

Biological and Ecological Studies of *Hydrotaea aenescens* (Wiedemann)
(Diptera: Muscidae), and Other Selected Arthropods of High-Rise
Cage Layer Poultry Houses

by

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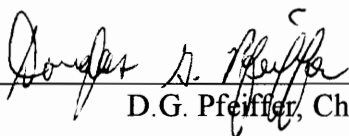
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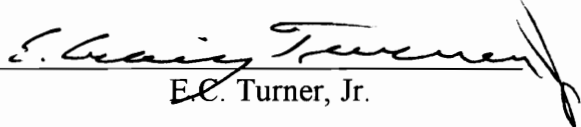
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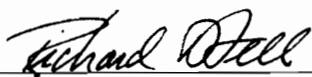
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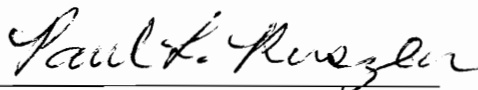
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Entomology

(ABSTRACT)

Laboratory and field studies of *Hydrotaea aenescens* were undertaken to provide information on this predator's biology and ecology under differing conditions, and to promote its use as a biocontrol agent for controlling house flies in poultry houses.

Taxonomic diversity of arthropods in a new high-rise cage layer poultry house was determined from manure samples collected from different manure moisture categories. Stabilization of taxonomic diversity and taxonomic evenness of manure-inhabiting arthropods occurred after the house had been operating for eight months. In contrast, overall diversity in high-rise poultry houses at a well managed, longer established farm was significantly higher than that observed at the new farm, even after 1 1/2 years of operation.

When the densities of selected manure-inhabiting predatory arthropods collected from the manure samples (including *Carcinops pumilio*, pseudoscorpions, a dermapteran species, an anthocorid species, and *H. aenescens*) were correlated with percent manure moisture, the results showed that, of these predators, only *H. aenescens* was positively correlated with both manure moisture and with densities of house fly larvae. This information emphasized that although predators such as *C. pumilio* may exhibit high predation rates on house fly eggs and first instars, their effectiveness is reduced by their spatial separation from their supposed prey. This contrasts greatly with *H. aenescens* performance.

Decreases in survival of house fly larvae occurred when the larvae were exposed to *H. aenescens* of higher larval stadia. This was dramatically demonstrated when 100 first instar house flies were exposed to 100 second instar *H. aenescens*. No house fly larvae survived.

Developmental times were determined at constant temperatures for egg, and larval *H. aenescens*. Developmental times decreased as temperature increased. Median time for egg and larval development ranged from 1.3 and 14.6 days at 22.2°C to 0.5 and 8.3 days at 35.0°C, for the respective stages.

All of this information, together with developmental times and mortality of *H. aenescens* immatures gathered in a study of temperature dependent development has enhanced understanding of the biotic interactions in accumulated poultry manure. These data will be invaluable in designing integrated pest management programs especially in the area of computer-aided decision making.

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Chapter 1

INTRODUCTION

Virginia ranks 20th-21st in the nation in egg production, with 894 million eggs (both commercial and hatching) being produced each year, and 64 million dollars in cash receipts generated annually. However, in Virginia as well as in the rest of the United States, continuing urbanization of rural farm lands, and increased awareness of environmental and health issues have generated rising concern about pests produced by modern farming methods. This is especially true for commercial egg-producers operating high density confinement facilities where manure accumulates in large amounts. In fact, C.V. Reddy in *Misset-World Poultry* (1992), estimated that a layer-farm housing 50,000 birds produces about six tons of fresh manure daily, with a total of more than 2,000 tons a year.

Manure which accumulates in these facilities serves as an ideal substrate for ovipositing females of the house fly *Musca domestica* L. (Diptera: Muscidae) which is recognized world-wide as one of the most important pests of livestock and humans. Typical complaints of residents near commercial egg producing facilities range from simple annoyance to concerns about potential disease and sanitation problems. Such concerns have resulted in serious financial and legal consequences for some producers, and a strong trend for most of them to almost exclusively rely on costly chemical control measures. Moreover, the dominance of insecticide control strategies has resulted in widespread development of insecticide resistance in house fly populations.

However, when properly managed, the manure environment of high rise, cage-layer houses contains a complex of numerous arthropod species which can prevent explosive outbreaks of house fly populations. Many studies have been conducted on the predators of house flies in poultry houses, with most of the work centering on *Carcinops pumilio* (Erichson) (Coleoptera: Histeridae), and *Macrocheles muscaedomesticae* (Scopoli) (Acarina: Machrochelidae). Flies in the genus *Hydrotaea* (formerly *Ophyra*), which also form part of this complex, are facultatively predaceous on house fly larvae, but until recently there has been a reluctance to recognize *Hydrotaea aenescens* as a key predator, with greater emphasis being placed on the advantages of *C. pumilio* instead. *H. aenescens* is normally found in low densities, but as part of an integrated pest management (IPM) program, it can be used to control house flies. A typical IPM program would involve manure management, house fly population monitoring, inundative releases of *H. aenescens*, and judicious use of insecticides. In fact, effective, economical house fly control has been achieved using this type of program, with significant reduction in insecticide application (Turner et al. 1992). This work has led to the establishment of *H. aenescens* colonies at commercial egg facilities in Virginia, Minnesota, New Jersey, Ohio, Tennessee, Florida, and Canada, and it is anticipated that interest in its use will grow.

To facilitate successful integration into control programs, further information is needed about *H. aenescens* biology and ecology under differing conditions. This study was designed to develop this essential information. Specific objectives were to:

- (1) determine the relationships of selected manure-inhabiting arthropods to manure moisture;

- (2) examine faunal succession and diversity of arthropods at a new high rise cage layer facility, and to compare these results with similar data from an older, well managed facility;
- (3) determine predation rates of *H. aenescens* on house flies under varying levels of prey density, predator and prey larval stadia, manure moisture, and in the presence or absence of the predator *C. pumilio*;
- (4) determine temperature-dependent development rates and survival of immature *H. aenescens*.

The information derived from these studies can be used to better understand what is needed in newly instituted high-rise cage layer poultry facilities to initiate successful house fly control programs. It can also be used in programs where *H. aenescens* is currently employed, in order to further understand the dynamics of the system. Added to this, measurements of development rates and mortality can be incorporated into computer simulation models for house fly management.

Chapter 2

LITERATURE REVIEW

Arthropod Fauna of Poultry Manure

Numerous studies have been conducted in many regions of the world on the fauna associated with poultry manure and other confined-livestock manure. Several of these studies are concerned with fauna present in terms of pests and their natural enemies (Peck & Anderson 1969, Axtell 1970a, Legner & Olton 1970, Green 1980, Hulley 1983). Most of the studies that were conducted document the distribution and abundance of many fly predators and parasites as well (Legner & Olton 1970, Legner et al. 1975, Pfeiffer & Axtell 1980, Green 1980, Hulley 1986, Hulley & Pfeleiderer 1988, Rueda et al. 1990). Additional work focused on the effect of various biotic factors such as competition, prey density, and alternative prey or hosts (Geden & Stoffolano 1988, Legner & Dietrick 1989). Other workers looked at abiotic factors such as temperature, humidity, manure moisture content, farm management practices, and manure accumulation time (Peck & Anderson 1969, Bills 1973, Legner et al. 1973, Dunning et al. 1978, Green 1982, Geden & Stoffolano 1987, Stafford & Collison 1987, Stafford & Bay 1987, Geden & Stoffolano 1988, Hulley & Pfeleiderer 1988, Morgan et al. 1988, Stafford et al. 1988, Wills & Mullens 1991).

Overwhelmingly, the predominant fly species noted were the house fly, *Musca domestica* L. (Diptera: Muscidae) and Sphaerocerid spp. (Peck & Anderson 1969, Hulley 1983, Armitage 1986), with the most numerous and important predators being *Carcinops* spp. [*C. pumilio* (Erichson) or *C. troglodytes* (Erichson)] (Coleoptera: Histeridae), and the mite *Macrocheles muscaedomesticae* (Scopoli) (Acarina: Macrochelidae) (Legner &

Olton 1970, Bills 1973, Pfeiffer & Axtell 1980, Hulley 1983, Armitage 1986, Hulley & Pfleiderer 1988). These studies have demonstrated that in poultry manure a relatively small number of key species make up the large population of predators. These species occur similarly in accumulated manure across broad geographical areas, and predictable patterns of arthropod succession occur (Geden 1990).

Another predator not normally occurring in large populations is the fly *Hydrotaea* (= *Ophyra*) *aenescens* (Weidemann) (Diptera: Muscidae) (Nolan & Kissam 1985). This predator is receiving increasing attention from both researchers and poultry operators due to the fly's ability to effectively control populations of house flies in high-rise cage layer houses when sustained augmentative releases of this predator are conducted.

The Black Dump Fly, *Hydrotaea aenescens* (Weidemann)

Taxonomic Status

According to Farkas and Papp (1991), close relationships between species in the genus *Hydrotaea* with those in *Ophyra* justified placing both groups into a single genus *Hydrotaea*. The genus *Ophyra* contained the species; *O. aenescens*, *O. capensis*, *O. anthrax*, and *O. leucostoma*. The first three have retained their species names and are now properly known as *Hydrotaea aenescens*, *Hydrotaea capensis*, and *Hydrotaea anthrax* (Farkas & Papp 1991). *O. leucostoma* has been renamed *H. ignava*. Although the genera were combined years ago, the use of the name *Ophyra* has persisted, especially in the United States.

