomial distribution, which is initially estimated approximately by :

$$k = \bar{x}^2 \,/\, (s^2 - \bar{x}),$$

where s^2 = sample variance ; and then accurately by the iterative maximum likelihood method (Bliss & Fisher, 1953).

The regression of $\overset{*}{x}$ on $\bar{x}$ is given by

$$\overset{*}{x} = a + b\bar{x},$$

where a may be termed the index of basic contagion and its value is related to the basic component of the distribution ($a = 0$, the basic component is an individual ; $a > 0$, the basic component is a positive association of individuals ; $a < 0$, the basic component is a negative association of individuals). The manner in which the basic components are distributed may be deduced from the value of b, the density contagiousness coefficient ($b = 0$, for a random distribution ; $b > 0$ for a contagious distribution ; $b > 0$ for a regular distribution).

It should be stated here that prior to this set of analyses it was found that there was never any significant difference in intensity of infection between male and female hosts ($p > 0.05$ in Mann Witney U Test), therefore the observations were pooled.

Results

SEASONAL VARIATIONS IN PREVALENCE AND RELATIVE DENSITY OF THE PARASITE POPULATION

The prevalence and relative density of infection rose from October to November, and then fell through the winter, presumably through the mortality of overwintering parasites ; minima occurred in March (prevalence) and April (relative density). During May and June the prevalence and relative density rose to a peak in June ; the observations for July showed reductions in both prevalence and relative density of infection *(fig. 1)*.

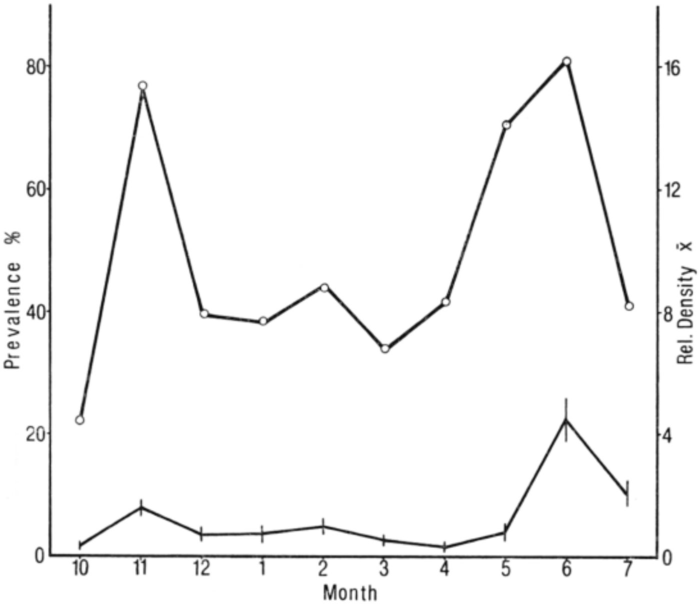

FIG. 1. — Seasonal variations in prevalence (upper line), and relative density (lower line) of *Thersitina gasterostei* infesting *Pungitius pungitius* (October 1979-July 1980). Vertical bars represent standard errors.

AGE STRUCTURE OF THE PARASITE POPULATION

Four distinct stages of maturity of adult female *T. gasterostei* have been identified and described above. The age structure of the *T. gasterostei* population changes seasonally *(fig. 2)*.

The relative density declined through the winter months ; during this period there was an increase in the percentage of ovigerous (Stage III) females (those actually bearing egg-sacs) within the population and a consequent decrease in the percentage of non-ovigerous (Stage II) females. Stage IV females first appeared in March and constituted 26.7 % of the total population at that time.

