

THE EFFECT OF AGE AND DIAPAUSE ON THE LONG-TERM INTAKE
OF PROTEIN AND SUGAR BY TWO SPECIES OF BLOWFLIES,
PHORMIA REGINA (MEIG.) AND *PROTOPHORMIA*
TERRAENOVAE (R. D.)

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Insects have proved to be good model systems with which to study feeding behavior. Some insects, in particular the black blowfly, *Phormia regina*, are capable of regulating their food intake. This species has been the subject of feeding studies in which both the maintenance of metabolic homeostasis and the underlying regulatory mechanisms have been investigated.

The principal food of the blowfly is carbohydrate (Dethier, 1969), although Strangways-Dixon (1961), Dethier (1961) and Belzer (1970) have demonstrated the existence of a specific protein hunger in flies. Female blowflies increase selectively their consumption of proteins prior to periods of egg maturation resulting in the appearance of cycles of protein intake concomitant with reproductive cycles. Spermatogenesis occurs independent of feeding in the blowfly (Cowan, 1932; Mackerras, 1933; Stoffolano, 1974a), although male specimens of *P. regina* require a protein meal for the maturation of the accessory reproductive glands (Stoffolano, 1974a). Male blowflies exhibit an initial peak of protein consumption but do not show subsequent peaks of protein intake (Belzer, 1970; Roberts and Kitching, 1974).

Studies on aging in insects (Clark and Rockstein, 1964; Rockstein and Miquel, 1973; Stoffolano, 1976) have indicated that numerous degenerative structural and functional changes occur in aging adult insects, such as mechanical damage to cuticle and wings, reduced fecundity, reduced levels of protein synthesis and respiration rates, and degeneration of the central nervous system. Changes occurring in the structure and function of the nervous system, specifically the peripheral chemoreceptor sense organs, are of particular interest since these receptors are intimately involved in the feeding process. A receptor system which showed age-related degenerative changes would imply that aged flies are less efficient feeders. Aged flies show increasing numbers of nonfunctional labellar sensilla and among those that remain functional, a decrease in the mean impulse frequency occurs (Rees, 1970; Stoffolano, 1973; Stoffolano, Damon and Desch, in preparation).

Stoffolano (1973) reported the occurrence of a facultative imaginal diapause in *P. regina* characterized by the lack of ovarian development in the female and by hypertrophy of the fat body in both sexes. In addition, the accessory reproductive glands are undeveloped in the diapausing fly. The occurrence of an imaginal diapause has also been reported for *Protophormia terraenovae* (Roubaud, 1927; Cousin, 1932; Danilevskii, 1965). Behavioral alterations such as migration, reduced

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mating activity, and changes in feeding behavior may also occur during insect diapause. Prior to entry into diapause many insects feed ravenously to build up large reserves of fat and glycogen in the fat body and other storage tissues (Lees, 1955). However, no work has been done on the selective intake of protein and sugar by diapausing flies, as compared to their nondiapausing counterparts (Stoffolano, 1974b).

This paper presents the results of an investigation aimed at elucidating the effects of age and diapause on the long-term intake of protein and sugar by male and female *P. regina* and *P. terraenovae* maintained under controlled environmental conditions.

MATERIALS AND METHODS

Adult specimens of *Phormia regina* and *Protophormia terraenovae* were collected in 1972: *P. regina* in Ithaca, New York, and *P. terraenovae* in College, Alaska. Rearing procedures for stock colonies of both species were identical, utilizing the techniques and diet described earlier (Stoffolano, 1973).

Rearing of experimental colonies

Experimental colonies of nondiapausing flies were obtained from larvae reared at 24° C, 24 hr light and 50–60% relative humidity. Pupae were transferred to an environmental chamber where subsequent emerging adults were reared. Nondiapausing *P. regina* adults were reared under June conditions of daylength and temperature for the Ithaca, New York, area in an attempt to simulate those conditions experienced by the insects in nature. Adults received a 16 hr photophase with a temperature of 24° C during the light phase and 13° C during the dark phase (8 hr). Diapausing *P. regina* adults were obtained from flies reared under conditions described earlier by Stoffolano (1973). Larvae were reared at 24° C and 24 hr dark. Pupae were transferred to an environmental chamber where subsequent emerging adults received a 9 hr photophase and a temperature of 18° C.

A concomitant low temperature and short photophase is required by *P. regina* adults for induction of the diapause condition, whereas in *P. terraenovae*, diapause appears to be temperature-independent (Greenberg, personal observation); diapausing flies can be reared at the same temperature as the nondiapausing population, thus eliminating a possible differential temperature effect which might confound the results. Therefore, *P. terraenovae* was used to repeat those studies made with *P. regina* on the intake of protein and sugar by diapausing and nondiapausing flies.

Nondiapausing adult specimens of *P. terraenovae* were obtained from larvae reared similarly to those of nondiapausing *P. regina*. Pupae were transferred to an environmental chamber where pupae and emerging adults received 24 hr light and 24° C. Diapausing flies were obtained from larvae reared in a manner similar to those of diapausing *P. regina*. Pupae were transferred to an environmental chamber, where emerging adults were kept at 24° C and a 9 hr photophase.

Large numbers of larvae were reared in order to obtain enough flies of approximately the same age. Only those flies emerging during one twelve-hour period were used; all others were discarded. The maximum age difference between any two flies was thus twelve hours.

Adult flies were maintained in approximately equal numbers in 25×50 cm wire mesh cages with front sleeving. Paper toweling, which was changed weekly, was placed on the bottom of the cages to absorb liquid wastes. The cages contained standard feeding solutions offered in separate glass vials stoppered with absorbant rolled cotton wicks. Feeding solutions consisted of 10% (w/v) yeast extract (Nutritional Biochemicals, Co) and 0.1 M sucrose prepared in distilled water. These solutions, offered as standard diet to the experimental colonies, were identical to the solutions used in the two-choice testing experiments. The feeding solutions were prepared fresh daily. Water was also available to the experimental colonies and was changed weekly. The artificial diet (Stoffolano, 1973) on which the larvae were reared was used as an oviposition medium in a manner similar to that described by Orr (1964). This eliminated an additional source of protein in the experimental colonies, since flies were observed to oviposit but not to feed on the medium. Flies were also observed to oviposit readily on the rolled cotton wicks saturated with feeding solutions.

Cages were housed in an environmental chamber equipped with four 20 watt fluorescent lights. Flies received a photophase and temperature regime as described earlier. Average light intensity in the chamber was 3121.63 lux. Humidity in the chamber fluctuated between 50–60% relative humidity.

Measurement of intake

Food intake was measured using a two-choice apparatus, consisting of individual feeding units housed in a clear plexiglass container, adopted and modified from Belzer (1970).

Individual feeding units consisted of two precision bore glass capillary tubes (internal diameter 0.889 mm, 30.5 cm long) inserted through holes drilled in a rubber stopper which in turn fitted into a cellulose nitrate centrifuge tube, 3 cm in diameter and cut to 7.5 cm in length with nylon mesh glued to the cut end. The glass tubes, calibrated externally in mm, were obtained from Ace Glass, Co., Vineland, New Jersey. The use of these tubes, rather than those used by Belzer (1970), provided more accurate measurements of intake. A further benefit of the calibrated capillary tubes was that it was no longer necessary to open the plexiglass container in which the tubes were housed to take readings. Readings could be taken through the plexiglass container, thus subjecting humidity inside the container to fewer fluctuations.