The genus *Hydrotaea* formerly contained 26 species (Huckett 1987). Of these, 15 species are Holarctic in distribution, and 7 species are considered exclusively indigenous to Nearctic (Huckett 1954). Adults of several species, including *H. irritans*, are known to cause discomfort to humans and livestock from the flies' persistent habit of attempting to feed on the secretions of the skin, eyes, nostrils, and lips (Huckett 1954, Greenberg

1971). The larvae of several species, including *H. dentipes*, and *H. irritans* are also known predators of other dipterous larvae, with the inception of predaceous behavior varying from second to third instar for the species (Greenberg 1971, West 1951).

Adults of *Hydrotaea* spp. (*Ophyra*), are small (6 mm), shiny, bluish-black flies. *H. aenescens* and *H. ignava* have the common names of black dump flies and black garbage flies, respectively (Axtell 1986a).

Hydrotaea aenescens

The black dump fly is found from Oregon to Arizona, and from Illinois to the East Coast, and is abundant in Central America where it is associated with human dwellings and excrement (Greenberg 1971). The adults are smaller than house flies and are separated morphologically from adults of other species by the presence of reddish-yellow palpi (Johnson & Venard 1957). Males are differentiated from females by the position of the eyes which are contiguous in the male and separated in the female. Wing disposition also differs between the sexes; resting males hold their wings crossed half-way between the fifth and sixth veins, while females hold theirs parallel (Johnson & Venard 1957).

Hydrotaea ignava

H. ignava is a common Holarctic species (Greenberg 1971). In its northern range, adults are heliophilous (Ball 1987). Larvae are found in feces of humans and livestock where the second and third instar larvae convert from a saprophagous lifestyle to predatory habits (Greenberg 1971).

Other *Hydrotaea* species

Other species include *H. capensis*, which is found in the southern regions of the Palearctic, *H. anthrax*, whose distribution is similar to *H. ignava* but extends further

north, and *H. nigra*, which is Australasian and was introduced into Hawaii from the Orient (Greenberg 1971).

Habitat and Distribution

All *Hydrotaea* species are considered hemisynanthropic to eusynanthropic, and predaceous on other coprophagous dipterous larvae (Greenberg 1971). *Hydrotaea* spp. are commonly found in poultry manure in many parts of world (Legner & Olton 1968, Peck & Anderson 1970,). *H. aenescens* is found in Europe (Adams 1984, Skidmore 1985), Chile (Ripa 1990), Texas (Robertson & Sanders 1979), South Carolina (Nolan & Kissam 1985), Florida (Hogsette 1979), Indiana (Hall & Williams 1980), and Pennsylvania (Stafford et al. 1988).

Predaceous Behavior

The predatory nature of *Hydrotaea* was reported as early as 1923 by Seguy (Hogsette 1979), and later by Keilin and Tate (1930) who described the cephalopharyngeal skeleton of *H. ignava* larvae as possessing characteristics indicative of both a saprophytic and carnivorous lifestyle. Subsequent studies by other researchers confirmed the predaceous, but not cannibalistic, behavior of *Hydrotaea* larvae (Anderson & Poorbaugh 1964b, Peck 1969, Peck & Anderson 1969, Hogsette 1979, Schumann 1982). Stafford et al. (1988) found that *H. aenescens* replaced the house fly as the principal fly species at one poultry farm. During this study, the *H. aenescens* population declined after a cleanout, but they reappeared in jug traps the next June and maintained dominance over the house fly population for the remainder of the year. In two high-rise poultry houses in South Carolina, Nolan & Kissam (1985) reported that *H. aenescens* replaced house flies as the dominant fly species after 1.5 ppm cyromazine was incorporated into the poultry feed, with dominance persisting several months after

cyromazine treatment was withdrawn. In studying manure removal schedules and selective application of adulticides on populations of house flies and little house flies, *Fannia canicularis* (L.) (Diptera: Muscidae) at several poultry houses in North Carolina, Axtell, (1970), found high populations of *H. ignava* and very few house flies or little house flies although no control program was instituted. In contrast early spring manure removal the following year resulted in few *H. ignava* and high house fly and little house fly populations. However, he still rejected the use of *H. ignava* for biocontrol of houseflies. Other workers have reported the potential of *H. aenescens* as a biocontrol agent of house flies in confined swine facilities (Mueller et al. 1981, Mueller 1982).

The House Fly

Economic Importance

The pest status of the house fly, *Musca domestica* L., in confined livestock facilities is well documented, particularly in cage layer poultry houses. This fly is generally considered the most abundant and economically significant pest at such facilities (West 1951, Anderson & Poorbaugh 1964, Axtell 1986a, 1986b). Control costs are substantial, and are manifested as both direct and indirect costs to the poultry producer (Axtell 1986a). The direct cost of fly control in cage layer hen operations, including expenses for insecticides, equipment, and labor, is estimated at \$40 million annually (Axtell 1985), or from 4.3-13 cents/bird/year (Axtell 1985, Turner et al. 1992). Indirect costs, usually the result of adult fly dispersion to neighboring residences and businesses, are incurred when complaints and/or violations of local public health regulations lead to expensive civil litigation (Axtell 1990). The producers may be subjected to fines or suspension of operation of the poultry facility pending mitigation of the fly problem.

Problems in confined-poultry houses persist despite declining farm numbers and relatively small amounts of pesticides used by the industry (Meyer 1990). This is attributed to: (1) Human dwellings situated in greater proximity to poultry houses due to expanding urbanization; (2) the development of resistance by house flies to insecticides (Iseki & Georgiou 1986, Shen & Plapp 1990), and (3) the continuous operation and emergence of large, multi-unit farms which house flocks of 1 million or more hens per farm (Anonymous, Poultry Digest 1991).

History and Hygienic Significance

The house fly has remained an offensive and formidable pest of humans and domestic animals for hundreds of years despite persistent attempts at control and eradication. Early accounts associating flies with human discomfort and suffering appear throughout recorded history (West 1951, Greenberg 1973, Cloudsley-Thompson 1976).

Although house flies are regarded primarily as nuisance pests, aspects of their behavior and morphology promote their suitability as mechanical vectors of disease (Greenberg 1971, 1973, Harwood & James 1979). These characteristics are: (1) their synanthropic, endophilous habits; (2) consumption of contaminated as well as uncontaminated food; (3) dispersal propensity and flight range; and (4) morphological adaptations of the proboscis and tarsi which facilitate bacterial pathogen transfer (Greenberg 1971, 1973, Harwood and James 1979, West 1951).

The number of bacteria on an individual fly is estimated to be as high as six million (Harwood & James 1979), and adult house flies under experimental conditions are shown to harbor the pathogenic microorganisms causing amoebic dysentery, typhoid fever, cholera, and salmonellosis (West 1951, Greenberg 1973, Harwood & James 1979). In addition, house flies are capable of serving as vectors of fowl cholera, poultry tapeworms, and Newcastle disease (Axtell 1985, Avacini & Ueta 1990). Fly defecation

and regurgitation also cause spotting on cages, walls, lighting equipment, and eggs, adding to the disease potential in poultry houses.

General biology

There is an abundance of literature on house fly biology, but discrepancies exist between the various references regarding longevity, fecundity, and survival and development rates. Disparity among these values can be attributed to variance in physical factors such as temperature, humidity, substrate, and nutrient quality, all of which regulate house fly development very precisely (Hogsette 1979, Skidmore 1985).

Distribution

Originally described by Linneaus in 1758, the house fly is considered a world-wide, cosmopolitan species with several subspecies described from the various geographic regions of the world (West 1951). In particular, two subspecies occur in the Nearctic region, namely *M. domestica domestica*, and *M. domestica vicina* (Greenberg 1971).

Life Cycle .

House flies develop rapidly under optimum conditions. In temperate regions of the USA (for example the southeastern states), during average summer conditions of 27°C and 75% relative humidity (RH), the time from egg to adult is 7-10 days (Harwood & James 1979, Axtell 1986b). Development time at different temperatures is shown in Table 1.

Table 1. Summary of development times for *M. domestica* at different temperatures (Axtell 1986b).

Temp °C	Stadium			Total average no. days
	Egg hours	Larva days	Pupa days	
16	49	11-26	18-21	44.8
18	33	10-14	12-15	26.7
20	23	8-10	10-11	20.5
25	14	7- 8	7- 9	16.1
30	10	5- 6	4- 5	10.4
35	8	3- 4	3- 4	7.0

The lethal temperature and lower temperature thresholds for larval development are about 46°C and 8°C, respectively, while the lower threshold for pupae is 11°C (Axtell 1986b). There are also differences in longevity at various temperatures and between the sexes (Fletcher et al. 1990). Those workers demonstrated that adult longevity varied inversely with temperatures from 20°-35°C. The mean longevity for males decreased from 33.8 days to 12.4 days, and for females from 44.6 days to 11.1 days. The fly is capable of over wintering in any of its life stages (Harwood & James 1979).

