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REPRODUCTION OF PTEROMALIDAE (HYMENOPTERA) PARASITIC ON FRESH AND FROZEN HOUSE FLY (*MUSCA DOMESTICA* LINN.) PUPAE

LEOPOLDO M. RUEDA* and RICHARD C. AXTELL
Department of Entomology, North Carolina State University, Raleigh, North Carolina 27607, U.S.A.

ABSTRACT

Fresh, less than 24-hour old house fly (*Musca domestica* Linn.) pupae and pupae frozen for 24 hours at -5°C were compared as hosts of 4 pupal parasites, *Muscidifurax raptor* Girault and Sanders, *Pachycrepoideus vindemiae* (Rondani), *Spalangia cameroni* Perkins and *Spalangia endius* Walker. Pupae which had been frozen were as suitable as fresh pupae for mass laboratory productions of *M. raptor*, *P. vindemiae* and *S. cameroni*, but not for *S. endius*. The net reproductive rate (R_0) estimates were slightly higher on fresh pupae than frozen pupae but the weight values of the emerged adults of all parasites tested, except *S. endius*, were not significantly different. The intrinsic rate of increase (r_m) values for *P. vindemiae*, *S. cameroni*, *M. raptor* and *S. endius* were 2.4, 3.4, 7.9 and 11.0%, respectively greater in fresh pupae than in frozen pupae. With fly pupae (frozen for 24 hours at -5°C, then placed in the culture chamber at 26.7°C up to 5 days before exposure to the parasites), there was a decrease in the number of progeny produced by *M. raptor*, *P. vindemiae*, *S. cameroni* and *S. endius*, but not by *M. zaraptor*. With pupae frozen for longer periods (up to 180 days at -5°C) before exposure to the parasites, there was a decrease in the number of progeny produced by *P. vindemiae*, *S. cameroni* and *S. endius*, but not by *M. raptor* and *M. zaraptor*.

INTRODUCTION

Mass releases of selected pteromalid parasites are an important component of an overall biological control strategy to suppress populations of house flies and other muscoid flies in poultry and livestock production facilities (Rutz and Axtell, 1979, 1981; Mourier, 1972; Olton and Legner, 1975; Morgan *et al.* 1975). To further increase the effectiveness of mass parasite releases, the periodic addition of frozen pupal hosts to poultry and livestock manure has been proposed by Pickens and Miller (1978). Theoretically this should maintain the number of parasites or even increase their hunting efficiency in periods of low host density and thus preventing the rapid increase of the host population (Pickens and Miller, 1978). The suitability of frozen pupae is a question, however. Also, in a mass release or augmentation program, large laboratory colonies of selected parasites are needed. Thus, the use of the frozen pupae would greatly facilitate

*Present address: Department of Entomology, University of the Philippines at Los Baños, College, Laguna 3720, Philippines. To whom correspondence should be addressed.

parasite production. Although Legner (1976, 1979) studied the effect of refrigerating the fly pupal host on the reproduction of some pteromalid wasps, no detailed studies have been reported on the use of frozen pupae as hosts. Therefore the reproductive potentials of four species of parasites were compared when reared on fresh and frozen pupae under laboratory conditions. The number of progeny produced by the female parasites when exposed to host pupae frozen for longer periods, and to frozen pupae allowed to thaw at various periods were also determined.

MATERIALS AND METHODS

Host and Parasite Cultures

House fly (*Musca domestica* Linnaeus) pupae and parasites used in these experiments were obtained from laboratory colonies at the Department of Entomology, N.C.S.U., Raleigh, North Carolina. House fly (CSMA) strains were reared in a room maintained at 26.7°C and 60 ± 10% RH as described by Rutz and Axtell (1979). The laboratory colonies, about 25-generation-old, of *Muscidifurax raptor* Girault and Sanders, *Spalangia cameroni* Perkins and *Pachycrepoideus vindemiae* (Rondani), were originally established from adults that emerged from laboratory-reared house fly pupae placed in poultry manure in North Carolina. The colony of *Spalangia endius* Walker, about 35-generation-old, was established from adults obtained from the Insects Affecting Man and Animals Research Laboratory, USDA, SEA, Gainesville, Florida. The colony of *M. zaraptor* Kogan and Legner, about 8-generation-old, obtained from E.F. Legner (University of California, Riverside), was a subculture whose stock parents originated from Denver, Colorado. Voucher specimens of these colonies were deposited in the N.C.S.U. Insect Collection.