The clear plexiglass container measured $25 \times 36 \times 38$ cm. The individual feeding units housed in the container were secured with rubber tubing stretched on removable plexiglass platforms. The platforms rested on adjustable wooden racks which could be tilted to produce an incline such that the fluid in the capillary tubes would flow downward as the fly fed. Relative humidity in the plexiglass container was maintained at 60–70% with a saturated NaCl solution prepared according to Winston and Bates (1960) and placed in a pan on the bottom of the container. The entire apparatus was housed in an environmental chamber equipped with four 20 watt fluorescent lights. Relative humidity in the chamber was maintained at 50–60%.

The standard feeding solutions were introduced into the glass capillary tubes using a hypodermic syringe and were then allowed to equilibrate for approximately

2 hr during which time evaporation among the capillary tubes was found to be the greatest. One tube of each feeding unit contained the 10% yeast extract solution ("protein" solution); the other contained 0.1 M sucrose or sugar solution. A "zero" reading was taken at the end of this period, and the cellulose nitrate centrifuge tubes containing the flies were fitted into place. Final readings were taken the next day (22 hr later); all readings were taken at the same time each day. At the end of each feeding trial capillary tubes were removed and replaced with clean ones, and the flies were discarded or dissected (see below). Used tubes were washed in detergent solution, rinsed in tap water and dried in an oven at 75° C.

Seven flies of each sex were used each day in calculating the daily mean intake. New flies were taken each day from the experimental colonies and briefly anesthetized with CO₂. The flies were separated as to sex, weighed individually and transferred to the cellulose nitrate centrifuge tubes, one fly to a tube. An equal number, seven, of evaporation controls (consisting of the individual feeding units without flies) were run concurrently. Evaporation among the capillary tubes was found to be least in those tubes closest to the salt bath and greatest among those farthest away. Controls and flies were therefore distributed as to their position on the plexiglass platforms using a table of random numbers to minimize variations in the results due to differential evaporation. Intake was measured from day 1 to day 40 for *P. regina* and from day 1 to day 25 for *P. terraenovae*. Flies were tested at the same temperature and photophase regime under which they were reared, except diapausing specimens of *P. regina* which were tested at the same temperature and photophase regime as the nondiapausing population.

Dissections

Female flies were dissected in an insect saline solution (Normann and Duve, 1969) at the termination of each day's feeding trial, and ovarian measurements taken to determine the relative stage of development. Diapausing flies were also dissected and examined for fat body hypertrophy and ovarian development.

Statistical analysis

Analysis of variance of the data was used to determine the effect of sex, age, diet and treatment (diapausing or nondiapausing) and their interactions on the intake of the fly. This included computer calculation of means, standard error of the mean, and F-values. An analysis of variance was also made of evaporation data, and these results were used in calculating adjusted daily mean intake values. Each point in Figures 1-4 represents the daily mean intake (in μ l) for seven flies minus the mean evaporation for that day, for that diet and for that treatment.

An analysis of covariance was carried out to determine the effect of fly weight on intake.

RESULTS

Nondiapausing and diapausing P. regina

The intake of protein and sugar by nondiapausing male and female *P. regina* was measured from day 1 to 40 days after emergence. The results are shown in Figure 1 and Table I.

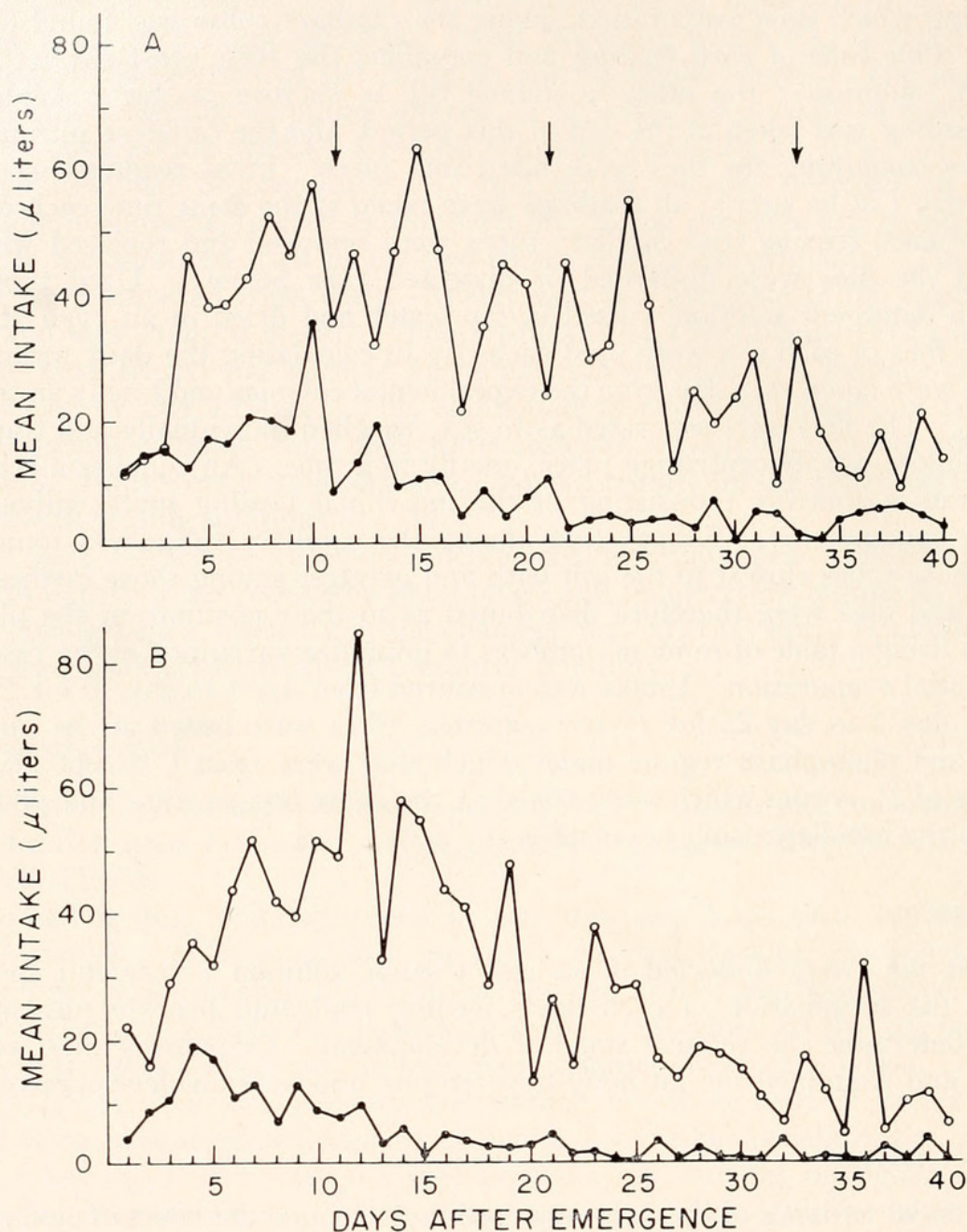


FIGURE 1. Total daily mean intake (μ l) of protein (solid circles) and sugar (open circles) by nondiapausing female (A) and male (B) *P. regina* from day 1 to 40 days after emergence. Each point on the graphs represents the daily mean intake for seven flies minus the mean evaporation for that day and for that diet. Arrows indicate days on which eggs were laid.