Fecundity and Larval Habits

Mating occurs 3-4 days after adult emergence (West 1951), with oviposition commencing 4-8 days later (Axtell 1986b). Fertilized females deposit white, elliptical eggs, each about 1 mm long, in batches of 120-150 (Axtell 1986b). The female produces 4-6 batches during her lifetime, with 2-4 days between successive batches (Service 1980, Axtell 1986b).

Females oviposit in a variety of decomposing and decaying organic materials which subsequently serve as both the larval food source and developmental medium. Animal manure is highly attractive to them, and females exhibit preferences for specific manure types (West 1951, Harwood & James 1979). In poultry manure, the levels of temperature and moisture content that are ideal for larval development are 27°C and 60%-75% respectively (Miller et al. 1974). Larvae progress through three instars. Mature third instar larvae (prepupae), typically 10-15 mm in length, become positively phototactic (Axtell 1986b), and migrate up to several meters to the lighter parts of the medium to pupate (Service 1980). Much additional information on the diversity of house fly breeding substrates has been reported by West (1951), Harwood & James (1979), Service (1980), and Skidmore (1985).

Adult Dispersal

Dispersal, like other life history phenomena, is influenced by physiological and ecological factors including age, sex, nutrition, and meteorological conditions. Release experiments show that dispersal is affected by conditions such as population density at time of release, and the time and place of adult release (West 1951, Greenberg 1971).

Within four days of emergence, adult flies may disperse to more favorable breeding areas (Pickens et al. 1967). Results from mark-and-recapture studies indicate dispersal rates varying from 2.3-11.8 km (Greenberg 1971) to a maximum distance of 20

km in 24 h (Bishopp & Laake 1921). Harwood & James (1979) reported a maximum dispersal distance of 32 km. Despite this ability, adult house flies tend to disperse to distances of only 1-3 km (Pickens et al. 1967, Harwood & James 1979). Interestingly, house flies have been reported to travel about 1 km past a clean farm facility to reach a dirty one (Pickens et al. 1976).

Adult Resting Sites

House flies use the inside of buildings for nocturnal resting sites (Anderson & Poorbaugh 1964a), unless indoor temperatures become excessive, in which case they can be found resting outdoors on vegetation (Oldroyd 1964). Inside poultry houses, the flies generally prefer vertical surfaces such as walls, partitions, and pipes, but can be found congregating on many other objects (Moon & Meyer 1985). This behavior has facilitated sampling and control methods such as the application of contact residual insecticides to resting sites (Scudder 1949). Unfortunately, this practice has produced rapid development of resistance in some cases (Hinkle et al. 1985).

Cage layer Poultry Production

High rise cage-layer houses are two-story, high density confinement systems that hold large hen populations of typically 20,000 to 100,000/house, and require a minimum amount of labor (Axtell 1986b). Hens are housed on the upper level in cages, usually three to four birds per cage measuring 0.30 m by 0.30 m by 0.46 m. These are arranged in a tiered design, with the cages stacked 3-4 high, such that each level is set back a distance from the layer below thus allowing manure to drop to the floor beneath the cages (Axtell 1986b). The tiered cages are arranged back-to-back for the length of the house, and there are normally three or four banks of these cages, with wooden walkways between the banks and along the outer walls (Axtell 1986b). The hens are provided with

water through an extensive piping system, with nipple-type drinking cups in each cage. Manure accumulates on the cement floor at ground level, in an area known as the manure pit. The pit areas are equipped with louvered fans that circulate air across the accumulating manure. Manure accumulates for months or years, and when properly maintained, forms cones that produce minimal odor and fly populations. Manure is removed by tractor-mounted front end loaders.

High rise cage layer houses can be environmentally controlled for temperature and photoperiod. Temperature is controlled by thermostats which govern the ventilator fans located in the walls of the pit. In addition to removing excess heat, the fans remove ammonia, carbon dioxide and water vapor, bring in fresh air, and create internal air circulation. It is this combination of an artificially manipulated environment together with the large quantity of accumulated manure which provides ideal conditions for production of potentially large populations of filth flies.

Integrated Pest Management (IPM) Strategies

It has been suggested that up to 98% mortality of house fly immatures result from abiotic and biotic factors which occur naturally under ideal conditions (Legner 1966). Thus, environmentally controlled, high rise cage layer poultry houses are particularly amenable to integrated pest management programs (Axtell 1991). As with any agricultural commodity, IPM programs for cage layer poultry houses are designed for practical and economical pest control by combining pest monitoring with a balance of cultural, chemical and biological control. Recently, pest management computer modelling programs have also been developed (Axtell & Stinner 1990, Axtell 1992).

Cultural Control

Manure management is essential. It forms the basis for a successful IPM program against filth flies in cage layer poultry houses. Proper management involves prevention of water intrusion from leaking waterers and drainage, supplying sufficient air flow over the manure to facilitate drying, and conducting appropriate manure removal (Axtell 1986b). All of these methods help produce a stable habitat that enhances the heterogeneous population of naturally-occurring predators and parasites (Dunning et al. 1978, Axtell 1986b, Barth 1986).

Accumulated poultry manure in cage-layer houses ranges from 70-80% moisture content, which is considered ideal for house fly larval development (Hart 1963, Miller et al. 1974, Card & Nesheim 1975). Manure moisture of less than 60% or greater than 80% reduces larval fly habitat, but the latter makes removal arduous, and renders manure conditions conducive to breeding of other pest fly species, e.g. soldier flies, *Hermetia illuscens* L. (Diptera: Stratiomyidae) (Miller et al. 1974, Axtell 1986b).

Scheduling manure removal so that it is staggered over 2-4 weeks, conducting removal during the winter months, and leaving a dry base of 13 to 15 cm deep helps to re-establish predators and parasites (Legner & Brydon 1966, Anderson et al. 1968, Peck & Anderson 1970). Total removal during summer months almost guarantees explosive fly populations within a few weeks (Axtell 1986b, Peck & Anderson 1970).

Chemical Control

Chemical control methods work well in conjunction with cultural and biological methods, but sole reliance on chemicals has repeatedly resulted in rapid development of resistance, as well as decimation of beneficial arthropods inhabiting the manure in the pits (Horton et al. 1985, Anderson et al. 1986, Meyer et al. 1989, Mandeville et al. 1990, Shen & Plapp 1990, Wills et al. 1990). Adult control may be achieved by insecticides

incorporated with baits (methomyl, naled), contact sprays (naled, pyrethrins, dichlorvos), and residual surface sprays (stirofos, malathion, permethrin) (Youngman 1990). Larval control typically may be achieved by using cyromazine (Larvadex®) as a feed additive, as well as spot-treatment of wet areas of manure. Selective use of such chemicals can help provide adequate fly control while being non-toxic to beneficial arthropods.

Cyromazine has been a particularly effective chemical that has a mode of action different from other larvicides used for fly control (Hall & Foeshe 1980, Williams & Berry 1980, Mulla & Axelrod 1983a, b). In addition it is compatible with the predatory mites and beetles inhabiting poultry manure (Axtell & Edwards 1983, Meyer et al. 1983). However, persistent use of cyromazine has led to the development of resistance by house fly populations (Bloomcamp et al. 1987).

Biological Control

Accumulated poultry manure when properly managed contains a variety of predatory and parasitic arthropods (Legner & Olton 1970, Axtell 1986b). Numerous studies have shown that the most effective predatory species in cage layer poultry houses are, (1) predaceous mites (Machrochelidae, Uropodidae, Parasitidae), (2) Coleoptera (Histeridae), and (3) Diptera (*Hydrotaea* spp.) (Peck & Anderson 1969, Pfeiffer & Axtell 1980, Green 1982, Morgan et al. 1983, Propp & Morgan 1985, Armitage 1986, Axtell 1986b). Other arthropods less commonly mentioned, although recognized as potential fly predators, include; (1) several beetles in the families Tenebrionidae and Staphylinidae, (2) Dermaptera, (3) Anthocoridae (Hemiptera), and 4) Pseudoscorpionida (Propp & Morgan 1985). Parasitic Hymenoptera in the family Pteromalidae constitute a further component of biological control (Legner & Brydon 1966, Legner 1971, Legner & Dietrick 1972, Olton & Legner 1975, Rutz and Axtell 1980, Morgan et al. 1981, Morgan & Patterson 1981 Rueda & Axtell 1985).

The role of biocontrol agents other than arthropods in regulating house fly populations has also been investigated. Examples are the fungus *Entomophthora muscae* (Cohn) Fresenius, and the bacterium *Bacillus thuringiensis* Berliner (Burns et al. 1961, Kramer & Steinkraus 1987, Mullens et al. 1987, Mullens 1990).

Acarina

Predatory mites, phoretic on flies and beetles, attack the eggs and first instars of various muscid larvae (Axtell 1963b, Rodriguez et al. 1970). Three important species which are highly effective predators of the house fly are *Macrocheles muscaedomesticae* (Scopoli), *Fuscoropoda vegetans* (DeGeer), and *Poecilochirus monospinosus* (Wise) in the families Macrochelidae, Uropodidae, and Parasitidae, respectively (Willis & Axtell 1965, Axtell 1981, 1986a, 1986b, Wise et al. 1988, Geden et al. 1989). These species are considered complimentary predators because each exhibits different preferences for prey and local habitat. These preferences include; (1) feeding preferences for eggs versus first instar house flies, (2) preferences for alternative prey, (3) preferences for fresh versus aged manure; and (4) preference for depth of manure colonized (Axtell & Rutz 1986, Wise et al. 1988, Geden 1990).