Parasite Reproductive Potentials on Fresh and Frozen Host Pupae

Fresh (<1-day-old) and frozen (<1-day-old, frozen for 24 hours at -5°C and allowed to thaw for 24 hours before experiment began) pupae of house fly were compared for suitability as hosts of the following parasites: *M. raptor, P. vindemiae, S. cameroni,* and *S. endius.* Cohorts of 15 mated females of each species were selected at random from the laboratory colonies. These females were allowed to feed and oviposit on 30 <1-day-old fresh pupae for 2 days prior to the beginning of the experiment. Each female was placed in a screen-capped plastic vial (4 cm diameter, 35 mL volume), lined with filter paper and its screen lid provided with honey. A batch of 20 house fly pupae randomly scattered over the base of the vial was furnished daily to each of the females until their death. In treatment 1, <1-day-old fresh pupae were provided, and in treatment 2, frozen pupae (<1-day-old, frozen for 24 hours at -5°C).

Each batch of host pupae, after 24-hour exposure to the parasites, were incubated at 26.7°C, 60 ± 5% RH for parasite (F_1 progeny) development and emergency. All parasite cultures were maintained under 12:12 hour light: dark photoperiod supplied by two 40-watt cool white fluorescent lamps positioned 0.54 m above the culture table. The longevity of the parasites, the number of offspring produced per female and sex ratio were recorded daily for each treatment. The net reproductive rate (R_o), and the

intrinsic rate of natural increase (r_m) values were estimated from an initial cohort of 15 ovipositing females as described by Birch (1948).

The net reproductive rate, R_0, was determined by taking the sum of $l_x m_x$ values where l_x is the survival rate based on the number of adult female survivors at the beginning of age unit x, and m_x is the daily fecundity rate based on the number of female progeny produced by a female parent during each of the age intervals, x (Birch 1948). The calculation of the intrinsic rate of natural increase (r_m or r) was based on trial and error substitutions of r to find the best fit in the formula:

$$\Sigma e^{-rx} l_x m_x = 1$$

where x is the pivotal age or the midpoint of each age group, e is the base of the natural logarithm, and l_x and m_x as described above (Birch 1948).

Differences between treatments were statistically analyzed for significance using the t-test.

Effects of Prolonged Freezing of Host Pupae

Five species of parasites, *M. raptor, M. zaraptor, P. vindemiae, S. endius* and *S. cameroni* were all tested to determine the effect of prolonged freezing of pupal hosts at -5°C on the number of offspring produced. Batches of laboratory-reared <1-day-old house fly pupae were frozen separately for 1, 30, 90 and 180 days. Frozen pupae were allowed to thaw for 24 hours before experiment began. Mated 4-day-old female parasites were randomly selected from laboratory colonies of each species. Each female was placed in a vial as described before. A batch of 20 pupae was randomly scattered over the base of the vial and was exposed to each female parasite for 24 hours. After a 24-hour exposure, pupae were incubated and all cultures were maintained as described before. Fifteen replicates were performed at each pupal freezing period regime. Data were analyzed for significant differences using Duncan's Multiple range test.

Effects of Varying Durations of Thawing Host Pupae

Five species of parasites, *M. raptor, M. zaraptor, P. vindemiae, S. endius* and *S. cameroni* were tested in this experiment. Laboratory-reared house fly pupae (<1-day-old) were frozen for 24 hours at -5°C and subsequently placed in the culture chamber (26.7°C, 60 ± 5% RH) for various periods of time: 1, 2, 3, 4 and 5 days. Mated 4-day-old female parasites were used in the experiment as described in the preceding section.

RESULTS AND DISCUSSION

Parasite Reproductive Potentials on Fresh and Frozen Host Pupae

Adult females of *P. vindemiae* (Figure 1) showed a 50% survival rate (l_x) about 18 days after emergence when exposed to fresh pupae compared to 14 days on frozen pupae; *M. raptor* (Figure 2) about 13 days compared to 7 days; *S. cameroni* (Figure 3) about 9 days compared to 7 days; and *S. endius* (Figure 4) about 10 days compared to 9 days.

The daily fecundity rates (m_x) or each parasite species when exposed to fresh pupae compared to frozen pupae are shown in Figures 1-4. *P. vindemiae*, when exposed to fresh pupae, showed its highest peak 2 days after adult (mother) emergence (average, 7.8 female progeny/adult female), slightly decreased during the next day (average, 6.5), then increased producing 3 distinct peaks during the 4th (average, 6.8), 7th (average, 6.2) and 14th (average, 3.8) days after adult emergence (Figure 1). When exposed to the frozen pupae, *P. vindemiae* showed its highest fecundity rate (average, 8.4) 2 days after adult emergence, abruptly decreased during the 4th day (average, 1.8), then increased producing 6 distinct peaks during the 6th (average, 5.2), 8th (average, 4.0), 11th (average, 5.0), 13th (average, 3.9), 15th (average, 3.6) and 17th (average, 3.4) days after adult emergence. *M. raptor* showed its highest fecundity rate during the 3rd day (average, 8.8) after adult emergence when exposed to fresh pupae and 5th day (average, 6.7) when exposed to frozen pupae (Figure 2). *S. cameroni* showed its highest fecundity rate during the 4th day after adult emergence, averaging 8.8 and 7.6 when exposed to fresh and frozen pupae, respectively (Figure 3). *S. endius* showed its highest peak of fecundity during the 4th (average, 4.6) and 5th (average, 3.4) days after adult emergence when exposed to fresh and frozen pupae, respectively.