Analysis of variance showed the total daily mean intake of protein and sugar by the population of flies was significantly influenced ($P < 0.01$) by the age of the fly. Intake was low during the first three days, increased on day 4 through 16, and then gradually decreased until day 40. Both sexes showed a similar trend in food intake with respect to age.

The total daily mean intake of sugar by the population of flies was significantly greater than protein intake. Females had a greater ($P < 0.01$) total daily mean intake compared to males and consumed both more sugar and protein than males.

On the average, female flies weighed more than male flies (see Table I).

Analysis of covariance of the effect of fly weight on intake showed that when intake was considered on a $\mu\text{l}/\text{mg}$ per day basis, sugar intake and total intake of protein plus sugar was not significantly different between male and female flies.

The intake of protein and sugar by diapausing male and female *P. regina* was measured from day 1 to 40 days after emergence. Diapausing flies were identified by lack of ovarian development in females and fat body hypertrophy in both sexes; the latter was not recognizable until day 15 after emergence. The results are shown in Figure 2 and Table I.

Analysis of variance showed age had a significant effect ($P < 0.01$) on the total daily mean intake of protein and sugar by the population of flies. Intake was low following emergence, increased rapidly on days 2 to 3, stayed high until days 27 to 28, then declined until day 40. Food intake by females was significantly more influenced ($P < 0.01$) by the age of the fly.

The total daily mean intake of sugar by the diapausing flies was significantly greater ($P < 0.01$) than protein. Females had a significantly greater ($P < 0.01$) total daily mean intake than males and consumed more of both protein and sugar compared to males.

On the average, diapausing female flies weighed more than diapausing males (see Table I). However, analysis of covariance of the effect of fly weight on intake ($\mu\text{l}/\text{mg}$ per day) gave the same results as the $\mu\text{l}/\text{day}$ analysis.

An analysis of variance conducted on data from nondiapausing versus diapausing *P. regina* showed that diapausing flies fed significantly more ($P < 0.01$) than nondiapausing flies. Intake of protein by the two populations of flies was not

TABLE I

Daily mean intake of protein and sugar by nondiapausing and diapausing *Phormia regina* and *Protophormia terraenovae*.

Experiment	Sex	Mean weight (mg)	Daily mean intake					
			Protein		Sugar		Protein and Sugar	
			(μl)	($\mu\text{l}/\text{mg}$)	(μl)	($\mu\text{l}/\text{mg}$)	(μl)	($\mu\text{l}/\text{mg}$)
ND, <i>P. regina</i>	M	43.67	4.35	0.0996	28.24	0.6466	16.29	0.3730
	F	50.97	8.98	0.1761	30.40	0.5964	19.68	0.3861
	M + F	47.32	6.66	0.1407	29.32	0.6196	17.99	0.3801
D, <i>P. regina</i>	M	40.38	2.59	0.0641	46.34	1.1475	24.47	0.6059
	F	42.59	8.07	0.1894	51.97	1.2202	30.02	0.7048
	M + F	41.48	5.33	0.1284	49.16	1.1851	27.18	0.6552
ND, <i>P. terraenovae</i>	M	42.63	3.65	0.0856	45.23	1.0609	24.45	0.5735
	F	50.63	7.42	0.1465	47.51	0.9383	27.47	0.5425
	M + F	46.63	5.65	0.1211	46.26	0.9920	25.96	0.5567
D, <i>P. terraenovae</i>	M	46.67	4.36	0.0934	39.26	0.8412	21.80	0.4671
	F	49.86	6.04	0.1211	36.96	0.7412	21.50	0.4312
	M + F	48.26	5.20	0.1077	38.11	0.7896	21.65	0.4486

ND, nondiapauses; D, diapause; M, males; F, females.

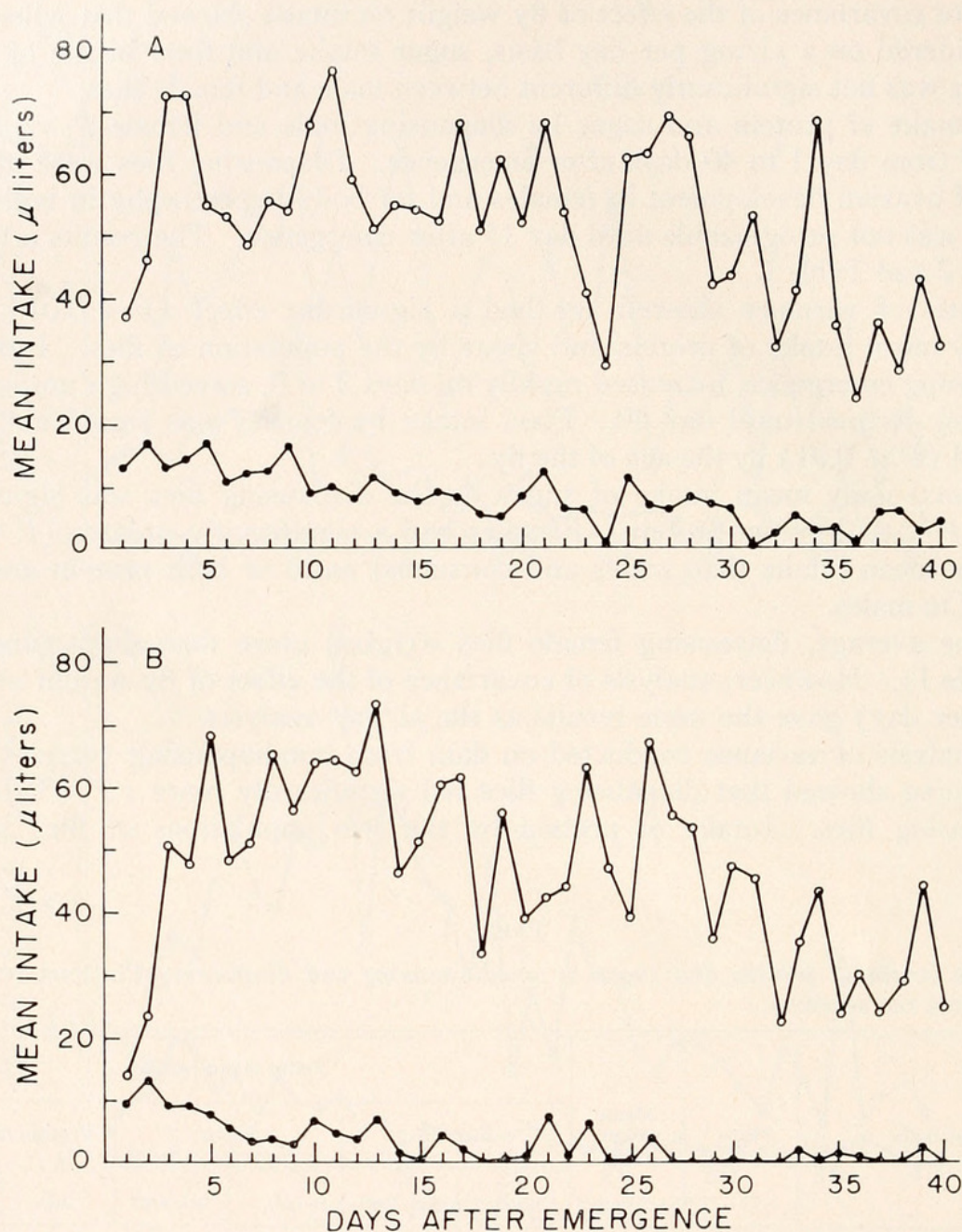


FIGURE 2. Total daily mean intake (μ l) of protein (solid circles) and sugar (open circles) by diapausing female (A) and male (B) *P. regina* from day 1 to 40 days after emergence. Each point on the graphs represents the daily mean intake for seven flies minus the mean evaporation for that day and for that diet.