The number of eggs and first instar larvae killed/predator/day is termed the relative predation rate, and the overall relative predation rates for the above three species are reported as 1.0 for *M. muscaedomesticae* adult females, 0.25 for *F. vegetans* adults and deutonymphs, and 0.6 for *P. monospinosus* adults and deutonymphs (Axtell 1991). In laboratory experiments on the predation rates of mites on house flies in the Philippines, Rueda et al. (1990) reported rates of 9.7 per day for *M. muscaedomesticae* adult females, 6.0 for deutonymphs and 0.7 for protonymphs. *M. muscaedomesticae* also caused 99% mortality of house fly eggs in poultry manure (Rodriguez et al. 1970), thereby substantially reducing the house fly population.

Coleoptera

Numerous coleopteran species commonly occur in poultry manure and are considered important predators of fly eggs and larvae (Peck 1969, Peck & Anderson 1969, Legner 1971, Pfeiffer & Axtell 1980). The Histeridae, specifically *Carcinops* spp., are typically the most abundant and are considered the most significant beetle predators (Peck 1969, Legner & Olton 1971, Bills 1973, Pfeiffer & Axtell 1980, Green 1982, Propp & Morgan 1985, Geden & Stoffolano 1987). The distribution of *C. pumilio* in accumulated poultry manure, as well as its predation on house flies has been studied extensively (Bills 1973, Geden and Axtell 1988, Geden et al. 1988, Geden & Stoffolano 1987, 1988, Geden et al. 1987). This information, along with other life history data, has been incorporated into a computer simulation model of house fly control in cage layer poultry houses (Axtell & Stinner 1990, Axtell 1992).

Life stages of the beetle are the egg, two larval stadia, pupa, and adult, with development time from egg to adult about 25 days, and adult longevity up to 200 days at 25°-30°C. (Geden 1984). *C. pumilio* adults are opportunistic predators, preying upon acarid mites and other dipterous larvae, as well as larvae of house flies. In moderate densities all life stages are cannibalistic, and the adults exhibit scavenging behavior, feeding on dead arthropods, dead chickens and broken chicken eggs (Geden 1990). Predation rates of *C. pumilio* adults on house fly eggs were 13 per day (Morgan et al. 1983), 30-50 fly eggs and larvae per day for adults, and 10-20 per day for larvae (Geden et al. 1988). They also noted that predation by adults was as much as 104 per day depending on prey density, and on whether the predators were starved or satiated at the time of testing.

Other Coleoptera reported as predators of house flies and other filth fly immatures in confined livestock facilities include, *Alphitobius diaperinus* (Panzer) (Tenebrionidae), *Ataenius californicus* Horn (Scarabaeidae), and *Aleochara* spp. (Staphylinidae) (Legner & Olton 1968). Rueda et al. (1990) surveyed natural enemies in poultry production systems in the Philippines, and found 35 species of arthropod predators and scavengers. They determined rates of predation on *M. domestica* eggs and first instar larvae for Staphylinidae, Histeridae, and Scarabaeidae. In laboratory experiments on predation, adult *Aleochara puberula* Klug (Coleoptera: Staphylinidae) produced the highest predation rate, destroying 255 egg and first instars/adult beetle/day, in comparison with *Carcinops* sp. which destroyed only 37.2 eggs per adult beetle per day. However, these tests were not conducted in field conditions, and most workers still regard *Carcinops* sp. as the more important predator.

Tremendous numbers of *A. diaperinus* are often found in both broiler and cage-layer poultry houses. In searching for pupation sites, the late instars tunnel through poultry house insulation causing as much as \$20,000 in damage to individual houses (Turner 1986). They are capable of harboring fungi, bacteria, protozoa, and viruses which cause Marek's disease, Newcastle disease, avian influenza and fowl pox (Axtell & Arends 1990). In spite of their destructive behavior, the beetles perform beneficial functions in the manure of cage layer houses by tunneling through the manure, and thus facilitating drying and reducing opportunities for fly breeding (Geden 1990). Adult and larval beetles are also facultative predators and will attack house fly eggs and larvae in the laboratory where both adults and late instars were shown to cause significant reduction of house fly emergence (Despins et al. 1988). The destructive habits of *A. diaperinus* are considered to outweigh its potential for biological control of house flies (Axtell 1986b).

Other Arthropods

Reports of other arthropods with potential for biological control are inconsistent in the literature, and although some of them have relatively high predation rates compared with key predators, their relative abundance varies. Typical of these are the staphylinid beetle *A. puberula*, and Hemiptera, family Anthocoridae (Rueda et al. 1990). In contrast, some Dermaptera are found in high numbers and are shown to have relatively high predation rates too. Examples are *Euborellia philippinensis* (Srivastava) (Dermaptera: Anisolabidae) (Rueda et al. 1990), and earwigs in the family Labiduridae (Legner & Olton 1970, Propp & Morgan 1985). Propp & Morgan (1985) also noted a high abundance of Pseudoscorpionida in poultry manure in Florida, and considered them to be among the most abundant predators found in their study. However, Wills & Mullens (1991), in a study conducted in California, found them to be present in small numbers. Their effectiveness as control agents is thus still unresolved.

Parasites

Due mainly to variations in manure conditions and strains of parasitic species, variable successes in controlling house fly populations at poultry farms have been achieved using augmentative releases of the pteromalids *Splangia endius* Walker, and *Muscidifurax raptor* (Girault & Sanders) (Morgan et al 1975a, b, 1981a, b, Peterson et al. 1983), which are available commercially. Morgan & Patterson (1990) reported 90% reduction in house fly pupal populations for one month following treatment of poultry feed with 0.5% cyromazine (Larvadex®). In five of the remaining six months they achieved more than 90% reduction of house flies by sustained releases of the parasites. Reduction of greater than 90% continued at a farm at which a 9 cm residual pad of manure was left in place at cleanout. In contrast, tests by Mullens et al. (1987) in

California, showed that parasitic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) failed to control fly larvae in poultry manure.

Pathogens

The fungus *Entomophthora muscae* (Cohn) Fresenius is known to produce epizootics in adult house fly populations in caged-layer operations in southern California (Mullens et al. 1987). The same study reported *E. muscae* epizootics in *H. aenescens* during the winter, spring, and fall. With infection levels reaching as high as 80%, Mullens et al. (1987) considered this pathogen to be the primary factor causing cool-season regulation of fly populations in some regions. In another study, Kramer & Steinkraus (1987) produced 100 % mortality of adult house flies by releasing infected flies into the house.

Beta exotoxins and delta endotoxins produced by varieties of the bacterium *Bacillus thuringiensis* are also toxic to fly larvae, disrupting molting and pupation (Hall & Arakawa 1959, Carlberg et al. 1985). *B. thuringiensis* var. *thuringiensis* (Berliner) was reported to be successful in killing house flies in laboratory trials (Hall & Aragawa 1959). When incorporated into poultry feed, *B. thuringiensis* var. *thuringiensis* not only reduced house fly survivorship but also produced unfavorable effects on food consumption, body weight, and egg production by hens (Burns et al. 1961). It has been suggested that some strains of *M. domestica* may develop resistance to the exotoxin, and sustained effective control in confined livestock facilities has yet to be demonstrated (Wilson & Burns 1968, Jepersen & Keiding 1990).

Hydrotaea Species as Biological Control Agents

Despite reluctance by some researchers to recognize the potential for using *H. aenescens* as a biological control agent for *M. domestica* (Axtell & Rutz 1986),

several researchers have begun integrating the former species into existing IPM programs at high rise cage layer facilities in the United States (Turner & Carter 1990, Turner et al. 1992, Williams, personal communication).

For several years, German researchers have been mass rearing and selling *H. aenescens* pupae for control of house flies in confined swine facilities, and they are presently expanding this market to include the poultry industry (Betke & Schultka unpublished, Schultka et al. 1986, Ellwanger, personal communication). In various studies, these researchers reported suppression of house flies by *H. aenescens* for many years after a single initial inundative release of *H. aenescens* pupae. Control was achieved without insecticides. In the United States, one commercial producer of natural enemies is also marketing *H. aenescens* (Daar et al. 1992)

Turner and Carter (1990) initiated a mass-release program at several high-rise poultry houses in Virginia - a program which caused a substantial reduction in house fly populations in cage layer houses receiving single and multiple releases of *H. aenescens* larvae and pupae. Cyromazine use was reduced in a seeded house compared with a control house at another location, with a cost saving of \$25 per 1000 birds.

When *H. aenescens* was mass-reared and incorporated into an IPM program in another study by Turner et al. (1992), effective house fly control was also achieved. The study included both on-farm rearing and inundative releases of *H. aenescens* into six, high rise, cage layer poultry houses in Virginia, together with manure management, monitoring of house fly populations, and judicious use of insecticides. Comparison of the IPM program with the farm's previous control program (which relied heavily on insecticide inputs), resulted in annual savings of over \$21,600 (about 3.8 cents per bird) and 80% reduction in insecticide input.