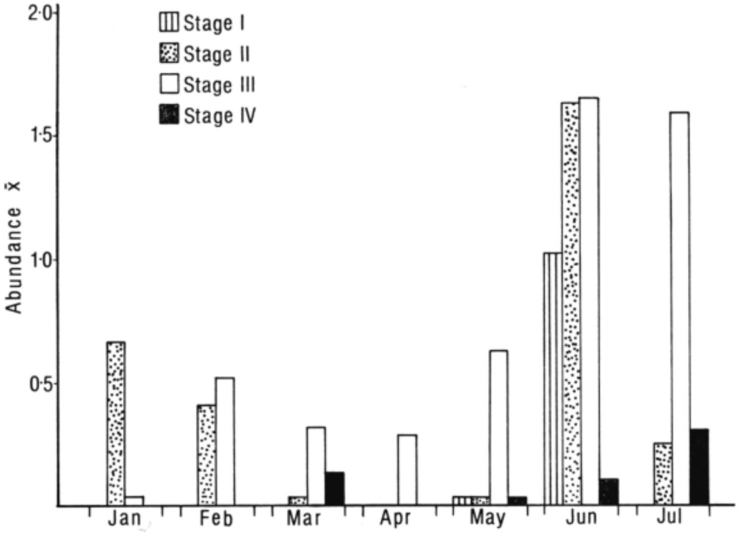

FIG. 2. — Seasonal variations in the age structure of the *Thersitina gasterostei* population infesting *Pungitius pungitius* (October 1979-July 1980).
x = mean number of individuals of each stage of maturity per host.

Through May and June the relative density of parasites increased. From February to May Stage III individuals formed at least 50 % of the population, reaching a peak in April. Stage I individuals first appeared in May and non-ovigerous females again formed the majority classes in June, when the total population of parasites reached a maximum.

There was a 2 month interval between the first appearance of Stage IV individuals and the first appearance of Stage I individuals, suggesting a development period for the free living stages of 7-11 weeks.

RELATIONSHIP BETWEEN HOST LENGTH AND RELATIVE DENSITY OF INFECTION

The number of parasites was significantly related to host length in only 3 of the 10 samples : in December, January and June ($p < 0.05$ in Spearman Rank Correlation).

FREQUENCY DISTRIBUTION AND DISPERSION PATTERN OF *T. gasterostei* ON *P. pungitius*

Histograms of the frequency distributions of *T. gasterostei* infecting *P. pungitius* are presented below *(fig. 3)*. The dispersion pattern of *T. gasterostei* in *P. pungitius*, was found to be contagious ($s^2 > \bar{x}$). In no sample did the Poisson distribution adequately fit the observed data. In 8 of the 10 samples (October to January ; March ; and from May to July) the negative binomial distribution was a good fit to the observed data ($p > 0.05$ in χ^2Goodness of Fit). The possibility that the Poisson log normal distribution (Cassie, 1962) described the data was investigated by χ^2 test for equality between variance to mean ratio of ln ($x + 1$) transformed data (as in Paperna, 1980). Agreement with this distribution was found in 7 of the 10 samples (October ; and December to May).

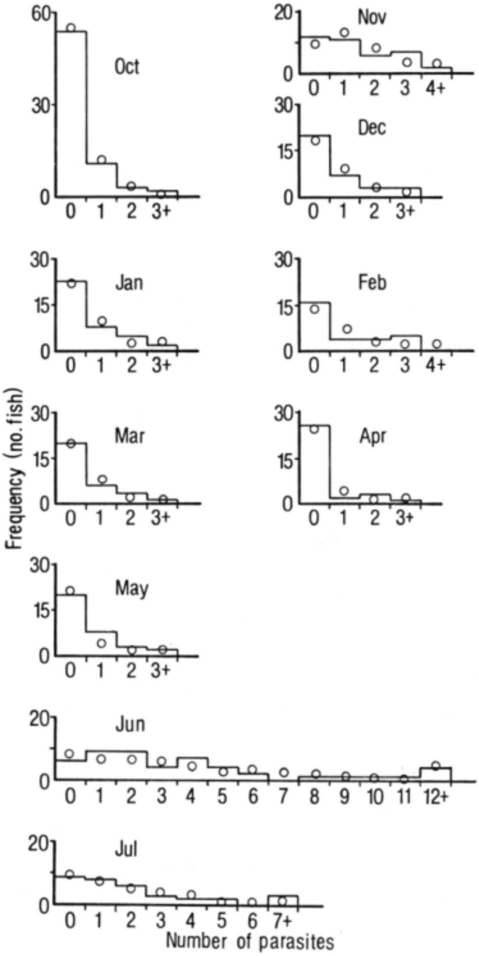

Fig. 3. — Frequency distributions of *Thersitina gasterostei* infesting *Pungitius pungitius*.
○ = calculated negative binomial.

