EFFECT OF TEMPERATURE, PREDATOR STARVATION, PREY DENSITY AND PREY INCUBATION ON FEEDING AND AGGREGATION ACTIVITIES OF PREDATORY NEMATODES, *MESODORYLAIMUS BASTIANI* AND *AQUATIDES THORNEI*

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Summary. Effects of temperature (5-40 °C), predator starvation (0-12 days), prey density (25-250 individuals), and prey incubation (4-24 h) were studied in *Mesodorylaimus bastiani* and *Aquatides thornei*. Six types of measurements viz., time and rate of site formation, durations of feeding and post-feeding aggregation, and number of predators feeding and showing post-feeding aggregation at feeding sites were made during this study. A minimum time to form feeding site, high rate of site formation and short feeding time were considered optimum attributes for predators. Temperatures of 25-35 °C, duration of predator starvation of 6 to 8 days, prey densities of 200-250 individuals, and duration of prey incubation of 12-16 h were the most suitable for predators in our experiments. All parameters were inter-dependent *e.g*, durations of feeding and feeding sites depended upon the number of predators feeding; number of predators showing aggregation at feeding sites depended upon duration of post-feeding aggregation, and rate of site formation depended upon time of site formation, duration of feeding, and aggregation activities of predators at feeding sites *etc*. Differential rates of feeding, attraction and aggregation by *M. bastiani* and *A. thornei* are attributed to different temperatures, prey densities, prey incubation and predator starvation. Such differences could also be attributed to differential rates of dispersion and degradation of prey attractants, and formation of minimum perceptible attraction gradients by attractants. Various temperatures, prey densities, and durations of prey incubation and predator starvation influence these characteristics.

Prey searching and aggregation activities of predatory nematodes are as important as their prey catching and feeding behaviour. However, very little is known about their behaviour in response to prey-emitted attractants. Attractants may be emitted through the hypodermis (Bilgrami and Jairajpuri, 1988), from body apertures of prey nematodes (Green, 1980) or released due to injury inflicted on the prey body (Bilgrami and Jairajpuri, 1989). Attraction to prey emitted attractants has been reported in dorylaim, nygolaim and diplogasterid predators but not in mononchs. Bilgrami *et al.* (2000) correlated feeding, attraction and aggregation activities in dorylaim and nygolaim predators and showed that the rate of predation depends on the degree of attraction and during- and post-feeding aggregation of predators at feeding sites. Further studies that correlate their prey searching, attraction and aggregation behaviour at feeding sites are required in order to assess the role of prey-emitted attractants in stimulating their predatory potentials.

In this paper, we report results of studies that were made to analyze and correlate activities involved in the feeding, attraction and aggregation behaviour of a dorylaim, *Mesodorylaimus bastiani* (Butschli) Andrassy and a nygolaim, *Aquatides thornei* (Schneider) Ahmad *et* Jairajpuri, predator at feeding sites under the influence of temperature, prey density and durations of predator starvation and prey incubation.

MATERIALS AND METHODS

In vitro cultures of predatory nematodes, *M. bastiani* and *A. thornei*, were maintained at 28±2 °C using *Rhabditis* sp. as prey. Rice root nematode, *Hirschmanniella oryzae* van Breda de Haan, was used as prey. Specimens of the nematode were surface sterilized with 0.01% streptomycin solution (3-5 min) and washed with distilled water (2-3 times) before use. Observations were made on during-feeding (i.e., after wounding and before feeding was completed) and post-feeding activities (i.e., when feeding was completed at a feeding site) of predators using the methods of Bilgrami and Pervez (2000). Feeding was considered as completed when the prey was completely ingested by predators.

Sets of five Petri dishes (5.5 cm-d), containing a 2 mm layer of 1% autoclaved water agar, were used to make observations on the feeding and aggregation behaviour of *M. bastiani* and *A. thornei* at feeding sites. Experimental temperature was 28°±2 °C. Fifty adult individuals of prey were released at different places on the agar surface and incubated for 8 h before releasing 50 adult individuals of *M. bastiani* or *A. thornei*. The Petri dishes were examined every 30 min by inverting over the stage of stereoscopic binocular microscope. The

point where prey was killed was marked on the bottom of the Petri dish by a circle (2 mm-d). This area, referred to as a feeding site, was given reference number. Other feeding sites were located and numbers given to facilitate subsequent examination of feeding sites. Experiments were terminated after 300 min (5 h). Temperatures of 5, 10, 15, 20, 25, 30, 35, 40 °C, prey densities of 25, 50, 75, 100, 125, 150, 175, 200, 225, 250 individuals, predator starvation of 0, 2, 4, 6, 8, 10, 12 days, and prey incubation of 4, 8, 12, 16, 20, 24 h were used for the analysis of feeding, attraction and aggregation activities of predatory nematodes. Time and rate of site formation, durations of feeding and post-feeding aggregation, number of predators feeding or showing post-feeding aggregation at feeding sites were measured to analyze and correlate feeding, attraction and aggregation activities of *M. bastiani* and *A. thornei* at feeding sites.