Chapter 3

Species Diversity in High-Rise Cage Layer Houses at Two Sites in Virginia, and the Relationship of Selected Arthropod Abundances to Manure Moisture

Introduction

Numerous field studies have been conducted on the ecology of the manure environment at livestock facilities, particularly at cage layer poultry farms. The focus of these studies ranges from arthropod faunal succession, and seasonality and faunal surveys (Anderson & Poorbaugh 1964, Peck & Anderson 1969, Legner & Olton 1970, Legner et al. 1975, Pfeiffer & Axtell 1980, Hulley 1983, 1986, Armitage 1986, Geden & Stoffolano 1987, Hulley & Pfleiderer 1988, Stafford et al. 1988), to the measurement of physical factors and their effects on the arthropod fauna (Bills 1973, Dunning et al. 1978, Stafford & Collison 1987, Geden et al. 1988, Stafford et al. 1988, Wills & Mullens 1991).

There is variation among these studies with respect to geographic region in which they were conducted, and the type of cage layer facilities investigated. Cage layer houses range from environmentally controlled, closed-sided, high-rise types with deep manure pits, to those with shallow-pits with or without open sides.

In spite of these differences, there are several arthropods, including house flies, (*Musca domestica* L) (Diptera: Muscidae), and certain of their predators, that are consistently reported as important and predominant members of the manure community. However, house flies are generally considered the most abundant and economically significant pests of confined livestock (Anderson & Poorbaugh 1964, Axtell 1986a, 1986b). It is reported too, that predators of house flies reduce a greater proportion of total house fly numbers than any other biotic regulatory agents (Legner & Brydon 1966), with reported reductions in fly populations of 50% to 95% (Axtell, 1963, Propp & Morgan 1985, Geden et al. 1988.)

In addition to predatory mites in the family Macrochelidae, *Carcinops pumilio* (Erichson) (Coleoptera: Histeridae) is the predator often reported as the most important fly predator in accumulated poultry manure (Legner & Olton 1970, Bills 1973, Hulley 1983, Armitage 1986, Hulley & Pfeleiderer 1988). This beetle usually occurs in high densities in poultry manure that is managed to minimize manure moisture (Pfeiffer & Axtell 1980, Propp & Morgan 1985). *C. pumilio* has a high attack rate on house fly eggs and larvae, and readily feeds on several alternative prey species (Geden et al. 1988). In groups, these small beetles can even attack and destroy later instar house fly larvae (Geden 1990).

Another predator not normally occurring in such large populations is the fly *Hydrotaea* (= *Ophyra*) *aenescens* (Weidemann) (Diptera: Muscidae) (Nolan & Kissam 1985). This predator is receiving increasing attention from researchers, and its utilization by poultry farmers as a biocontrol agent is growing each year (Turner et al. 1992, personal observation). *H. aenescens* has similar environmental requirements to the house fly, and is inexpensive and easy to rear. *C. pumilio*, however, is very difficult to mass rear due to its long generation time and propensity for cannibalism (Geden & Axtell 1988, Geden 1990).

Of the abiotic factors operating in poultry houses, manure moisture is probably the most important factor affecting populations of manure-inhabiting arthropods (Bills 1973, Barth 1986, Axtell 1986a, 1990). Therefore, the following study was conducted to obtain a better understanding of the interactions between selected manure arthropods and manure moisture in high-rise cage layer poultry houses. The study was designed to take advantage of a unique opportunity to sequentially sample the arthropod fauna in a newly constructed high-rise house in Virginia (the only house at the site), shortly after the initial stocking of the house with hens. Other workers have examined the effect of manure accumulation time and manure removal schedules on arthropod abundance and succession (Peck & Anderson 1970, Geden & Stoffolano 1988). However, those studies

were conducted at poultry farms with established houses that were situated adjacent to each other. Whether total or partial manure removal was conducted in a particular house, the possibility existed for immigration of arthropods from adjacent houses.

The specific objectives of the study were: (1) to develop a sampling procedure according to moisture classes defined by physical characteristics of the manure, (2) to correlate the abundance of selected arthropods with manure moisture, and (3) to compare arthropod species diversity in new houses to the faunal diversity at older well-managed houses.

It was decided that moisture classes would be used as opposed to randomly-selected samples, to ensure that a broad range of moisture levels would be sampled on each date. If random sampling was conducted, the possibility existed that many of the samples would be collected from similar conditions, instead of from the full range of moisture which extends from ca. 20% to 90% (Stafford & Bay 1987). In previous studies in which manure moisture was investigated, sampling was either random, or samples were collected from the same sites on each sample date (Peck & Anderson 1969, Bills 1973, Armitage 1985, Stafford & Bay 1987, Geden et al. 1988).

The arthropods of primary interest were house fly larvae, dung gnats, *H. aenescens* larvae, *C. pumilio* adults and larvae, lesser mealworm adults and larvae [*Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae)], earwigs (Dermaptera), pseudoscorpions (Pseudoscorpionida), and anthocorid nymphs and adults (Hemiptera: Anthocoridae). All of the non-dipteran arthropods listed have been reported to be either obligate or facultative predators on house fly eggs, or larvae, or both (Legner & Olton 1968, Peck & Anderson 1969, Legner et al. 1975, Stafford & Collison 1987, Despins et al. 1988, Rueda et al. 1990). Sphaeroceridae were tabulated because they are an important alternative prey for *C. pumilio* (Geden et al. 1988), and because they were observed to be extremely numerous in preliminary studies.

Materials and Methods

Manure samples were collected from, high-rise cage layer poultry houses at Glenwood Farms, Jetersville, Amelia Co., Va., and at Nottoway Farms, Blackstone, Nottoway Co., Va. At both farms, hens were housed on the upper level in five banks of four-tiered cages separated by four walkways. Manure accumulated on the first floor in a concrete pit which had ventilator fans located on the pit walls.

The houses at Glenwood Farms, described by Turner et al. (1992) were situated parallel to each other, and ca. 15 m apart. Houses #1 through #5 (a numbering system designated by the operator) were constructed between 1984 and 1987, and had dimensions of 17.1 m by 154.2 m. Each held 102,000 hens and used nipple waterers. House #6 was built in 1978, was 12.2 m by 158.2 m, and housed 65,000 hens. The houses at Nottoway Farms both measured 152.4 m x 56 m, and each housed 115,200 hens.

An initial study was made to determine the feasibility of collecting manure samples by visual assessment of moisture level. In order to determine whether sampling would be accurate between houses, sampling was conducted from houses #5 and #6 at the Glenwood Farms on four dates during 1987 (1, 9, 25 Sept., and 29 Oct.). Four moisture categories were established by which visual separation could be made by the sample collector, based on physical characteristics of the manure. The categories and their description were:

Dry- manure light-gray in color, clumped and hard, and often mixed with feathers and spilled feed.

Medium-Dry- medium-gray in color, lightly clumped, some feathers mixed in.

Medium-Wet- dark color, bread dough texture, thickly clumped, usually few feathers present.

Wet- dark color, thick, slurry-like, often located under currently, or recently leaking water pipes, usually no feathers present.

Manure samples were collected as follows: the collector searched the manure surface, looking for the specified manure moisture category e.g. "wet". A metal spoon was inserted into the manure to the depth of the spoon handle (ca. 10 cm), and the contents were scooped into a plastic ice-cream container 8.2 cm high, 9.3 cm bottom diameter, and 600 ml liquid capacity. Additional scoops were taken from the manure adjacent to the first scoop until the container was almost filled. The container was then covered with a vented lid. Sampling was restricted to the upper 10 cm of manure because Willis & Axtell (1968) and Geden & Stoffolano (1987b), showed that the majority of manure arthropods occur in approximately the upper 5 cm of manure. For the next replicate sample, the collector selected a different location of the pit with apparently similar physical characteristics. Collection of all samples from each moisture category proceeded in the same manner, with four replicates collected per moisture category on each sample date.

After collection, the samples were transported to the laboratory, where the weight of each container was recorded. Then each manure sample was emptied onto a wire-mesh screen and placed into a Tullgren funnel. The funnels were fitted with 60W light bulbs which served as the heat and light source to evacuate the arthropods into a collecting jar containing 70% ethyl alcohol. The funnels were taped shut to prevent the escape or intrusion of arthropods. The samples were held in the funnels for about 2 weeks after which the remaining manure was weighed, and the weights were recorded. The manure from the "wet" category was dried further prior to final weighing by holding the samples in an oven at 38^o C for 24 h. Manure moisture (percent water content) was calculated for each sample. Differences in moisture categories between houses were compared using *t*-tests (Gustafson 1991).

The results obtained from the initial study indicated that it was best to collect the samples using only three categories - "dry", "medium", and "wet". The adjustment was made after the collectors expressed difficulty in visually distinguishing between the

"med-dry" and "med-wet" categories. Therefore the physical characteristics of these two categories were combined to yield the category "medium".