significantly different; but diapausing flies fed significantly more ($P < 0.01$) on sugar than the nondiapausing flies. In both experimental situations, females had a greater total food intake than males. There was a significantly greater ($P < 0.01$) effect of age on the total daily mean intake of the diapausing population compared to the nondiapausing population.

Diapausing individuals of *P. regina* weighed less, on the average, than the nondiapausing populations. An analysis of covariance of the effect of fly weight on intake (μ l/mg per day) gave results similar to those for the μ l/day analysis.

Nondiapausing and diapausing P. terraenovae

The previous experiments were repeated with modifications using the arctic blowfly, *P. terraenovae*. The results on the intake of protein and sugar by nondiapausing *P. terraenovae* from day 1 to 25 days after emergence are shown in Figure 3 and Table I.

Analysis of variance showed age had a significant effect ($P < 0.01$) on the total daily mean intake of protein and sugar by the population of flies. Intake was low during the first day after emergence, increased on days 2 to 3, remained high

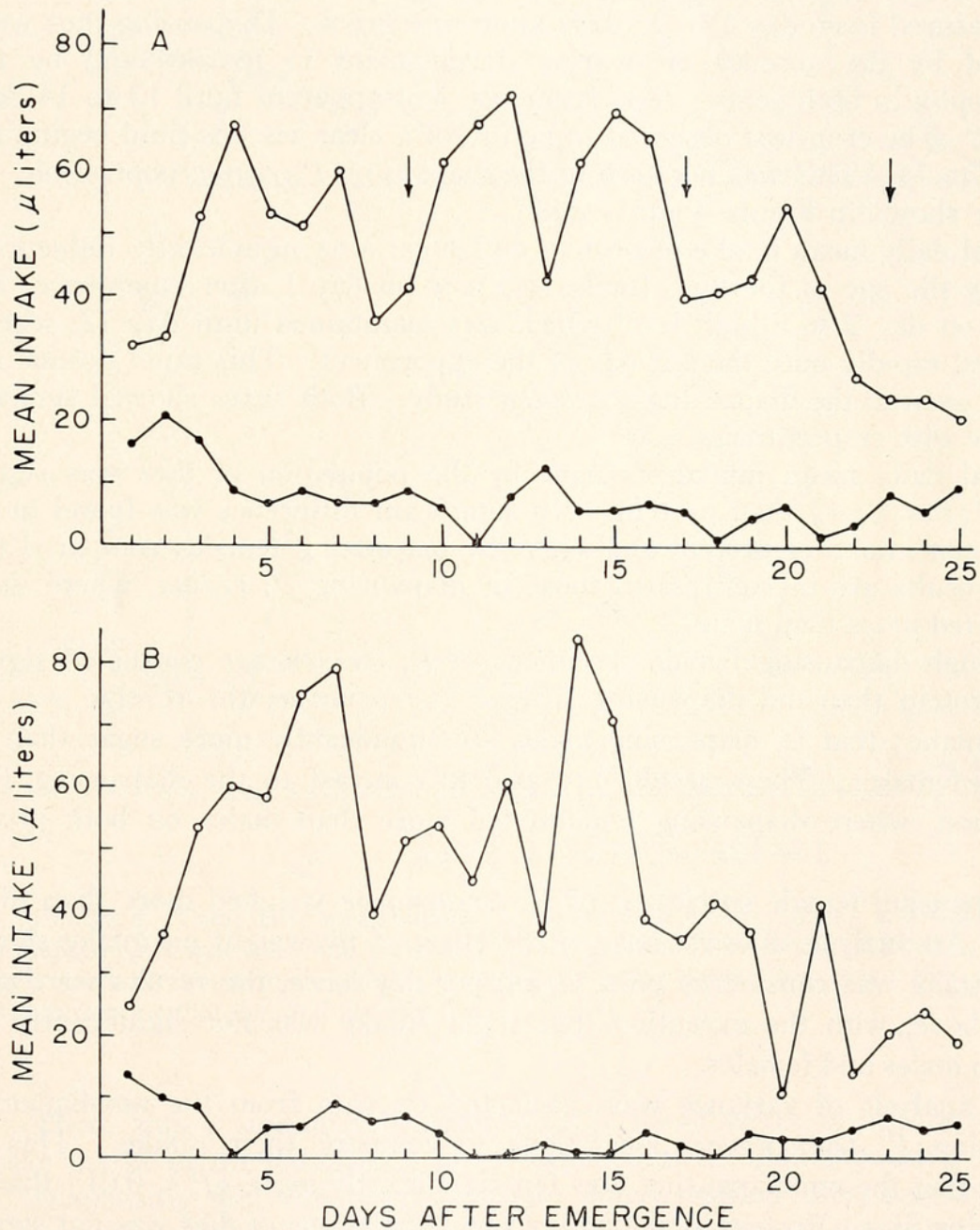


FIGURE 3. Total daily mean intake (μ l) of protein (solid circles) and sugar (open circles) by nondiapausing female (A) and male (B) *P. terraenovae* from day 1 to 25 days after emergence. Each point on the graphs represents the daily mean intake for seven flies minus the mean evaporation for that day and for that diet. Arrows indicate days on which eggs were laid.

until days 14 to 15 and then declined steadily to day 25. Both sexes showed a similar trend in intake with respect to age. The total daily mean intake of sugar by the population of flies was significantly greater ($P < 0.01$) than protein. Females had a significantly greater total daily mean intake than males and consumed more of both protein and sugar compared to males.

In general, the results of analysis of covariance were similar to those for the nondiapausing *P. regina* population. That is, when intake was considered on a $\mu\text{l}/\text{mg}$ per day basis, sugar intake and total intake of protein plus sugar was not significantly different between male and female flies.

The intake of protein and sugar by diapausing male and female *P. terraenovae* was measured from day 1 to 25 days after emergence. Diapausing flies were characterized by the absence of ovarian development in females and by fat body hypertrophy in both sexes. The latter was not apparent until 13 to 14 days after eclosion. The crop was observed to be full of a clear viscous fluid beginning about days 13 to 14. This was not seen in the diapausing *P. regina* population. The results are shown in Figure 4 and Table I.