The estimated values of net reproductive rate (R_0) and intrinsic rate of natural increase (r_m) also are shown in each graph. For the calculation of R_0 and r_m values the pivotal age for each group was taken as the average length of development (egg to adult) of females at 26.7°C and 60 ± 5% RH plus 2 days for mating and host feeding. All adult females were about 2 days old when the experiment was started, and their average pivotal age was 19.0 days for *M. raptor*, 20.0 days for *P. vindemiae* and 23.0 days for both *S. endius* and *S. cameroni*. The survival rate of immature females from oviposition was estimated to be 90%. Legner (1976, 1979) also made the same estimate of survival rate of immature females of some pteromalid species. Under laboratory condition, it was expected that low mortality occurred in the egg, larval or pupal stages of the parasites because these stages were well protected within the puparium. In nature, however, desiccation, predation and superparasitism are the main obvious causes of the mortality of immature stages (Coats, 1976). The density of the host (20 pupae/female parasite) exposed to the parasite in the present experiment minimized higher mortality rates due to superparasitism (Markwick, 1974; Legner, 1979). The values of l_x were calculated by multiplying the number of adult female survivors by 0.90. The values of m_x were computed using the number of emerged female offspring/female parent in the age interval x. All the statistical values calculated here are applied mainly to the adult stage since no attempts were made to include actual mortality rates of the immature stages.

M. raptor, *S. endius*, and *S. cameroni* produced a significantly greater number of female offsprings , an average of 64.2, 20.4 and 26.9, respectively, when exposed to fresh pupae as compared to an average of 48.5, 11.4 and 21.2 female offspring, respectively, when exposed to frozen pupae (Table 1). In *P. vindemiae*, no significant differences in reproduction were observed between those exposed to either fresh or frozen pupae. However, considering the total number of offspring (both females and males), only *S. endius* produced a significantly greater number of offspring when exposed to fresh pupae than in frozen pupae.

In the mass production of parasites under laboratory conditions, the R_0 values are more useful than r_m as they relate to the net or actual reproduction of parasites in the culture (Legner, 1979). Parasites exposed to fresh, non-frozen pupae produced slightly higher R_0 values than those in frozen pupae. For fresh and frozen pupae, the averages of the oven-dry weights of individual *M. raptor* progeny were 10.1 and 8.9 x 10^{-2} mg, respectively; *P. vindemiae*, 7.2 and 9.0 x 10^{-2} mg; *S. endius*, 15.9 and 12.4 x 10^{-2}; and *S. cameroni*, 18.9 and 18.3 x 10^{-2}. With the exception of *S. endius*, the oven-dry weight comparisons of the progeny of all species exposed to either fresh or frozen pupae did not differ significantly. In *M. raptor, P. vindemiae*, and *S. cameroni*, the lower R_0 values on frozen pupae in laboratory cultures may be given less consideration since the emerged parasite (F_1) offspring did not differ significantly from those emerged on fresh pupae. Legner (1979) also gave less emphasis on R_0 values when similar weight data of parasite progeny reared on refrigerated and non-refrigerated host pupae were observed. Frozen pupae, therefore, can support and provide adequate nourishment for normal development of the progeny in these three parasites. In practical mass cultures of parasites in the laboratory frozen pupae can be used to a certain extent for *M. raptor, P. vindemiae*, and *S. cameroni* but not for *S. endius*. Nevertheless, other evaluations are needed before using frozen pupae for these parasite species because different strains of the same species may react differently when exposed to the same host pupae.

Concerning the intrinsic rate of natural increase (r_m), the calculated values for the parasites exposed to pupae which had been frozen were slightly lower than those in fresh pupae. In *P. vindemiae, S. cameroni, M. raptor* and *S. endius*, differences of 2.4, 3.4, 7.9 and 11.0%, respectively, for frozen and fresh pupae values were calculated. This suggests that frozen pupae may not heavily affect the performance of adult female parasites especially during the early part of their oviposition period. The method of calculating r_m values used in the present data favored greatly the early ovipositional output of the female parasite (Birch, 1948; Legner, 1979). Under field conditions, e.g., in poultry and livestock barns, the relative abundance of the parasites and degree of parasitism on house fly pupal hosts decrease tremendously during the colder months of the year (Ables and Shepard, 1976; Rutz and Axtell, 1980). This could be explained possibly to a certain extent by the longer developmental period of various stages of the house fly host as pointed out by Legner (1979) and availability of only few suitable pupal stages in the manure.