Manure samples from the categories "dry", "medium", and "wet" were collected from a newly constructed high-rise, cage layer house (Nottoway #2), over a period of 1 1/2 years, starting three weeks after the house was stocked with birds (13 Oct. 1987). This was the only house located on the farm, which had another house (#1) under construction. Samples were collected from #2 approximately twice monthly from, 13 Oct. 1987 to 4 Feb. 1988, with two replicates per moisture category. Sampling was initiated in the second house (Nottoway #1) after its completion date, and after stocking with birds during the last week of Jan. 1988. (The houses were assigned numbers by the producer, and they are referred to by these numbers during this study.)

Sampling was discontinued in house #2 when an equipment malfunction caused all of the laying hens in the house to suffocate in mid-February, 1988. House #1 had constantly leaking waterers from the outset, and the manure became semi-liquid in consistency. Because it was impossible to find samples in the medium and dry categories, sampling was discontinued and a decision was made to resume sampling when house #2 was fully functioning. This would allow sufficient time for the producer to repair the watering system in house #1. Sampling from both houses resumed on 3 May 1988. Table 1 gives the sampling dates for houses #1 and #2.

Samples were collected and processed in the same manner described above for the preliminary samples from Glenwood Farms. On each sampling date, and after samples were taken, the collectors also conducted mass-releases of laboratory-reared *H. aenescens* into the manure pits as part of the farm's IPM program. The approximate number of fly larvae released into each house on each date was recorded. These data were not included in this study.

To compare the fauna from the newly-established houses at Nottoway Farms with that from houses at a longer-established, well managed facility, samples were collected.

Table 1. Sample dates from house #1 and #2 at Nottoway Farms, Nottoway Co., Va.

House #2		House #1 and House #2	
Date		Date	
1987	1988	1988	1989
n=6		n=9	
13-Oct	7-Jan	3-May	2-Mar
22-Oct	14-Jan	25-May	30-Mar
5-Nov	21-Jan	9-Jun	11-Apr
12-Nov	4-Feb	21-Jun	27-Apr
3-Dec		12-Jul	
10-Dec		19-Jul	
17-Dec		2-Aug	
		31-Aug	
		7-Sep	
		14-Sep	
		6-Oct	

from houses #3 and #4 at Glenwood Farms. Manure samples were collected monthly for six months in 1988 and 1989, on the following dates: 10 Nov., 15 Dec., 20 Jan., 16 Feb., 16 Mar. and 13 Apr. These dates did not correspond to the sampling dates at Nottoway Farms as they were intended to provide a baseline for comparison of diversity independent of time. Nine samples were collected per sample date from each of the three moisture categories. The samples were collected and processed as previously described.

Kolmogorov-Smirnov tests (Gustafson 1991) were used compare the cumulative frequency distributions of percent moisture in manure samples between one house and the other at each location. The tests were also used to compare the pooled data of both houses at Glenwood with the pooled data of both houses at Nottoway Farm for the same sampling period. The null hypothesis for the tests is "that the two distributions of moisture samples are statistically similar, and that any difference observed (in any aspect of the distributions) is due to chance". These tests were used to verify the accuracy in sampling between houses at each location. Differences in moisture category between houses was compared using *t*-tests (Gustafson 1991).

Taxonomic Diversity

Arthropods extracted from the manure samples were collected from the funnels, sorted and counted, and selected taxa were identified to family. Spiders, mites, and lepidopteran specimens were noted, but not identified further. Reference specimens are located in the Entomology Department at VPI&SU. Because the mass of each manure sample differed due to collection by volumetric means, the number of individuals in each taxon was multiplied by 100 and divided by the dry weight, in order to yield the number of individuals/100 g of manure. The arthropods of primary interest to the study, i.e. house flies, *H. aenescens*, *C. pumilio*, lesser mealworms, earwigs, pseudoscorpions, and Anthocoridae were identified to the lowest possible taxon. Pseudoscorpions were not identified below the order level.

Simpson's diversity index (D_s), and Heip's evenness index were calculated for each sample using the "Ecological Measures" software package (Kotila 1986). Simpson's diversity indices were calculated for the overall taxon diversity for each house. In these calculations, larvae of lesser mealworms and *C. pumilio* were considered as separate taxa from the adults because the larvae are known to exhibit slightly different preferences in moisture level from the adults. *t*-tests were used to test for significant differences in diversity between the two houses at each farm (Brower & Zar 1984).

Simpson's diversity index was also calculated for taxon diversity for the entire period during which Nottoway Farms house #2 was sampled. This value was compared to the total diversity at Glenwood Farms (both houses), using a *t*-test, to determine whether diversity after 1 1/2 years at the newly-established farm (Nottoway) was similar to the diversity at the longer-established farm (Glenwood).

Morisita's similarity indices, and percent similarity values (Kotila 1986), were both used to determine the similarity of taxa between houses #1 and #2 at Nottoway Farm for the same time period, and for the similarity in taxa between the two farms. Both measures of similarity were also used to compare the similarity in taxa between house #2 at Nottoway #2 (over the entire sample period), and Glenwood #3, and that between Nottoway #2 (again over the entire sample period), and Glenwood #4 at Glenwood. The latter comparisons were made to determine whether house #2 at Nottoway, after operating for over one year, would have a similar arthropod community to the houses at Glenwood Farms which had been in operation for several years, and where good integrated pest management (IPM) practices had been consistently used by the producer. Values for Morisita's index range from zero (when no similarity exists), to 1 (when the communities are identical) (Brower & Zar 1984).

Relationship of Selected Arthropods to Manure Moisture.

To determine the relationship between the more numerous predator per scavenger, and prey species, and the moisture content of manure sampled from Nottoway and Glenwood Farms, Canonical Correlation Analysis (PROC CANCORR, SAS Institute 1985) was used. Canonical correlation was used because it was thought that abundances of manure-inhabiting arthropod species are not determined solely by the moisture content of the manure.

Canonical correlation analysis determines the linear relationships between two sets of variables, in this case; (1) manure moisture, and (2) arthropod density. Densities were transformed using $\log \text{density} + 1$. The results of the analysis include Pearson's correlation coefficients between each taxon, and between taxon density and manure moisture. The canonical correlation measures the overall correlation between moisture and taxon density. Also included in the results are Wilks' Lambda (the likelihood ratio that the canonical correlation in the population is 0.0), an F -statistic based on the distribution of the likelihood ratio, and the P -value associated with the F statistic.

Canonical correlation analyses were performed on the pooled data from both houses at Glenwood, and on the pooled data from both houses at Nottoway Farms. Correlations among taxa, and between each taxon and manure moisture were tested for significance using t -tests ($\alpha=0.05$) (Zar 1984).

Results and Discussion

Mean percent manure moisture of each category from the initial study ranged from 17.76% to 67.15% (Table 2). For each category there were no significant differences in mean percent moisture between Glenwood #5 and #6, therefore the samples were pooled (Table 1) to arrive at an overall mean for each category. An analysis of variance (ANOVA) (Gustafson 1991) determined that there were significant differences in mean percent moisture between the moisture categories ($F=74.00$, $df=3,31$;

Table 2. Mean percent manure moisture \pm standard error (SE), of the four moisture categories collected from Glenwood Farms house #5, and #6, and results of t tests for each category between the two houses.

Category	House							
	#5		#6		Pooled			
	\bar{X}	SE	\bar{X}	SE	t^*	P		
Dry	20.98	(3.5)	17.76	(5.8)	0.4737736	0.6524089	19.37	(3.2)
Med-Dry	45.69	(2.9)	47.43	(3.7)	-0.3713437	0.7231413	46.56	(2.2)
Med-Wet	58.54	(2.3)	54.64	(3.2)	1.0037832	0.3542325	56.59	(1.9)
Wet	64.72	(2.5)	67.15	(1.8)	-0.7799894	0.4650439	65.94	(1.5)

* t -crit = 2.446, df = 6, α = 0.05

$P < 0.01$), and a Tukey's HSD test ($\alpha = 0.05$) showed each category was significantly different from the others. It was determined from these results that sampling was accurately conducted between houses.

Accuracy of Sampling Using Three Categories

Figs. 1 and 2 show the frequency distributions of percent moisture of manure samples from each house at both Glenwood and Nottoway farms. Also shown in these figures are the frequencies of occurrence of the samples at 10-unit intervals, as well as the percent of the total number of samples collected that fall into each interval. At Nottoway Farms, manure samples ranged from 5.86% to 86.05% moisture with both the highest and lowest moisture level collected from house #1. The samples collected from Glenwood #3 and #4 ranged from 1.22% to 74.09%. All of the figures depict a bimodal distribution of samples in each house, with the majority of samples collected containing greater than 40%-50% moisture. Geden & Stoffolano (1988) reported that the majority of their manure samples from cage layer houses were greater than 50% moisture content, and Stafford & Bay (1987) reported greater than half of their sample moisture values lay in the 70%-79% range in shallow-pit houses in Texas.

Of interest is the fact that a total of 16.1% of the samples from the pooled data of Nottoway #1 and #2 held greater than 70% moisture, as opposed to 6.5 % of the samples at Glenwood Farms. This probably occurred because sampling from Nottoway Farms was initiated shortly after stocking with hens. Samples were therefore taken initially from manure which accumulated on the floor instead of on a residual pad of manure which is commonly left in place after cleaning the houses at older facilities. The pad absorbs moisture and also provides harborage to many manure-inhabiting arthropods which, through their tunneling behavior, facilitate drying of the manure (Axtell 1970).