The time taken by predators to form a feeding site was obtained by dividing the time from inoculation of predators to site formation (i.e., when prey was wounded) by the total number of feeding sites formed in the Petri dish. The rate of site formation was determined by dividing the total number of feeding sites formed in all Petri dishes by the number of replicates (n = 5). Average duration of feeding was determined by dividing the time between commencement (i.e., prey wounding) and termination of feeding (i.e., prey consumed) by the total number of feeding sites in the Petri dish. Average duration of post-feeding aggregation was obtained by dividing the sum of intervals between total period the nematode had spent at a feeding site and time of feeding by total number of feeding sites formed. Number of predators feeding upon prey at a feeding site was obtained by dividing total number of predators feeding at all sites by total number of feeding sites in the Petri dish. The average number of predators showing post-feeding aggregation at feeding sites was obtained by dividing the number of predators present at all feeding sites after consuming a prey by total number of feeding sites formed in Petri dish. The post-feeding aggregation was the aggregation of predators at the feeding site after feeding was completed and prey completely consumed. It was measured in terms of the number of predators aggregating at the feeding site. Coefficients of correlation, regression equations and significance of the results were obtained by using Ky-Plot version 2.

RESULTS

Time of feeding site formation

The average time of site formation by *M. bastiani* (r = - 0.84) and *A. thornei* (r = - 0.74), related to temperature (P < 0.05) (Fig. 1a), was shortest for *M. bastiani* at 25-35 °C and for *A. thornei* between 20 and 35 °C. The time was longest at 5 °C (P < 0.05) for both predators. Both species showed a negative correlation (r = - 0.86 to - 0.88) between time of site formation and duration of their starvation (P < 0.05) (Fig. 1b). Fresh predators

(well fed with prey nematodes) took the longest time to form feeding sites and the time of site formation decreased significantly with increase in the duration of starvation (P < 0.05). Ten-day-starved predators took the shortest time to form feedings site but 12-days-starved predators took longer than predators starved for 4-10 days (P < 0.05). The time of site formation by *M. bastiani* (r = - 0.74) or *A. thornei* (r = - 0.84) decreased with increase in prey density (P < 0.05) (Fig. 1c) and the time was shortest when 200-250 prey individuals were used (P < 0.05). The average time taken by *M. bastiani* (r = - 0.84) or *A. thornei* (r = - 0.74) to form feeding sites also depended upon the duration of prey incubation (Fig. 1d). Predators took the shortest time when prey was incubated for 12-16 h (P < 0.05) and the greatest time when incubation was 4 or 24 h (P < 0.05).

Rate of site formation

The temperature influenced rate of site formation by *M. bastiani* (r = 0.84) and *A. thornei* (r = 0.81) (P < 0.05) (Fig. 2a). Most feeding sites were recorded at 30 °C and least at 5 °C (P < 0.05). Rate of site formation by *M. bastiani* (r = 0.90) and *A. thornei* (r = 0.79) increased significantly over a 0 to ten day period of starvation (P < 0.05) (Fig. 2b). Fresh unstarved predators formed the fewest sites, while ten-day-starved predators formed the most. The rate of site formation by *M. bastiani* (r = 0.70) and *A. thornei* (r = 0.72) also depended upon prey density (P < 0.05) (Fig. 2c), with fewer sites formed with 25 and most with 250 prey individuals (P < 0.05), and the duration of prey incubation (r = 0.70-0.71) (Fig. 2d), with most sites recorded with 12-16 h- and least with 4- or 24 h-incubated prey individuals (P < 0.05).

Duration of feeding

Temperature governed the duration of feeding by *M. bastiani* (r = - 0.83) and *A. thornei* (r = - 0.69) (P < 0.05) (Fig. 3a). Feeding duration by *M. bastiani* was longest at 5 °C and shortest at 25-30 °C, whilst that of *A. thornei* was least at 25 °C (P < 0.05). Feeding duration was also influenced by the period of starvation of *M. bastiani* (r = 0.94) and *A. thornei* (r = 0.87) (P < 0.05) (Fig. 3b). Fresh and 2-day-starved predators completed feeding within a much shorter period than other groups of starved predators (P < 0.05). Both *M. bastiani* (r = 0.85) and *A. thornei* (r = 0.88) showed a positive correlation between duration of feeding and prey densities at feeding sites (P < 0.05) (Fig. 3c). Feeding was shortest and longest with 25 and 250 prey individuals respectively (P < 0.05). Feeding by *M. bastiani* (r = 0.88) and *A. thornei* (r = 0.85) also depended upon duration of prey incubation (P < 0.05) (Fig. 3d), being longest with prey incubated for 24 h (P < 0.05).