Parameters of the negative binomial distributions and their probabilities in χ^2 Goodness of Fit are presented in *Table I*.

The regression analysis of mean crowding ($\overset{*}{x}$) on mean density ($\bar{x}$) demonstrates

TABLE I. — Parameters of the negative binomial distribution for the dispersion pattern of *T. gasterostei* in *P. pungitius*.

	n	$\bar{x}$	k	χ^2	DoF	P
Oct.	68	0.33	0.582	0.19	1	> 0.5
Nov.	38	1.39	6.659	3.03	2	> 0.2
Dec.	33	0.67	1.059	0.56	1	> 0.3
Jan.	38	0.68	0.903	0.69	1	> 0.3
Feb.	29	0.93	1.144	9.70	2	< 0.01
Mar.	30	0.50	1.234	0.36	1	> 0.5
Apr.	32	0.34	0.259	9.01	1	< 0.01
May	33	0.79	0.496	1.10	2	> 0.5
Jun.	51	4.45	1.247	5.13	9	> 0.8
Jul.	33	2.12	1.348	2.20	5	> 0.8

the contagious nature of the distribution of *T. gasterostei*, the regression coefficient, b, being greater than unity. The regression and correlation coefficients are expressed below.

$$\overset{*}{x} = 1.65\,\bar{x} + 0.357,\ r = 0.970,\ p < 0.001$$

The positive value of the intercept, a, suggests that the basic component of the distribution is a clump, or a positive association of individuals.

Discussion

The relative density of the overwintering population of *T. gasterostei* decreases from November to December and remains low (< 1 parasite per fish) until May. No fresh infection took place during the winter months. During the overwintering period the percentage of ovigerous females increased from < 10 % in January to > 75 % in March. In contrast, overwintering females of other ergasilid species do not develop egg sacs until April or even later in spring (Gnadeberg, 1948 ; Tedla & Fernando, 1970 ; Zmerzlaya, 1972 ; Cloutman & Becker, 1977). Bauer (1962) describes a gradual accumulation of eggs in the ovaries of *E. sieboldi* during the winter but merely states that egg sacs are produced when the water becomes warmer. Female *T. gasterostei* with fully ripened egg sacs (the eggs possessing bluegreen centres) first appeared in March.

The overwintering population continued to produce egg sacs during March, April and May, and the first individuals of the summer generation were found in May. This suggests a development period of 7-11 weeks from hatching to infection (the relevant sampling dates being 7.3.1980, 24.4.1980, 21.5.1980 ; infection may have started any time after 24th April).

This compares with the hatching to infection development period of 4-5 weeks for *E. confusus* (Tedla & Fernando, 1970), although their data applied to the period late May to late June and may reflect development at higher temperatures. Zmerzlaya (1972) quotes a development period for *E. sieboldi* under experimental conditions at 17.7-20.1 °C of 22 days. She also states that the summer generation first appears in mid-late June, an observation which agrees with that of Gnadeberg (1948).

The bulk of the first summer population which is present in June has attained full maturity and is producing egg sacs 4 weeks later, a period from the peak of new infection to the peak of egg production similar to that observed by Tedla & Fernando (1970) and Zmerzlaya (1972) for other ergasilid species. Unfortunately, because sampling had to stop after July, it was impossible during this study to estimate the number of summer generations produced in this population of *T. gasterostei*. Rawson (1977) found that peaks of abundance occurred at 6-8 week intervals in three species of *Ergasilus* during the summer. The pattern terminated with peaks of abundance in November : the overwintering generation. Since the development period, egg-gravid adult takes a similar time in *T. gasterostei* it might be assumed that a similar pattern occurs in this species.