Figure 3 shows the cumulative frequency distributions of the manure samples mentioned above. Results from Kolmogorov-Smirnov tests show that there were no significant differences between the cumulative frequency distributions of percent

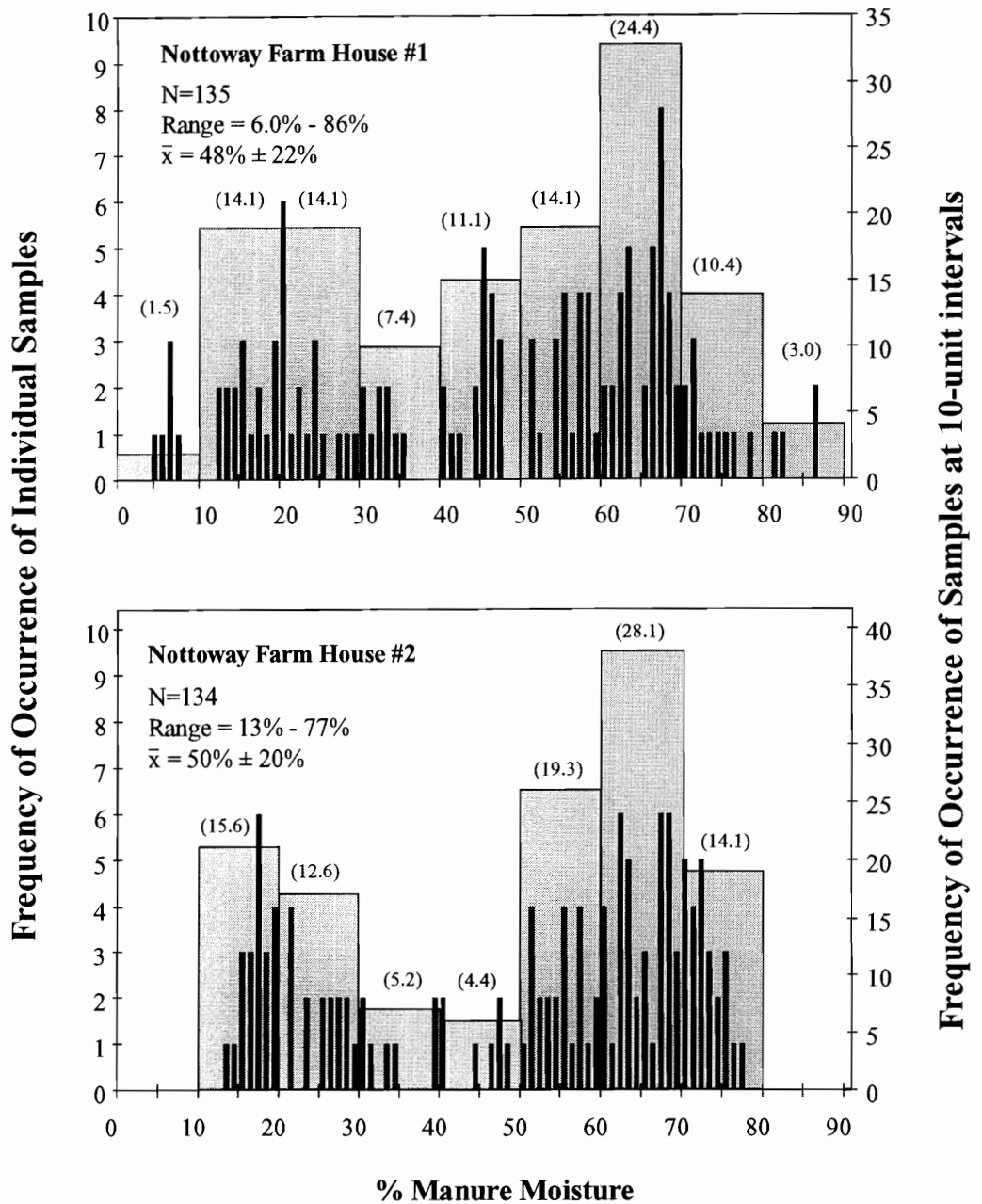


Fig. 1. Frequency distribution of percent moisture for manure samples from Nottoway Farms, Blackstone, Va., plotted to the nearest whole number (black bars, n= total sample number). Background bars show the frequency of occurrence at 10-unit intervals (number in parentheses is the percent of the total number of samples collected).

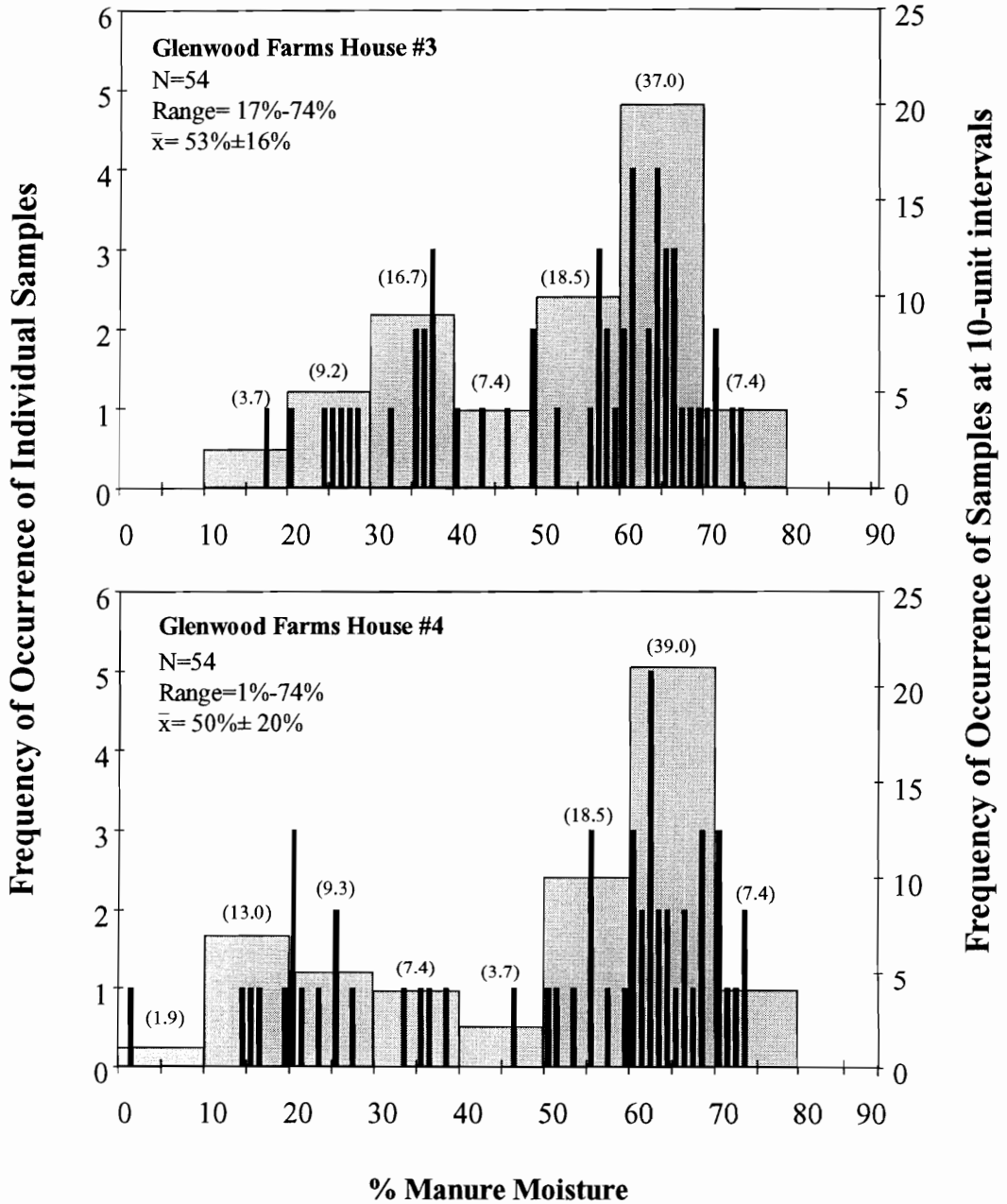


Fig. 2. Frequency distribution of percent moisture for manure samples from Glenwood Farms, Jetersville, Va., plotted to the nearest whole number (black bars, n= total sample number). Background bars show the frequency of occurrence at 10-unit intervals (number in parentheses is the percent of the total number of samples collected).