Duration of post-feeding aggregation

The duration of post-feeding aggregation by *M. bastiani* (r = 0.60) and *A. thornei* (r = 0.76) was governed by temperatures (P < 0.05) (Fig. 4a), with maximum
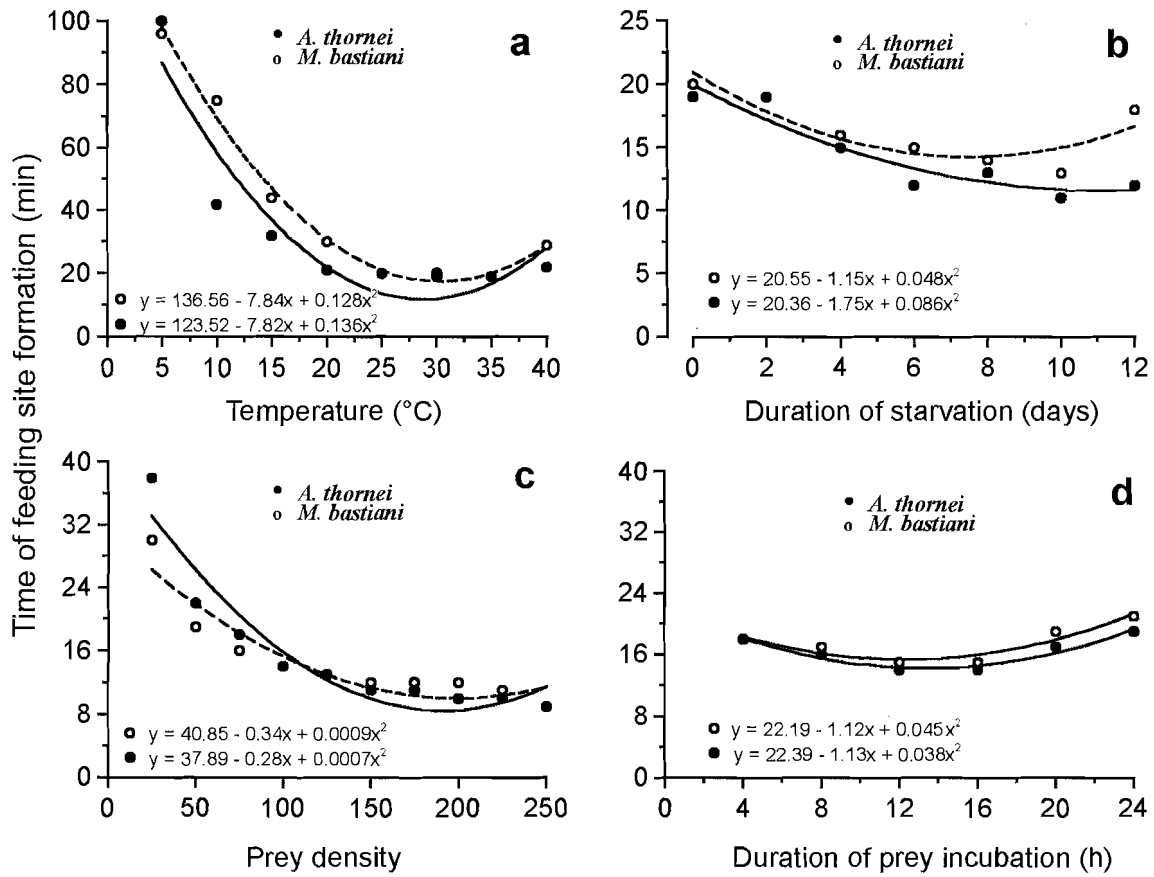
Fig. 1. Effect of temperature (a), duration of starvation (b), prey density (c), and duration of prey incubation (d) on the time of feeding site formation by predators.
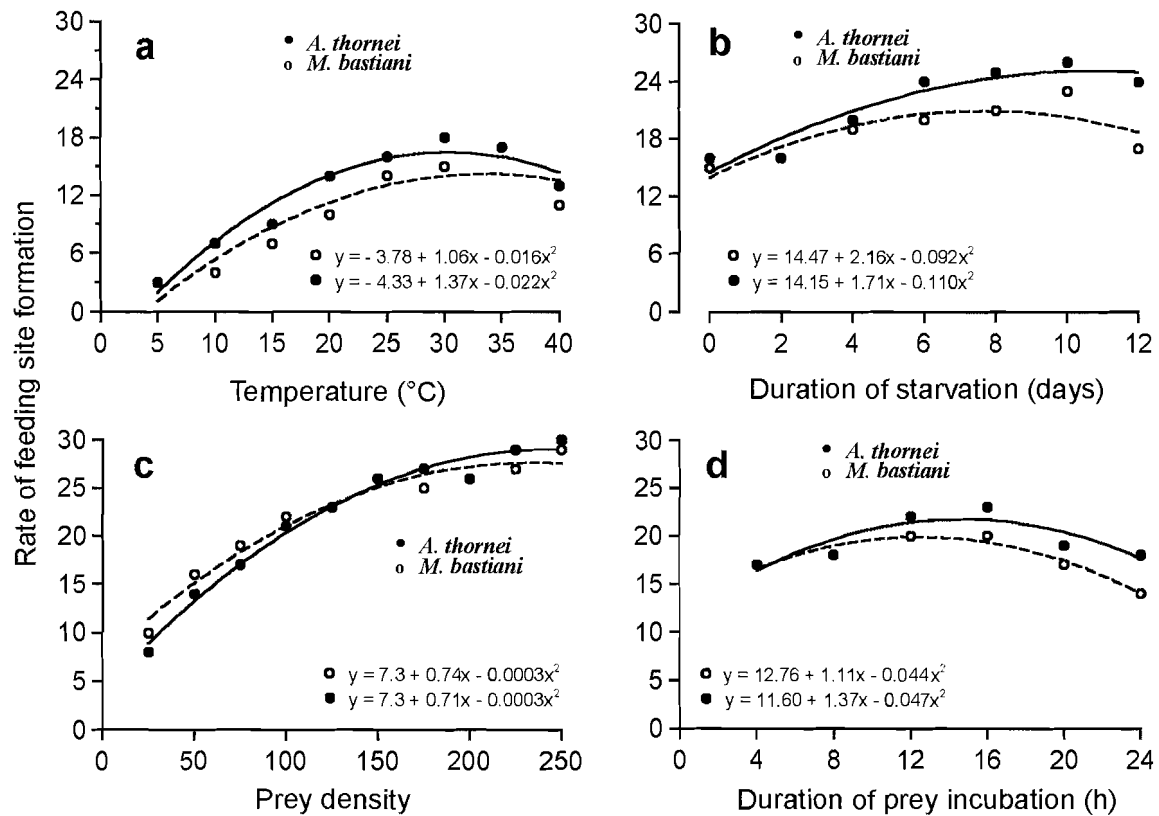