Effects of Prolonged Freezing of Host Pupae

With the exception of *M. raptor* and *M. zaraptor*, pupae which had been frozen for longer periods before exposure to the parasites, provided a less favorable environment insofar as the number of progeny produced by the parasites is concerned. No adults of *S. endius* and *S. cameroni* emerged from the pupae frozen for 180 days. Pupae frozen for 90 days had a significantly lower number of parasite progeny (Table 2) compared to those frozen for shorter periods. In *P. vindemiae*, pupae frozen for 1 and 30 days produced significantly greater number of offspring compared to those frozen for 90 and 180 days. Number of *M. raptor* and *M. zaraptor* progeny were not affected by freezing of pupal host up to 180 days. Legner (1979) noted that lower temperatures cause pupal desiccation and mortality of housefly, *M. domestica* and this may have affected the development of the parasites which depend exclusively for food on the insect host. House fly

pupae refrigerated at 10°C for more than 21 days had progressive chitinization rendering them unacceptable to the parasites (Legner, 1979). If eggs of the parasites were ever laid on frozen host pupae, they were unable to complete development up to adult stage due to poor nourishment. In the cases of *M. raptor* and *M. zaraptor*, immature stages of these parasites in the host pupae frozen even up to 180 days, were able to nourish and survive to adult stage. Under extreme host deterioration, only a fraction of the available food is utilized by the parasites (Legner, 1969). However, studies are needed to find out the maximum period of time (i.e. beyond 180 days) host pupae could be frozen without much detrimental effects to the parasites, especially *M. raptor* and *M. zaraptor*. In biological control programs, especially in mass releases of the selected parasites, prolonged freezing of the host pupae for subsequent use when needed could save enormous time and effort. Host pupae could be produced, in large numbers, at one time and be available, either as propagation hosts in the laboratory or as sentinel hosts to be spread in the field during times when the natural population of house flies are at very low level, especially during the colder months. These sentinel hosts could act as supplemental hosts to maintain parasite populations at a sufficient level that would prevent extreme upsurges of host populations (Pickens and Miller, 1978).

Effects of Varying Durations of Thawing Host Pupae

In *M. raptor*, frozen pupae thawed and exposed to parasites 1 and 2 days after produced significantly greater numbers of adults than those thawed and exposed to parasites 3 to 5 days after (Table 3). In *P. vindemiae, S. endius* and *S. cameroni* significantly greater numbers of offspring were exhibited by frozen pupae thawed and exposed to parasites 1 day after than those thawed and exposed to parasites at longer period after. The results possibly could be explained by the fact that frozen pupae thawed and exposed to parasites at longer periods (e.g. several days) after deteriorated faster rendering their contents less suitable for immature stages of the parasites to derive nourishment and develop normally. The disintegration of the insect cells (cytolysis) normally occurs after slow thawing (Losina-Losinsky, 1967). Salt (1961) reported the effects of thawing on the survival of frozen insects. *M. zaraptor*, unlike the other 4 species tested, was not affected by the longer period of thawing of frozen pupae as shown by the number of progeny that it produced. This can be explained by the highly cannibalistic behavior of *M. zaraptor* (Wylie, 1971a), and its ability to consume effectively its own exuviae (Coats, 1976) and possibly other dead tissues of the host pupae. In time of stress for normal food, such as during deterioration and decreased nutritional value of pupal host, *M. zaraptor* is able to practice cannibalism as well as feed on dead tissues to survive. Wylie (1971b, 1972) suggested that cannibalism and its ability to feed on larvae of other parasites enable *M. zaraptor* to compete more successfully with those parasites such as *S. cameroni* and *Nasonia vitripennis*. *S. endius* and *S. cameroni* reproduction was reduced greatly in frozen pupae thawed and exposed to the parasites 1-5 days after.

In actual field condition, the degree of deterioration of frozen pupae during prolonged thawing process might vary depending upon the prevailing environmental condition. This should be given important consideration when using frozen pupae as surrogate hosts to augment mass parasite release programs.

ACKNOWLEDGEMENT

This work was supported by North Carolina Research Service (Project NC 1181), Regional Project S-181 and US-AID-National Crop Protection Center, University of the Philippines at Los Baños.

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Table 1. Average number of emerged adults of four species of pteromalid parasites (Hymenoptera) when allowed to oviposit on fresh and frozen pupae of house fly, *Musca domestica* Linn.

Species	Condition of pupae	Mean No. adult emerged[a]		
		Female	Male	Total
Muscidifurax raptor	Fresh[b]	64.2*	35.5	100.3
	Frozen[c]	48.5	43.1	94.7
Pachycrepoideus vindemiae	Fresh	62.5	31.4	94.6
	Frozen	44.5	22.9	70.3
Spalangia endius	Fresh	20.4*	15.1	35.5*
	Frozen	11.4	9.3	22.1
Spalangia cameroni	Fresh	26.9*	11.9	38.8
	Frozen	21.2	9.6	30.8

*Differs significantly from the mean number from frozen pupae at 5% level, by t-test.
[a]Mean number of progeny emerged when 2-day-old mated, adult females were allowed to oviposit on laboratory-reared house fly pupae (20 pupae/vial/adult/day, 15 adults/treatment) throughout the oviposition period.
[b]Less than 1-day-old fresh laboratory-reared house fly pupae.
[c]Less than 1-day-old fresh, laboratory-reared house fly pupae frozen for 1 day at -5°C.