Total daily mean intake of protein and sugar was significantly influenced ($P < 0.01$) by the age of the fly. Intake was low on day 1 after emergence, increased rapidly on day 2 to a high level which was maintained until day 12, when intake decreased rapidly until the last day of the experiment. This rapid decline in intake was not seen in the diapausing *P. regina* study. Both sexes showed similar trends in intake with respect to age.

Total daily mean intake of sugar by the population of flies was significantly greater ($P < 0.01$) than protein. No significant difference was found in the total daily mean intake of protein and sugar by diapausing females compared to males. These results are in contrast to those of diapausing *P. regina*, where diapausing females fed more than males.

Though diapausing female specimens of *P. terraenovae* consumed significantly more protein than did diapausing male *P. terraenovae*, the reverse was true for sugar intake; that is, diapausing males ate significantly more sugar than the diapausing females. These results are also in contrast to the diapausing *P. regina* population, where diapausing females fed more than males on both protein and sugar.

Diapausing female specimens of *P. terraenovae* weighed more than diapausing males. An analysis of covariance of the effect of fly weight on intake showed that when intake was considered on a $\mu\text{l}/\text{mg}$ per day basis, the results were similar to those above, with the exception that sugar intake was not significantly different between males and females.

An analysis of variance was conducted on data from the nondiapausing and diapausing *P. terraenovae* populations to compare their intake. This analysis showed that the nondiapausing flies fed significantly more ($P < 0.01$) than did the diapausing flies. Protein intake by the two populations of flies was not significantly different, but nondiapausing flies consumed significantly more ($P < 0.01$) sugar than the diapausing flies. These results are different from those obtained with *P. regina*, where the diapausing population fed more than the nondiapausing population.

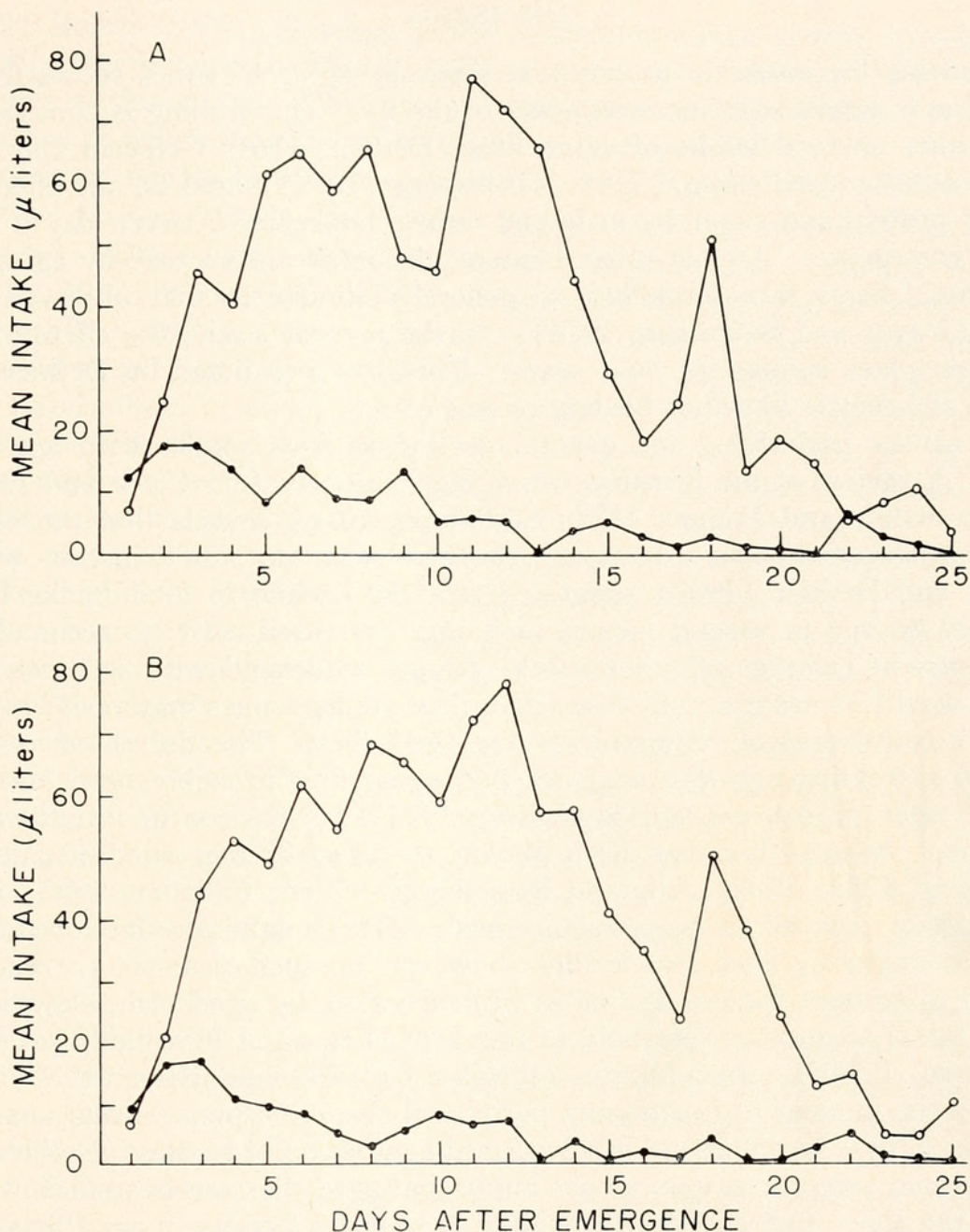


FIGURE 4. Total daily mean intake (μ l) of protein (solid circles) and sugar (open circles) by diapausing female (A) and male (B) *P. terraenovae* from day 1 to 25 days after emergence. Each point on the graphs represents the daily mean intake for seven flies minus the mean evaporation for that day and for that diet.

There was a significantly greater ($P < 0.01$) effect of age on the total daily mean intake of the diapausing flies compared to the nondiapausing flies.

Diapausing flies weighed more than nondiapausing flies, on the average. An analysis of covariance of the effect of fly weight on intake showed that when intake was considered on a μ l/mg per day basis, total intake of protein plus sugar was not significantly different between diapausing and nondiapausing flies. This is in contrast to what was seen for *P. regina*, where nondiapausing flies fed significantly less than diapausing flies on a μ l/mg per day basis.

DISCUSSION

Measuring the intake of protein and sugar by aging blowflies shows that there is a decline in intake with increasing age of the fly. This finding is consistent with other studies on food intake of aging flies (Dethier, 1961; Gelperin and Dethier, 1967; Roberts and Kitching, 1974). Greenberg (1959) found no age effect on the intake of protein and sugar by male and female houseflies between day 6 and day 20 after emergence. Among other Diptera, the intake of sucrose by aging female mosquitoes, *Aedes taeniorhynchus*, is generally similar to that observed for the blowfly (Nayar and Sauerman, 1974). In the present study, the effect of age on food intakes was similar for both sexes. This was also noted by Dethier (1961) for male and female blowflies feeding on sugar.