Fig. 2. Effect of temperature (a), duration of starvation (b), prey density (c), and duration of prey incubation (d) on the rate of feeding site formation by predators.
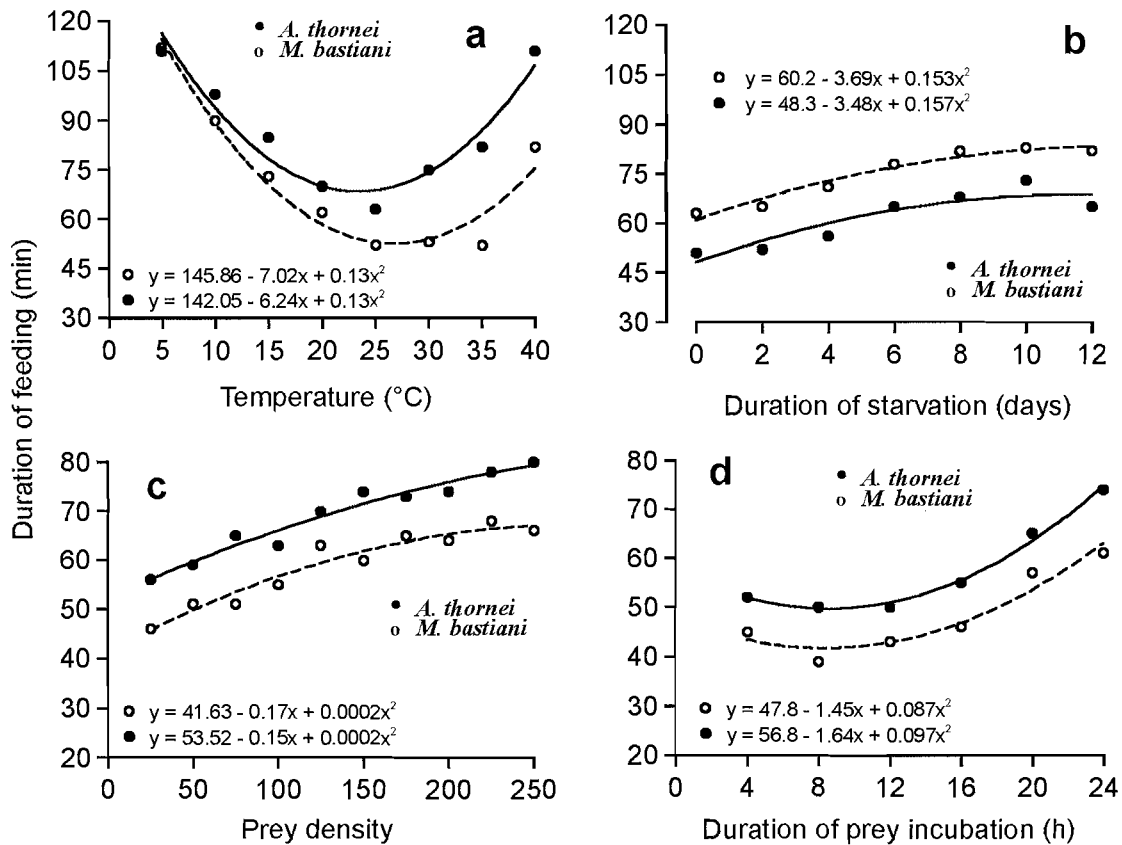
Fig. 3. Effect of temperature (a), duration of starvation (b), prey density (c), and duration of prey incubation (d) on the duration of feeding by predators at feeding sites.