Table 2. Average number of adults of five species of pteromalid parasites that emerged from house fly pupae after being frozen at -5°C for 0-180 days before exposure to oviposition by the parasites.

Duration of freezing[b] (days)	Mean number of adults emerged[a]									
	M. raptor		*M. zaraptor*		*P. vindemiae*		*S. endius*		*S. cameroni*	
	Female	Total	Female	Total	Female	Total	Female	Total	Female	Total
0 (fresh)[c]	7.7a	12.5a	8.6a	12.1a	7.1a	11.1a	3.3a	5.9a	4.3a	6.7a
1	7.1a	10.3ab	7.3a	11.0a	7.6a	10.3a	2.8b	4.1b	1.5b	3.1b
30	7.3a	10.4ab	6.3a	8.9a	8.3a	11.2a	1.2c	3.1b	1.3b	1.9b
90	5.6a	9.3ab	5.9a	9.2a	5.8b	8.1b	0.5c	0.9c	0.7b	1.3c
180	4.8a	8.2b	6.9a	10.4a	4.9b	7.5b	0.0	0.0	0.0	0.0

[a]Mean number of adult parasites emerging from laboratory-reared house fly pupae after a 24-hour exposure. Data transformed to $\sqrt{X + 0.5}$ for statistical analysis. Means in the same column followed by the same letter are not significantly different from each other (5% level) (Duncan's multiple range test).
[b]Less than 1-day-old fresh, laboratory-reared house fly pupae frozen for various lengths of time.
[c]Less than 1-day-old fresh laboratory-reared house fly pupae.

Table 3. Average number of adults of five species of pteromalid parasites (Hymenoptera) that emerged from house fly pupae exposed to oviposition by the parasites after freezing at -5°C for 24 hours and different durations of thawing.

Duration of thawing[b] (days)	Mean number of adults emerged[a]									
	M. raptor		*M. zaraptor*		*P. vindemiae*		*S. endius*		*S. cameroni*	
	Female	Total	Female	Total	Female	Total	Female	Total	Female	Total
0 (fresh)[c]	7.9a	11.1a	6.5a	9.7ab	7.6a	10.2a	4.5a	6.3a	7.9a	10.3a
1	6.9a	10.9a	6.3a	6.8a	8.1a	11.0a	0.9b	1.7b	4.8b	6.3b
2	8.1a	11.8	6.3a	7.5ab	2.5b	5.7b	0.7bc	1.1c	2.1c	3.0c
3	2.6b	4.3b	5.4a	6.3a	1.7b	3.1c	0.4cd	0.7c	3.3bc	4.3c
4	3.4b	6.1b	7.9a	9.7ab	6.3c	7.8b	0.5bc	0.6c	2.3c	3.2c
5	2.9b	4.1b	8.9a	10.7b	4.5c	6.7b	0.2d	0.3d	0.8d	1.5d

[a]Mean number of adult parasites emerging from laboratory-reared house fly pupae after a 24-hour exposure. Data transformed to $\sqrt{X + 0.5}$ for statistical analysis. Means in the same column followed by the same letter are not significantly different from each other (5% level) (Duncan's multiple range test).

[b]Less than 1-day-old fresh, laboratory-reared house fly pupae frozen for various lengths of time.

[c]Less than 1-day-old fresh, non-fresh laboratory-reared house fly pupae.