The causes underlying the general decline in food intake with age are not known. A review of the literature on aging in insects (*cf.*, Clark and Rockstein, 1964; Rockstein and Miquel, 1973; Stoffolano, 1976) reveals that numerous degenerative structural and functional changes occur in adult insects with age. Gelperin and Dethier (1967) suggested that the decline in food intake by aging flies could be due to several factors including decreased activity, accumulation of stored nutrient reserves or other subtle changes associated with senescence. The fact that aged flies are generally less active than younger ones may result in reduced energy or carbohydrate requirements for aged flies. The decreased respiratory rate with increasing age also suggests that aging flies probably need less food as they get older (Calabrese and Stoffolano, 1974). A decline in fecundity and in the number of eggs laid has been shown to occur among aged female insects (Greenberg, 1955; Woke, Ally and Rosenberger, 1956; Callahan, 1962) implying concomitant reduced nitrogen requirements. This might account for the decline in protein intake by aged female flies; however, no such clear-cut correlation can be found to account for the decline in protein intake by aged male blowflies.

The tarsal acceptance threshold of nondiapausing adult blowflies rises with age (Stoffolano, 1974b). In addition, Stoffolano (1973) and Rees (1970) noted a decline in the number of functioning peripheral chemoreceptor sensilla among aged flies. Since these receptors are involved in the initiation of feeding (Dethier, 1969), a degenerated receptor system would imply that aged flies are less efficient feeders. Each of the above factors could contribute to reduced feeding in aged flies. In the final analysis, however, this decline in food intake is probably due to several factors, no specific one of which is more important than the others.

Females of both species have a greater total intake of both protein and sugar compared to males (when compared on a $\mu\text{l/day}$ basis). However, when total daily mean intake and total daily mean sugar intake by males are compared to that of females on a $\mu\text{l/mg}$ per day basis, there is no significant difference between the two. Female flies weigh more than males and tend to feed more, though not significantly more. To our knowledge, this study is the first to compare intake values on a $\mu\text{l/mg}$ basis. Previously, Greenberg (1959) found no significant difference in sucrose intake for female compared to male houseflies, and Roberts and Kitching (1974) found that female specimens of *Lucilia cuprina* consumed more sugar and protein compared to males.

In the present study, the finding that female flies consumed significantly more protein compared to males is consistent with previous studies (Greenberg, 1959;

Dethier, 1961; Belzer, 1970), although peaks of protein intake closely correlated with female reproductive cycles (Belzer, 1970; Roberts and Kitching, 1974) were not as apparent in this study. This was probably due to the different environmental conditions at which the flies in this study were reared and tested.

Vitellogenesis in female blowflies is dependent on a protein meal (Rasso and Fraenkel, 1954; Harlow, 1956; Orr, 1964; Belzer, 1970; Bennetová-Řežabová, 1972; Stoffolano, 1974a), while spermatogenesis is independent of feeding in *P. regina* (Cowan, 1932; Mackerras, 1933, Stoffolano, 1974a) and also in other flies (Chaudhury and Ball, 1973). Thus, it would seem obvious that the protein intake of females would be greater than that of males. However, as shown in this study (see Fig. 1B) and others (Dethier, 1961; Belzer, 1970), male blowflies also show a distinct protein selection. A protein meal is necessary for male *P. regina* to develop the reproductive accessory glands and may be required to activate the centers that influence the development of this gland (Stoffolano, 1974a).

The intake of sugar by male and female blowflies is overwhelmingly greater than the intake of protein. Dethier (1961) and Roberts and Kitching (1974) also found that flies having free access to both protein and sugar always consumed greater volumes of the carbohydrate. Dethier (1969) further concluded that the principle food of the blowfly is carbohydrate.

In the present study sugar intake fluctuated markedly. Dethier (1961) attributed these fluctuations to differences in the activity of the fly which correlated with variations in the climate of the laboratory (temperature and humidity were not held constant). Gelperin and Dethier (1967) noted that even under constant temperatures, some fluctuation in sugar intake remains. Our results show that even when temperature and humidity are controlled, these fluctuations in sugar intake still persist.

The second part of the present study dealt with the question of feeding in diapausing flies. The evidence reported in this paper indicates that flies reared in the laboratory under diapause-inducing conditions and exhibiting characteristics indicative of imaginal diapause (arrested ovarian development and hypertrophied fat body) do feed on both protein and sugar.

Reports in the literature concerning insect feeding during diapause are contradictory. Although many insects have been reported to feed while in a reproductive arrest, this activity may be reduced and limited to nonprotein sources and to a "pre-diapause" period (de Wilde, 1954; El-Hariri, 1965; Mitchell and Taft, 1966; Stoffolano, 1968; Hodek, 1971; Brown and Chippendale, 1974).

Mansingh (1971) has divided diapause into three periods and defines "pre-diapause" as a preparatory period when food taken in by the insect is accumulated and stored. These nutrient reserves are used as an energy source to maintain basal metabolic functions during the succeeding "diapause period," when protein synthesis and basal metabolism are reduced. Growth and development are resumed during the "post-diapause" period. Feeding behavior observed for the diapausing flies in this study may represent food intake during a "pre-diapause" period. In both species, and especially *P. terraenovae*, total intake declined during the course of the experiment. This decline could represent the onset of the "diapause" period when feeding is reduced. Forty-day old diapausing specimens of *P. regina* had significantly reduced sucrose intake compared to 40-day old nondiapausing flies

(Stoffolano, 1975). The observation made in the present study that fat body hypertrophy develops in diapausing flies after 12–15 days of feeding suggests that this food taken in by the insect is being accumulated and stored.

In nature, *P. regina* and *P. terraenovae*, entering reproductive diapause in response to shortened fall photoperiods, may continue to feed actively during this time when environmental conditions still permit activity and food sources are still abundant. The adult may feed and even engorge on food to build up metabolic reserves (such as glycogen and fat) to be used as an overwintering energy source. It seems obvious that such a period of feeding when large accumulations of food are set aside for winter should exist (Stoffolano, 1974b).

As already stated, nondiapausing female blowflies require a protein meal to develop their ovaries and therefore will selectively increase their protein intake prior to vitellogenesis. Procedures which disrupt normal ovarian development also disrupt protein feeding (Dethier, 1961). During diapause the ovaries do not develop and yet the evidence presented in this study indicates that the amount of protein taken by diapausing blowflies compared to nondiapausing flies was not significantly different (Table I).

In mosquitoes, the term gonadotrophic dissociation (Swellengrebel, 1929) is commonly applied to any situation where ovaries remain undeveloped in females that have taken a full blood meal (Eldridge, 1966). In such mosquitoes, a large fat body often develops. The function of ovarian diapause may be to divert nutrients from the ovary to the fat body which serves as a storage depot (Spielman and Wong, 1973). In this study, both species of diapausing flies showed fat body hypertrophy. It is not known whether a diapausing female fly taking a protein meal in the fall needs to feed on protein in the spring to develop ovaries.

The feeding behavior of the two species of diapausing blowflies differed in several respects when compared to each other and their nondiapausing counterparts. Intake by diapausing *P. terraenovae* was not significantly different from the nondiapausing population (on a $\mu\text{l}/\text{mg}$ per day basis), while diapausing specimens of *P. regina* fed significantly more than nondiapausing flies. The protein intake of both diapausing and nondiapausing populations of flies was not significantly different; differences in sugar intake accounted for the differences seen in total intake. In addition, an enlarged crop full of a clear viscous fluid was seen in diapausing *P. terraenovae* but not in the diapausing *P. regina* population. These differences in feeding behavior could be the result of confounding effects of differences in temperature regimes experienced by the nondiapausing and diapausing *P. regina* populations as already discussed. They may also be related to differences in the ecology of the two species of flies.