duration at 25 °C and shortest at 5 °C (P < 0.05). It increased with duration of predator starvation (P < 0.05) (Fig. 4b), with fresh and 10-day-starved predators showing the shortest and longest durations of post-feeding aggregation respectively (P < 0.05). The responses of 12-day-starved predators decreased. Both *M. bastiani* (r = - 0.90) and *A. thornei* (r = - 0.99) showed negative correlations between duration of post-feeding aggregation and prey densities (P < 0.05) (Fig. 4c). Duration of post-feeding aggregation was longest with 25 prey individuals and shortest with 250 prey individuals (P < 0.05). The duration of post-feeding aggregation of *M. bastiani* (r = - 0.96) and *A. thornei* (r = - 0.97) depended upon period of prey incubation (P < 0.05) (Fig. 4d), with post-feeding aggregation longest for both species of predators when prey was incubated for 4 h and shortest when the prey incubation period was 24 h (P < 0.05).

Number of predators feeding at feeding site

The numbers of *M. bastiani* (r = 0.57) and *A. thornei* (r = 0.57) individuals feeding at a site increased up to 35 °C (P < 0.05) (fig. 5a) and temperatures lower or higher than 35 °C resulted in fewer feeding individuals. The number of predators feeding upon prey also increased significantly with the duration of starvation of *M. bastiani* (r = 0.81) and *A. thornei* (r = 0.89) (P < 0.05) (Fig.

5b), although the number decreased again if starvation was for 12-days. Correlations between feeding individuals of *M. bastiani* (r = - 0.90) and *A. thornei* (r = - 0.96) and prey densities were also significant (P < 0.05). Most predators fed together at a density of 25 prey individuals (P < 0.05) (Fig. 5c) and least at 250. Both *M. bastiani* (r = - 0.95) and *A. thornei* (r = - 0.85) showed a negative correlation of number of feeding nematodes with the duration of prey incubation. The number of feeding individuals was greatest when prey was incubated for 4 h and least when duration was 24 h (P < 0.05) (Fig. 5d).