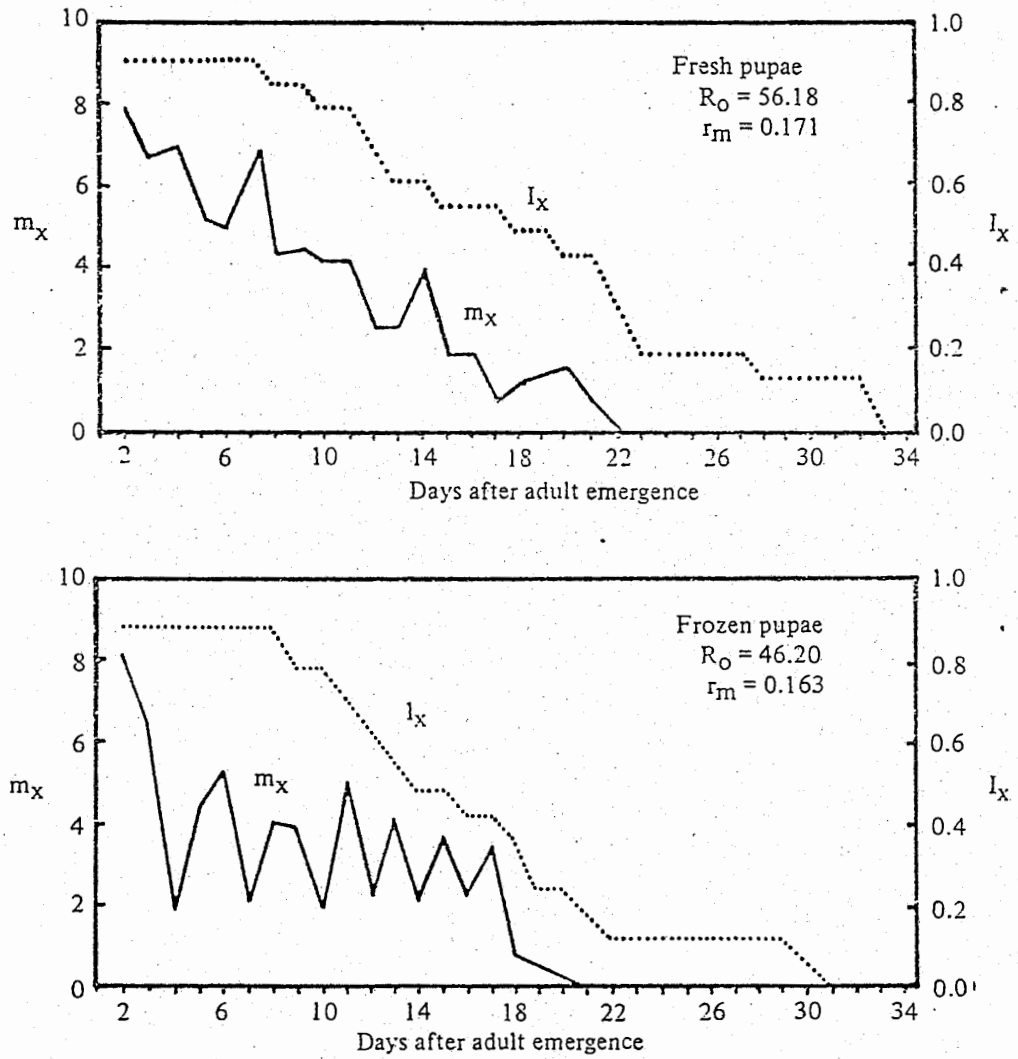

Fig. 1. Survival rate (% adult surviving x 100), l_x and daily fecundity rate (mean number of female progeny produced/day), m_x for females of Pachycrepoideus vindemiae *(Rondani) when ovipositing on fresh, less than 24-day-old* Musca domestica *pupae and pupae frozen at -5°C for 24 hours. R_0 = net reproductive rate, and r_m = intrinsic rate of natural increase.*