Since diapause represents a physiological adaptation evolved to overcome adverse environmental conditions of a particular climatic zone (Mansingh, 1971), the two species may have evolved different overwintering strategies in response to conditions peculiar to their own ecology. *P. regina*, a temperate region species in which diapause is temperature-dependent, may continue to feed while the weather permits. *P. terraenovae* is an arctic species and enters diapause apparently independent of the temperature. Other cues, such as the enlarged crop, may control feeding behavior in this species. Knowledge of the winter biology of these two

species is limited, and consequently further study on the behavior of these flies in nature is needed.

Intake is more affected by the age of the fly in the diapausing populations than in the nondiapausing population of flies. This effect was greater for *P. terraenovae* than for *P. regina*. In diapausing specimens of *P. terraenovae*, the decline in feeding was coincident with the appearance of fat body hypertrophy and a full crop during days ± 14 . As the abdomen of the diapausing fly becomes distended due to fat hypertrophy, and in the case of *P. terraenovae* a full crop, body wall stretch receptors may send impulses to the central nervous system resulting in a decline of food intake. This inhibition may occur at the central level of the nervous system rather than at the peripheral level, since the tarsal acceptance threshold of diapausing flies is not different from that of nondiapausing flies (Stoffolano, 1975). Thus it may *not* be the same system of negative feedback operating in the nondiapausing replete fly to inhibit further feeding (Gelperin, 1971) as is operating in the diapausing fly to reduce feeding. However, rather than a real aging effect, which is irreversible, these other factors may have resulted in the decline in food intake and thus the statistically greater aging effect seen in diapausing *P. terraenovae* compared to the nondiapausing populations. Forty-day old diapausing specimens of *P. regina*, removed from diapause conditions and held at nondiapause conditions for 13 days, show an increase in food intake comparable to that of 40-day old nondiapausing flies (Stoffolano, 1975).

The feeding behavior of these flies in nature before, during, and after the overwintering diapause is not known. However, our results are consistent with the idea that diapause in *P. regina* and *P. terraenovae* may occur in three stages as outlined by Mansingh (1971). The first stage, characterized as a preparatory stage, is accompanied by trophic levels of metabolism and arrested ovarian development. Though the insect continues to feed, due to reduced levels of protein synthesis and the occurrence of gonadotrophic dissociation, nutrients are not metabolized along reproductive pathways but along so called "diapause pathways". This accumulation of nutrient reserves results in fat body hypertrophy which may act on the central nervous system *via* stretch receptors to inhibit feeding. During the second stage of diapause, the insect ceases feeding, and respiration drops to the level of basal metabolism (Slama, 1964). The first stage of diapause or prediapause would be synchronized to occur with the still favorable environmental conditions existing in the fall. The insect could take advantage of these conditions to feed and store this food as reserves to be used later during the severe conditions of freezing temperatures and lack of food sources associated with winter. The lower rate of metabolism during the winter presumably leads to a slow utilization of food reserves. As the food reserves are utilized, neural inhibition may be released and the post-diapause adult may feed again when warmer temperatures in the early spring permit activity.

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SUMMARY

1. The long-term intake of protein and sugar by nondiapausing and diapausing male and female *P. regina* and *P. terraenovae* was determined using a two-choice apparatus with statistical analysis of the results (both $\mu\text{l/day}$ and $\mu\text{l/mg per day}$).

2. Results of studies using nondiapausing *P. regina* and *P. terraenovae* were found to be similar. Intake was significantly influenced by the age of the fly, aged flies tending to feed less. Males and females exhibited similar trends in food intake as they aged. Sugar intake by male and female blowflies was significantly greater than protein intake. Female flies consumed significantly more protein than males. However, on a $\mu\text{l/mg}$ basis there was no significant difference in either sugar intake or total intake of protein plus sugar between male and female flies.

3. Diapausing flies were found to feed on both sugar and protein, although sugar intake was significantly greater than protein intake. Protein intake by both species of diapausing flies did not differ significantly from that of nondiapausing flies. However, diapausing specimens of *P. regina* had a significantly greater sugar intake than the nondiapausing *P. regina* population; while diapausing specimens of *P. terraenovae* fed significantly less on sugar than the nondiapausing *P. terraenovae*. These results were the same when considered on a $\mu\text{l/mg}$ basis. In the *P. terraenovae* population, intake was found to be more influenced by age among diapausing flies compared to nondiapausing flies.

4. An analysis of covariance showed that the effect of fly weight on intake was not significant.

LITERATURE CITED

- BELZER, W., 1970. The control of protein ingestion in the black blowfly, *Phormia regina* (Meigen). Ph.D. Dissertation, University of Pennsylvania, Philadelphia, Pennsylvania, 195 pp. (Diss. Abstr., **31**(6): 3579B; order no. 70-25,626.)
- BENNETOVÁ-ŘEŽABOVÁ, B., 1972. The regulation of vitellogenesis by the central nervous system in the blowfly, *Phormia regina* (Meigen). *Acta Entomol. Bohemoslov.*, **69**: 78-88.
- BROWN, J. J., AND G. M. CHIPPENDALE, 1974. Migration of the monarch butterfly, *Danaus plexippus*: energy sources. *J. Insect Physiol.*, **20**: 1117-1130.
- CALABRESE, E. J., AND J. G. STOFFOLANO, JR., 1974. The influence of age and diet on respiration in adult male and female blowflies, *Phormia regina*. *J. Insect Physiol.*, **20**: 383-393.
- CALLAHAN, R. F., 1962. Effects of parental age on the life cycle of the house fly *Musca domestica* Linnaeus (Diptera: Muscidae). *J. N. Y. Entomol. Soc.*, **70**: 150-158.
- CHAUDHURY, M. F. B., AND H. J. BALL, 1973. The effect of age, nutritional factors, and gonadal development on the mating behavior of the face fly, *Musca autumnalis*. *J. Insect Physiol.*, **19**: 57-64.
- CLARK, A. M., AND M. ROCKSTEIN, 1964. Aging in insects. Pages 227-281 in M. Rockstein, Ed., *The Physiology of Insecta*, Vol. I. Academic Press, New York and London.
- COUSIN, G., 1932. Étude expérimentale de la diapause des insectes. *Bull. Biol. Fr. Belg. Suppl.*, **15**: 1-341.
- COWAN, F. A., 1932. A study of fertility in the blowfly, *Phormia regina* Meigen. *Ohio J. Science*, **32**: 389-392.