Number of predators showing post-feeding aggregation at feeding site

Most individuals of *M. bastiani* and *A. thornei* showed post-feeding aggregation at 25-30 °C (P < 0.05) (Fig. 6a) but *A. thornei* showed no aggregation at 5 or 40 °C and *M. bastiani* at 5-15 or 40 °C. Four day-starved *A. thornei* (r = - 0.84) and 6-8 day-starved *M. bastiani* (r = - 0.65) showed the greatest post-feeding aggregation (P < 0.05) (Fig. 6b). Greatest numbers of *A. thornei* (r = - 0.82) showed post-feeding aggregation when tested with 125 prey individuals (P < 0.05) (Fig. 6c), whilst prey densities less than 125 individuals resulted in less aggregation. The greatest number of *M. bastiani* (r = - 0.88) showing post-feeding aggregation was at a density of 100 prey individuals. Neither *M. bastiani* nor *A.*
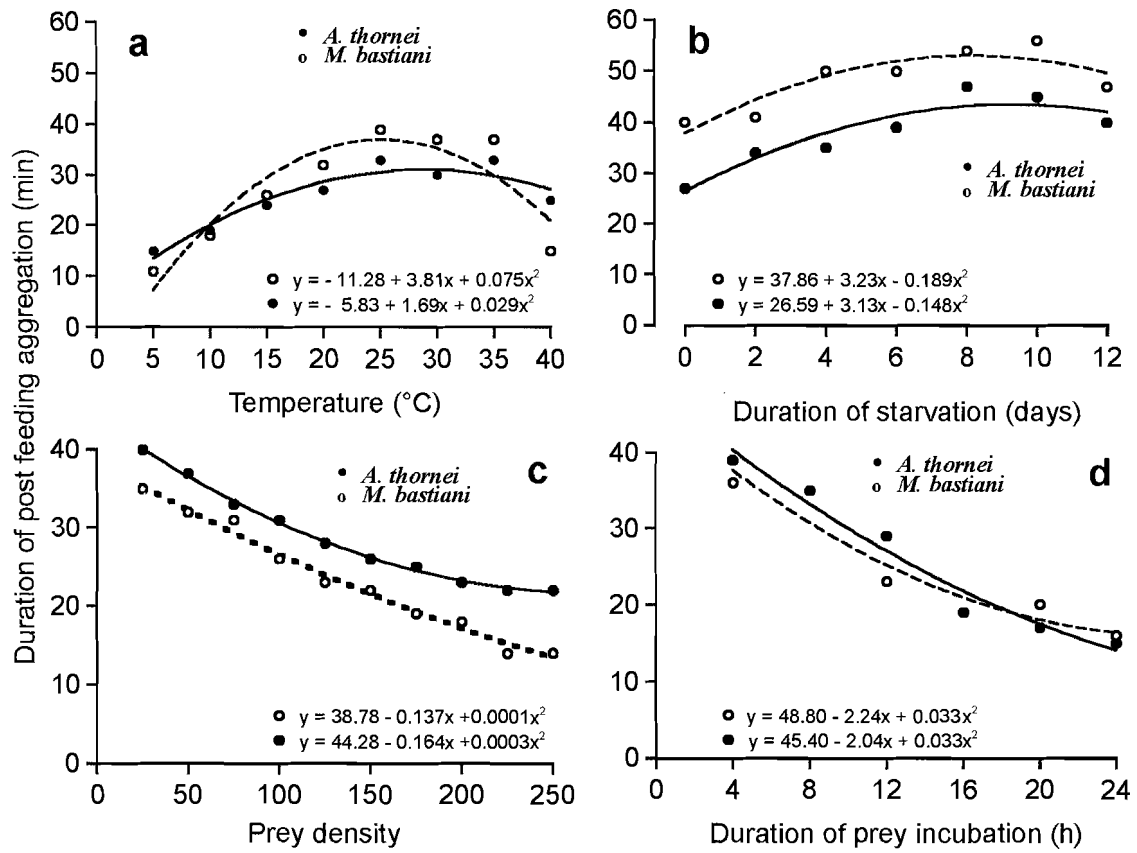
Fig. 4. Effect of temperature (a), duration of starvation (b), prey density (c), and duration of prey incubation (d) on the duration of post-feeding aggregation by predators.
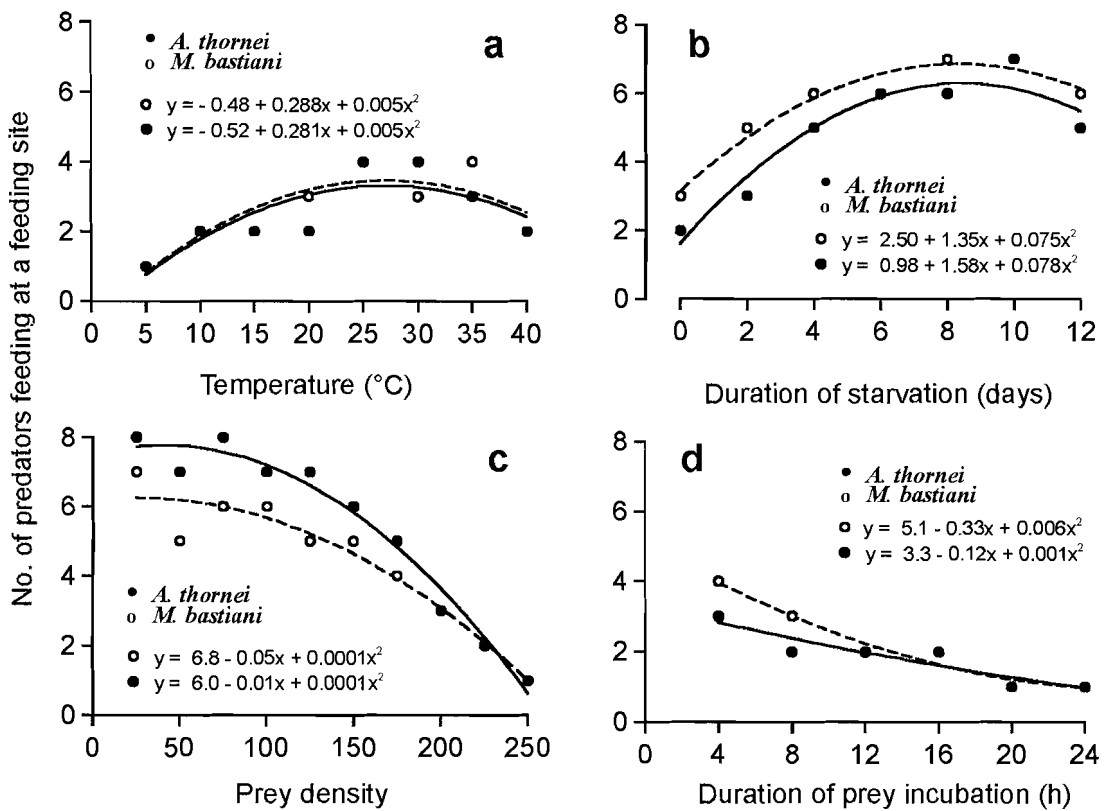