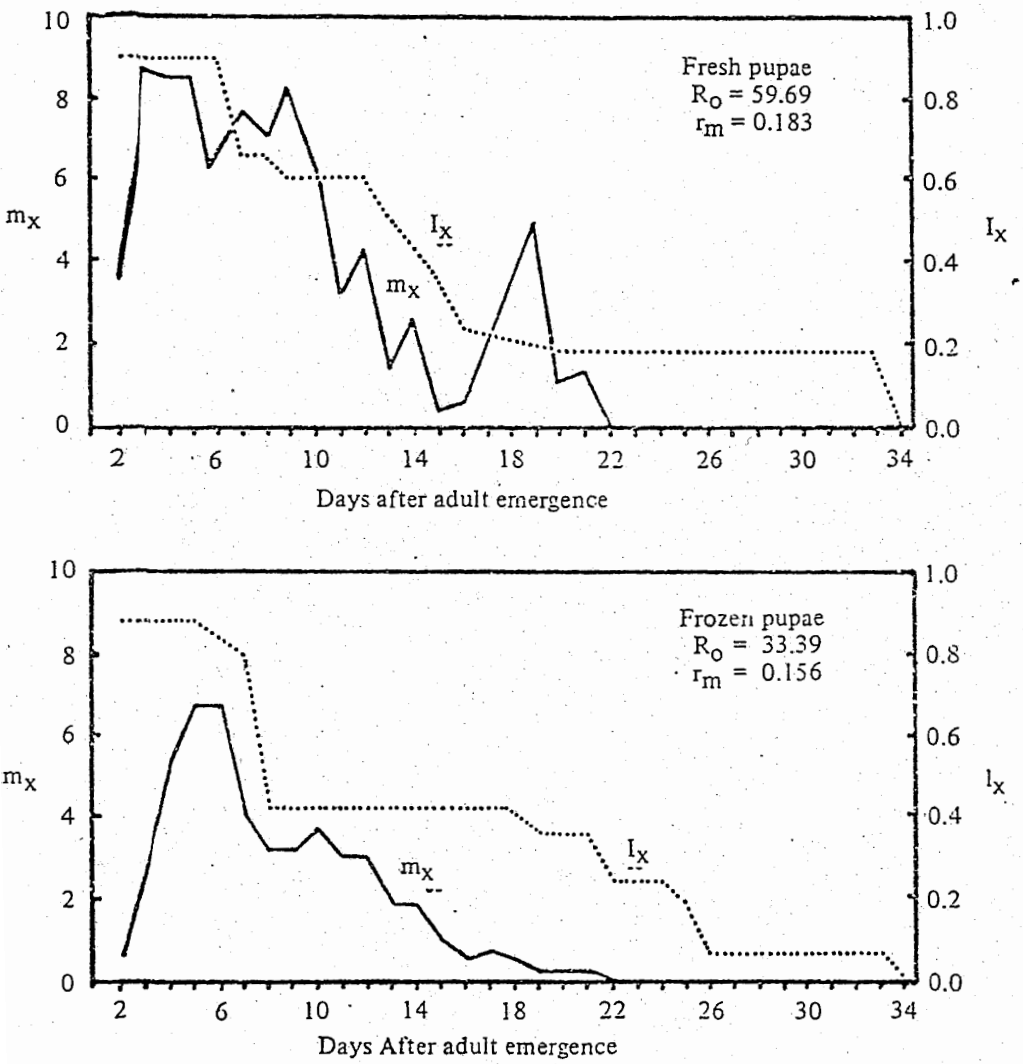

Fig. 2. Survival rate (% adult surviving x 100), l_x and daily fecundity rate (mean number of female progeny produced/day), m_x for females of Muscidifurax raptor *Girault and Sanders when ovipositing on fresh, less than 24-day-old* Musca domestica *pupae and pupae frozen at -5°C for 24 hours. R_0 = net reproductive rate, and r_m = intrinsic rate of natural increase.*