Many authors have described parasite populations as being over-dispersed (e.g. Li & Hsu, 1951 ; Kennedy & Hine, 1969 ; Paperna, 1980). Fryer (1966) has discussed gregariousness in parasitic crustaceans, and cites numerous examples of clumping in populations of parasitic copepods. The negative binomial distribution was shown by Crofton (1971) to be a generally suitable model for parasite populations. This distribution has been successfully fitted to populations of parasitic helminths (Pennycuick, 1971 ; Anderson, 1974 ; MacKenzie & Liversidge, 1975 ; Kennedy & Burrough, 1977). Boxshall (1974b) and Evans *et al.* (1983) have applied the negative binomial distribution to parasitic copepods.

In this study the negative binomial has been shown to fit the frequency distributions of *Thersitina gasterostei* in 8 of 10 samples. Where it was not a good model for the *T. gasterostei* population infesting *P. pungitius* (in the February & April samples) the Poisson distribution was not a suitable model either. The apparent differences in distribution during these months may be due to low infection rates or possibly to seasonal factors. Walkey *et al.* (1970), in a preliminary analysis, suggested that the negative binomial would be a good model for a population of *T. gasterostei* infesting *G. aculeatus* in Norfolk. Their data only applied to the month of May, and this present study shows that the distribution is more generally applicable to a population of *T. gasterostei* throughout the year.

The method of Iwao (1970) involving the regression of $\overset{*}{x}$ on $\bar{x}$ was used in an attempt to clarify the factors generating the observed distributions of *T. gasterostei*. The index of basic contagion (the intercept, a) was greater than zero ($a = + 0.357$). According to Iwao (1970) this indicates that a clump or colony of individuals is the basic component of the distribution : individuals are positively associated. The density contagiousness coefficient (the regression line, b) is 1.65, greater than unity and therefore indicating that the clumps are themselves contagiously distributed. It is likely that infective females aggregate in the plankton in a manner conforming to Cassie's Poisson log normal distribution, which would explain the agreement between this distribution and the dispersion pattern of the parasite within the host in 7 of the samples. Boxshall (1974b) showed that *Lepeophtheirus pectoralis* had an overdispersed distribution and his regression analysis showed that this had been generated by host fish encountering randomly distributed clumps of infective larval parasites. It appears that the contagious distribution of *T. gasterostei* is caused by a similar mechanism (the clumps here being contagiously distributed).

Further analysis of the data by Spearman Rank Correlation showed that the numbers of young (Stage I) individuals are positively correlated with the numbers of old (Stages III & IV) individuals : In June, when the first major wave of infection by young (Stage I) parasites occurred there was a significant correlation between the numbers of Stage I and the numbers of Stage III & IV individuals per host ($r_s = 0.372$, $p < 0.01$, $n = 51$ in Spearman Rank Correlation). Fish which were already infected were prone to further infection by a new generation of parasites. Fryer (1961) reported that young adults of *Lernaea cyprinacea* were often found where old individuals were already present on the host, in this case two different species of *Tilapia*. A similar pattern occurred in a population of the lernaeopodid copepod *Tracheliastes polycolpus* infesting dace, *Leuciscus leusiscus* in Yorkshire rivers (Aubrook & Fryer, 1965). This phenomenon is now shown to occur in *Thersitina gasterostei*, although the cause is unclear. It is probably most likely that infected fish are simply weakened and therefore more prone to further infection than uninfected fish ; alternatively, established adult copepods may attract young females to a settling site (an already infected host) by means of an aggregation pheromone. A further possibility might be that the development of some individuals is inhibited or retarded in cicumstances of high population density.

The density of infection was found to be related to host length in only 3 of 10 samples of *P. pungitius*. It is possible that the absence of any relationship in the remaining 7 samples provides evidence of some immunity amongst larger fish. For the moment this is speculation, however little is known regarding immunity of fishes to parasitic copepods generally (Kabata, 1981) and the subject as a whole would appear to warrant further study. Where there is a relationship between length of host and the relative density of the parasite it is a positive relationship : larger fish tending to be more heavily infected. The reasons for this are probably twofold. Length is proportional to age in fish and older fish have had a higher probability of encountering parasites. Also, larger fish present a larger area for attachment of parasites, although the majority (72 %) of hosts examined were infected by less than 2 parasites so crowding of attachment space in small fish is unlikely to have been a problem for the parasite.

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