Fig. 5. Effect of temperature (a), duration of starvation (b), prey density (c), and duration of prey incubation (d) on the number of predators feeding at feeding sites.
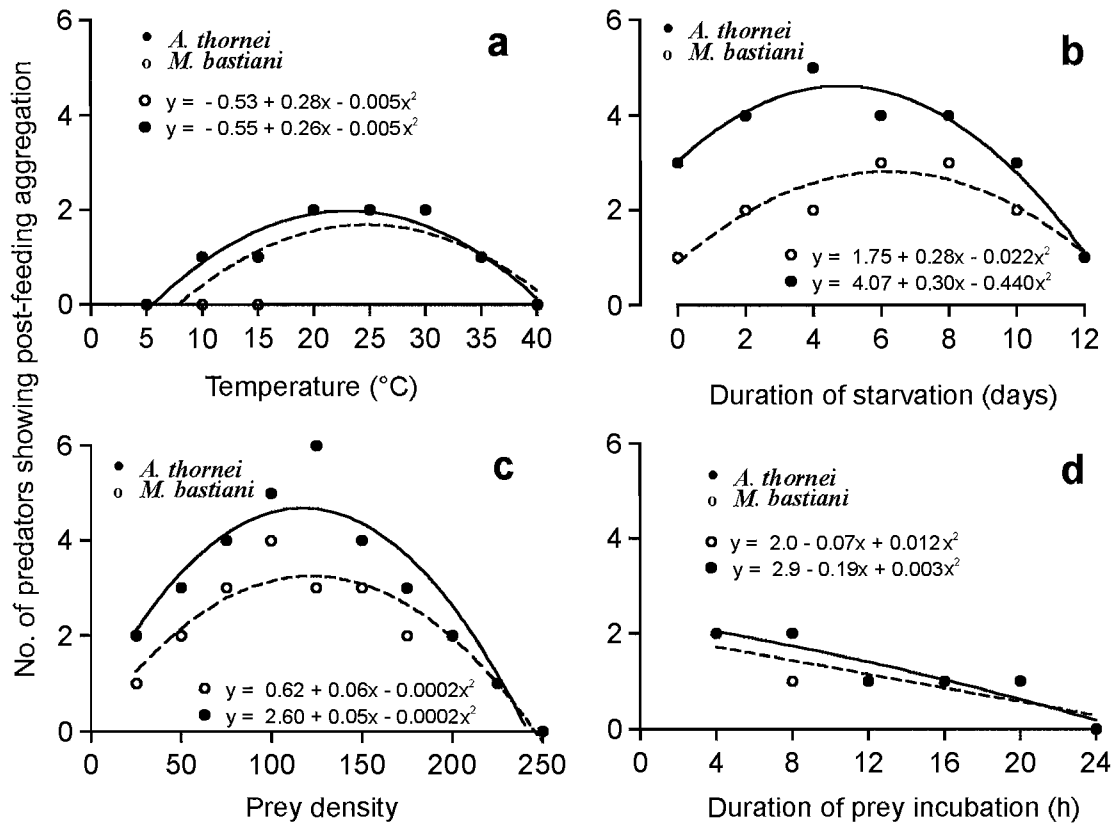
Fig. 6. Effect of temperature (a), duration of starvation (b), prey density (c), and duration of prey incubation (d) on the number of predators showing post-feeding aggregation at feeding sites.

thornei showed post-feeding aggregation when prey density was 250. The greatest number of *M. bastiani* (r = - 0.52) and *A. thornei* (r = - 0.54) individuals showing post-feeding aggregation were when prey was incubated for 4 h (P < 0.05) (Fig. 6d). No such aggregation was shown by predators when prey nematodes were incubated for 24 h.