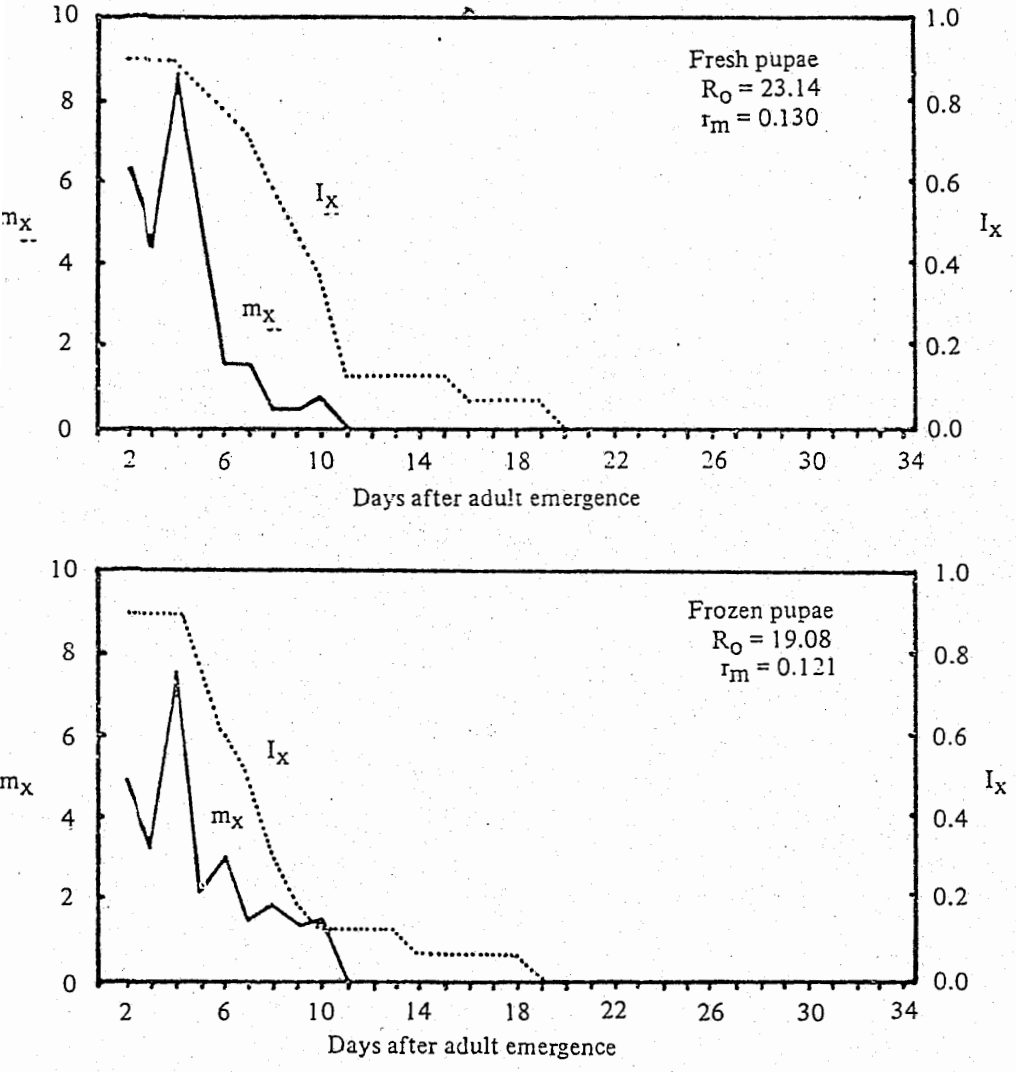

Fig. 3. Survival rate (% adult surviving x 100), 1_x and daily fecundity rate (mean number of female progeny produced/day), m_x for females of Spalangia cameroni *Perkins when ovipositing on fresh, less than 24-day-old* Musca domestica *pupae and pupae frozen at -5° C for 24 hours. R_0 = net reproductive rate, and r_m = intrinsic rate of natural increase.*

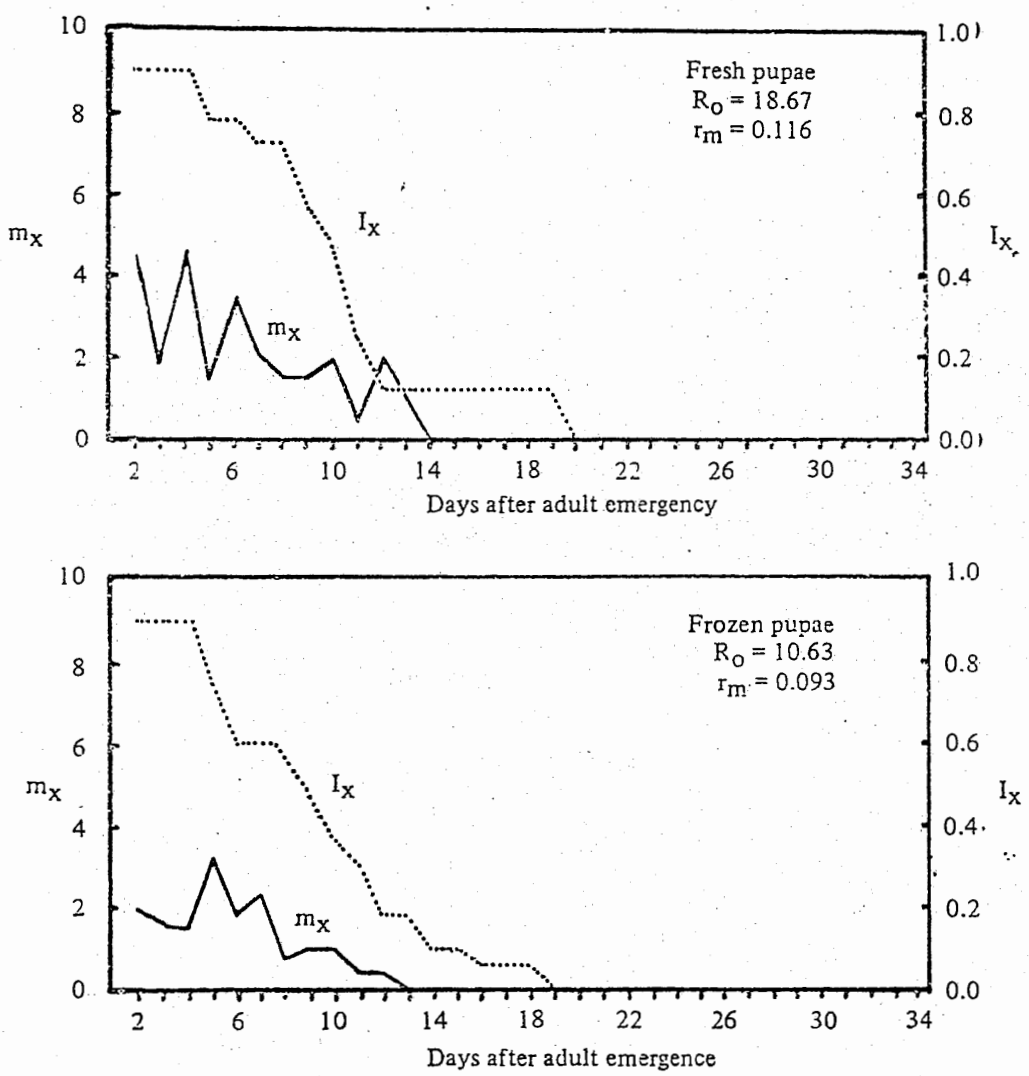

Fig. 4. Survival rate (% adult surviving x 100) l_x and daily fecundity rate (mean number of female progeny produced day), m_x for females of Spalangia endius *Walker when ovipositing on fresh, less than 24-day-old* Musca domestica *pupae and pupae frozen at -5°C for 24 hours, R_0 = net reproductive rate, and r_m = intrinsic rate of natural increase.*