- DANILEVSKII, A. S., 1965. *Photoperiodism and seasonal development of insects*. Oliver and Boyd, London, 283 pp.
- DETHIER, V. G., 1961. Behavioral aspects of protein ingestion by the blowfly *Phormia regina* Meigen. *Biol. Bull.*, **121**: 456-470.
- DETHIER, V. G., 1969. Feeding behavior of the blowfly. Pages 111-266 in D. S. Lehrman, R. A. Hinde, and E. Shaw, Eds., *Advances in the study of behavior*, Vol. 2. Academic Press, New York.
- ELDRIDGE, B. F., 1966. Environmental control of ovarian development in mosquitoes of the *Culex pipiens* complex. *Science*, **151**: 826-828.
- EL-HARIRI, G., 1965. Studies of the physiology of hibernating Coccinellidae (Coleoptera): changes in the metabolic reserves and gonads. *Proc. R. Entomol. Soc. London*, **40**: 133-144.
- GELPERIN, A., 1971. Regulation of feeding. *Annu. Rev. Entomol.*, **16**: 365-378.
- GELPERIN, A., AND V. G. DETHIER, 1967. Long-term regulation of sugar intake by the blowfly. *Physiol. Zool.*, **40**: 218-228.
- GREENBERG, B., 1955. Fecundity and cold survival of the housefly. *J. Econ. Entomol.*, **48**: 654-657.
- GREENBERG, B., 1959. Housefly nutrition. *J. Cell Comp. Physiol.*, **53**: 169-177.
- HARLOW, P. M., 1956. A study of ovarian development and its relation to adult nutrition in the blowfly *Protophormia terraenovae* (R. D.). *J. Exp. Biol.*, **33**: 777-797.
- HODEK, I., 1971. Termination of adult diapause in *Pyrhocris apterus* (Heteroptera: Pyrrhocoridae) in the field. *Entomol. Exp. Appl.*, **14**: 212-222.
- LEES, A. D., 1955. *The physiology of diapause in arthropods*. Cambridge University Press, London, 151 pp.
- MACKERRAS, M. J., 1933. Observations on the life histories, nutritional requirements and fecundity of blowflies. *Bull. Entomol. Res.*, **24**: 353-362.
- MANSINGH, A., 1971. Physiological classification of dormancies in insects. *Can. Entomol.*, **103**: 983-1009.
- MITCHELL, E. R., AND H. M. TAFT, 1966. Starvation method for obtaining diapausing boll weevils able to survive the winter hibernation. *J. Econ. Entomol.*, **59**: 55-57.
- NAYAR, J. K., AND D. M. SAUERMAN, JR., 1974. Long-term regulation of sucrose intake by the female mosquito, *Aedes taeniorhynchus*. *J. Insect Physiol.*, **20**: 1203-1208.
- NORMANN, T. C., AND H. DUVE, 1969. Experimentally induced release of neuroendocrine influencing hemolymph trehalose level in *Calliphora erythrocephala* (Diptera). *Gen. Comp. Endocrinol.*, **12**: 449-459.
- ORR, C. W. N., 1964. The influence of nutritional and hormonal factors on egg development in the blowfly *Phormia regina* (Meig.). *J. Insect Physiol.*, **10**: 53-64.
- RASSO, S. C., AND G. FRAENKEL, 1954. The food requirements of the adult female blowfly, *Phormia regina* (Meigen), in relation to ovarian development. *Ann. Entomol. Soc. Am.*, **47**: 636-645.
- REES, C. J. C., 1970. Age dependency of response in an insect chemoreceptor sensillum. *Nature*, **227**: 740-742.
- ROBERTS, J. A., AND R. L. KITCHING, 1974. Ingestion of sugar, protein and water by adult *Lucilia cuprina* (Wied.) (Diptera, Calliphoridae). *Bull. Entomol. Res.*, **64**: 81-88.
- ROCKSTEIN, M., AND J. MIQUEL, 1973. Aging in insects. Pages 371-478 in M. Rockstein, Ed., *Physiology of Insecta*, Vol. I. Academic Press, New York and London.
- ROUBAUD, E., 1927. Sur l'hibernation de quelques mouches communes. *Bull. Soc. Entomol. Fr.*, pp. 24-25.
- SPIELMAN, A., AND J. WONG, 1973. Studies on autogeny in natural populations of *Culex pipiens* III. Midsummer preparation for hibernation in anautogenous populations. *J. Med. Entomol.*, **10**: 319-324.
- SLAMA, K., 1964. Hormonal control of respiratory metabolism during growth, reproduction and diapause in female adults of *Phyrrhocris apterus* L. (Hemiptera). *J. Insect Physiol.*, **10**: 283-303.
- STOFFOLANO, J. G., JR., 1968. The effect of diapause and age on the tarsal acceptance threshold of the fly, *Musca autumnalis*. *J. Insect Physiol.*, **14**: 1205-1214.
- STOFFOLANO, J. G., JR., 1973. The effect of age and diapause on the mean impulse frequency

- and failure to generate impulses in chemoreceptor labellar sensilla of *Phormia regina*. *J. Gerontol.*, **28**: 35-39.
- STOFFOLANO, J. G., JR., 1974a. Influence of diapause and diet on the development of the gonads and accessory reproductive glands of the black blowfly *Phormia regina* (Meigen). *Can. J. Zool.*, **52**: 981-988.
- STOFFOLANO, J. G., JR., 1974b. Control of feeding and drinking in diapausing insects. Pages 32-47 in L. Barton Browne, Ed., *Experimental analysis of insect behavior*. Springer-Verlag, New York, Heidelberg, and Berlin.
- STOFFOLANO, J. G., JR., 1975. Central control of feeding in the diapausing adult blowfly *Phormia regina*. *J. Exp. Biol.*, **63**: 265-271.
- STOFFOLANO, J. G., JR., 1976. Insects as model systems for aging studies. Pages 408-427 in M. F. Elias, B. E. Eleftheriou, P. K. Elias, Eds., *Special review of experimental aging research*. EAR, Inc., Bar Harbor, Maine, 481 pp.
- STRANGEWAYS-DIXON, J., 1961. The relationship between nutrition, hormones and reproduction in the blowfly *Calliphora erythrocephala* (Meig.). *J. Exp. Biol.*, **38**: 225-235.
- SWELLENGREBEL, N. H., 1929. La dissociation des fonctions sexuelles et nutritives (dissociation gonotrophique) d'*Anopheles maculipennis* comme cause du Paludisme dans les Pays-Bas et ses rapports avec "l'infection domiciliaire." *Ann. Inst. Pasteur (Paris)*, **43**: 1370-1389.
- DE WILDE, J., 1954. Aspects of diapause in adult insects. *Arch. Neerl. Zool.*, **10**: 375-385.
- WINSTON, P. W., AND D. H. BATES, 1960. Saturated solutions for the control of humidity in biological research. *Ecology*, **41**: 232-237.
- WOKE, P. A., M. S. ALLY, AND C. R. ROSENBERGER, JR., 1956. The number of eggs developed related to the quantities of blood ingested in *Aedes aegypti* (L.) (Diptera: Culicidae). *Ann. Entomol. Soc. Am.*, **49**: 435-441.



Greenberg, Sharon L and Stoffolano, John G. 1977. "THE EFFECT OF AGE AND DIAPAUSE ON THE LONG-TERM INTAKE OF PROTEIN AND SUGAR BY TWO SPECIES OF BLOWFLIES, PHORMIA REGINA (MEIG.) AND PROTOPHORMIA TERRAENOV AE (R. D.)." *The Biological bulletin* 153, 282–298.

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