DISCUSSION

Mesodorylaimus bastiani and *A. thornei* showed differential feeding, attraction and aggregation activities during and after feeding when tested at different temperatures, prey densities, and durations of prey incubation and predator starvation. This reflects differing requirements of predators for food and feeding, and attraction and aggregation at feeding sites. The variations may occur because the two species of predators belong to different predatory groups and require different conditions (Bilgrami and Jairajpuri, 1988; Yeates and Wardley, 1969), which are governed by their ageing and starvation (Reversat, 1981). A short time taken by predators to form feeding sites, high rates of site formation, and short durations of feeding sites and feeding by predators can probably be considered as optimum attributes for efficient predation.

Temperature influences not only sensory behavioural responses of predatory nematodes but also produces thermodynamic effects on various activities (Jairajpuri and Bilgrami, 1990). Taking these facts into account, the differential aggregation responses of predators at various temperatures may be explained, which have altered aggregation parameters at feeding sites. Changes in sensory behavioural responses (Green, 1980), the physical state of the predators (Bilgrami and Jairajpuri, 1989), and locomotion, which is governed by temperature (Wallace, 1963), could be other factors responsible for differential aggregation behaviour. Predators may have spent more time feeding when fewer sites were formed for the above reasons, in addition to any effect of small numbers of feeding sites on the opportunities for predators to feed and show aggregation. Dispersion of attractants and formation of minimum perceptible attraction gradients as affected by low or high temperatures might have restricted the number of predators at sites for prolonged feeding. Temperatures between 25-30 °C were suitable for predators because the time of site formation, and the duration of feeding sites and feeding were least and the rate of site formation and number of predators showing feeding or aggregation at feeding sites were greatest.

Food requirements of predatory nematodes are density dependent (Shafqat *et al.*, 1987; Moens *et al.*,

2000). Their aggregation at feeding sites is also governed by prey densities as is evident with M. *bastiani* and *A. thornei*. When preys were plentiful, attractants emitted by them had a favourable influence on the aggregation of predatory nematodes relative to when prey was scarce. This phenomenon may be related to a high concentration of attractants produced by the high prey densities (Green, 1980), and the reason why maximum aggregation was found at prey densities of 200-250 individuals. The reason why predators required more time to form fewer sites with shorter feeding but longer post-feeding aggregation duration may be related to a lower mean concentration of attractants emitted due to fewer prey nematodes. Increased numbers of predators feeding or aggregating at feeding sites with low prey densities might have resulted from fewer sites being formed in the test arena. The numbers of predators at feeding sites decreased as the number of feeding sites increased with prey density.

Increased starvation of predators also increased their minimum response threshold (Doncaster and Seymour, 1973). Thus, starving predators could sense weaker stimuli formed by attractants emitted by fewer prey nematodes. This phenomenon could be substantiated by early formation of feeding sites, increased rate of predation, longer durations of feeding and post-feeding aggregation, and by the fact that greatest numbers of predators aggregated at feeding sites when 10-day-starved predators were used. The reason why values for various parameters decreased when predators were starved for less or more than ten days may be due to their habituation to prey non-availability and loss of appetite respectively. The loss of aggregation responses in predators starved for ten days or more may also have resulted from the loss of energy as starving nematodes utilize their food reserves (Reversat, 1981).

The formation of minimum attraction gradients around predators (Pervez and Bilgrami, 2000) and the dispersion of attractants emitted by prey nematodes (Bilgrami *et al.*, 2001) are important attributes, both of which may be influenced by prey incubation. The differential behaviour shown by *M. bastiani* and *A. thornei* at feeding sites may be attributed to such effects. The optimum values for time of site formation, site number and duration of feeding sites, feeding and post-feeding aggregation found with 12-16-h-incubated prey individuals could be due to formation and dispersion of attraction gradients at high intensities. In contrast, short periods of incubation of prey resulted in weak attraction gradients for predators to respond to. The predator's own threshold for perception of attractants at low intensities could be an other possibility. The attractants starts to degrade (Heuttel, 1986) after long periods of prey incubation (20-24 h), which could decrease the rate of site formation, extend the time of site formation, and the time of feeding by predators.

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