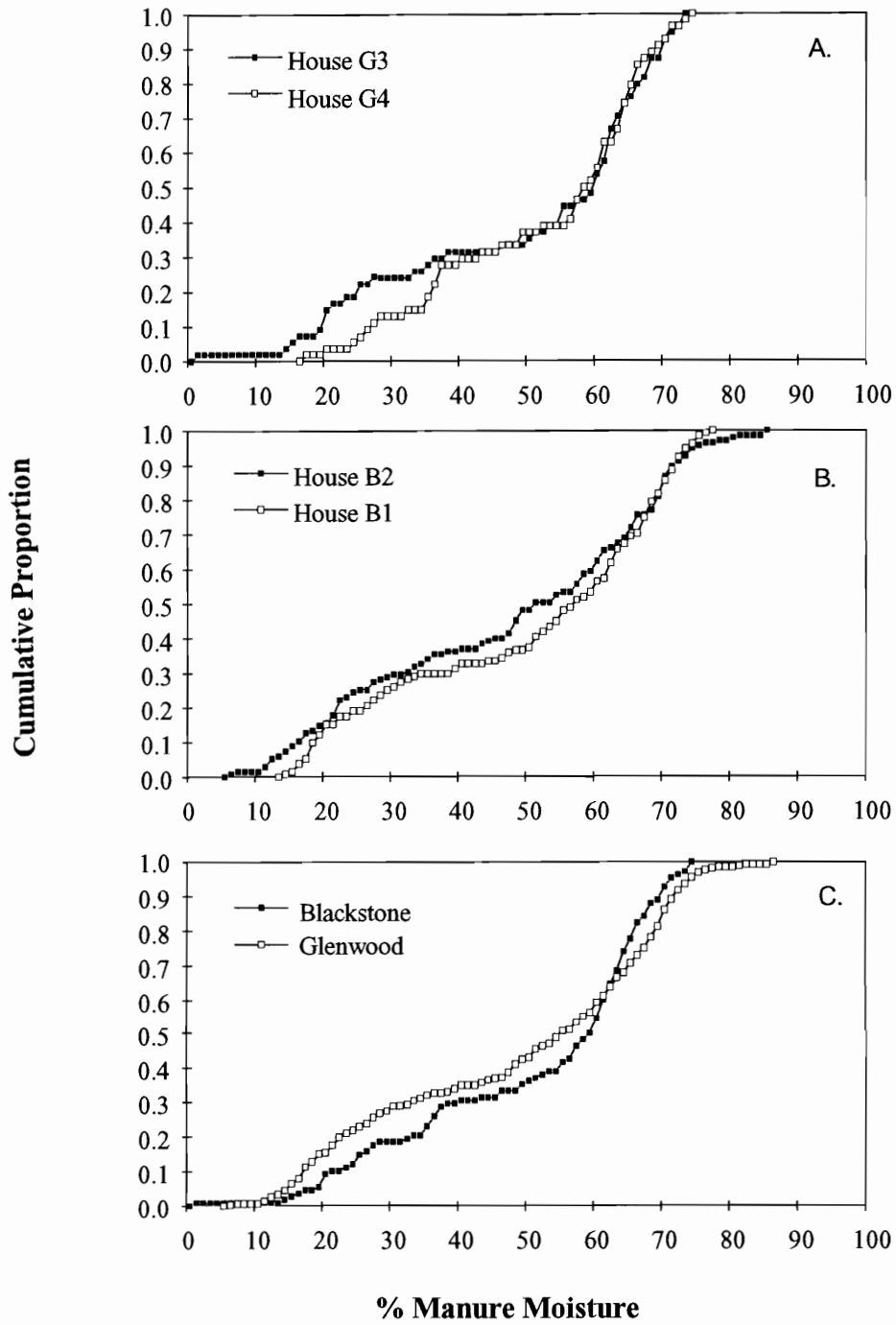


Fig. 3. Cumulative frequency distribution of percent moisture of manure samples collected from house #1 and #2 at Nottoway Farms, and house #3 and #4 at Glenwood Farms.

moisture in the manure samples from Glenwood #3 and #4 (Maximum deviation=0.148, $P= 0.59$) (Fig 3a), and houses #1 and #2 at Nottoway Farms (Maximum deviation=0.115, $P= 0.34$) (Fig 3b). When the samples from each house were pooled to compare the distributions between the farms, the distributions were still not significantly different (Maximum deviation = 0.108328, $P= 0.328$) (Fig. 3c).

These results indicate that sampling accuracy was achieved not only between houses at one farm but also between farms. This is important because it demonstrates that this sampling scheme will yield similar distributions of manure moisture when the investigator wants to obtain similarly representative samples from different locations.

Species Diversity

A list of the arthropods which were extracted from the manure samples collected from both high-rise cage-layer poultry houses at Glenwood and Nottoway Farm is given in Table 3. The total number of individuals in each taxon from all houses over the entire sampling period appears in parentheses in the table next to the taxa names. These numbers represent the sum of the number of individuals/100 g dry manure for all four houses, across all sample dates.

Some interesting and unexpected taxa were collected, including Phthiraptera, Thysanoptera, *Graphocephala* sp. (Hemiptera: Cicadellidae), Elmidae (Coleoptera), Otitidae and Scatopsidae (Diptera), Chalcididae and Ichneumonidae (Hymenoptera). However, the most abundant taxonomic group enumerated was the Diptera. Dung gnats (Sphaeroceridae) and house flies comprised the greatest numbers of Diptera, with over 250,000 dung gnats and 7000 house flies collected. A very large number (47,000) of *H. aenescens* were also collected, probably due to mass releases. Lesser mealworm larvae were the most abundant Coleoptera followed by *C. pumilio* adults, lesser mealworm adults, and *C. pumilio* larvae, in that order (Table 3).

Table 3. Arthropods collected from manure samples from two high-rise cage layer poultry houses at Glenwood Farms, Jetersville, Va., and from two houses at Nottoway Farms, Blackstone, Va., during 1987-1989. Numbers in parentheses are the total number collected.

ARTHROPODA	COLEOPTERA CONT.
ARAENEAE*†	Cleridae (72 A)
ACARI*†	<i>Necrobia ruficollis</i> Fabricius
PSEUDOSCORPIONES (1342)	Elmidae (2 A)
HEXAPODA	LEPIDOPTERA*
DERMAPTERA (836 A & I**)	DIPTERA (332491)
Labiidae	Mycetophilidae (76 A)
HEMiptera (1580)	Sciariidae (2253 A)
Anthocoridae (1571 A & I)	Cecidomyiidae (730 A)
Cicadellidae (4 A)	Psychodidae (742 A)
Aphididae (5 A)	Scatopsidae (4 A, 14 I)
THYSANOPTERA (6)	Chironomidae (397 A)
PHTHIRAPTERA (45)	Stratiomyidae (251 I)
COLEOPTERA (47,387)	Lonchopteridae (3 A)
Histeridae (9686A, 5163I)	Phoridae (88 A, 17 I)
<i>Carcinops pumilio</i> (Erichson)	Otitidae (2 A)
Demestidae (603A, 1779 I)	Heleomyzidae (66 A)
<i>Dermestes</i> sp.	Sphaeroceridae (268938 A)
Staphylinidae (235)	Curtonidae (56 A)
<i>Philonthus</i> sp. (171 A)	Drosophilidae (1280 A, 1383 I)
<i>Staphylinus</i> sp. (64 A)	Muscidae (56013)
Tenebrionidae (29778)	<i>Fannia</i> sp. (1455 I)
<i>Alphitobius diaperinus</i> (Panzer)	<i>Musca domestica</i> L. (7134 I)
(9260 A, 20363 I)	<i>Hydrotaea aenescens</i> (Weidemann) (47376 I)
<i>Tribolium</i> sp. (155 A)	Calliphoridae (181)
Anthicidae (69 A)	<i>Phaenicia</i> sp. (142 A)
	<i>Phormia</i> sp. (39 A)
	HYMENOPTERA (561)
	Pteromalidae† (478 A)
	Braconidae (79 A)
	Ichneumonidae (1 A)
	Chalcididae (2 A)

* not identified or counted

** A=adults, I=immature

† more than one type collected

Figs. 4 & 5 show the total number of taxa collected on each date for each house at both farms. Arrows in the figures indicate the sample dates when the associated taxa were first encountered in the manure samples. Although on a given date (e.g. 19 July 1988), Staphylinidae, Cleridae, and Otitidae were collected for the first time from house #1 (Fig 4), other taxa collected on previous dates (e.g. house flies, *H. aenescens* and *A. diaperinus*, etc.) were likely to have been collected from the same sample. In fact, many of the taxa shown in the figures occurred infrequently, and were included to show when the taxa were first found.

Sphaeroceridae was the only taxon collected during the first two weeks of sampling from house #2 at Nottoway Farms, and shortly thereafter, several dipterans were collected, including house flies and *H. aenescens*. *H. aenescens* occurrence was the result of prior mass releases of larvae and pupae. Except for the collection of a few *C. pumilio* larvae, these beetles were not conspicuous until after six months of manure accumulation, when both the adults and larvae became abundant. The total number of taxa per sample date increased with time in house #2 to between 25 and 30 by the summer of 1988, and leveled off between 20 and 25 per sample date for the remainder of the sample period.

In Nottoway #1 (Fig. 4), a considerable number of taxa were present at outset of sampling, with the taxonomic assemblage consisting of several dipterans, including the expected dung gnats, and *H. aenescens*. However, house flies were not collected until approximately one month later. Subsequently, the number of taxa increased slightly during the summer months, and dropped as cooler weather approached. The next spring (Mar 1989) the total number of taxa collected (20) was back down to the level seen the previous year, and remained between 20 and 25 taxa per sample date. At the Glenwood Farms houses (Fig. 5) the samples collected throughout the six months yielded a consistent number of taxa across the sampling dates.

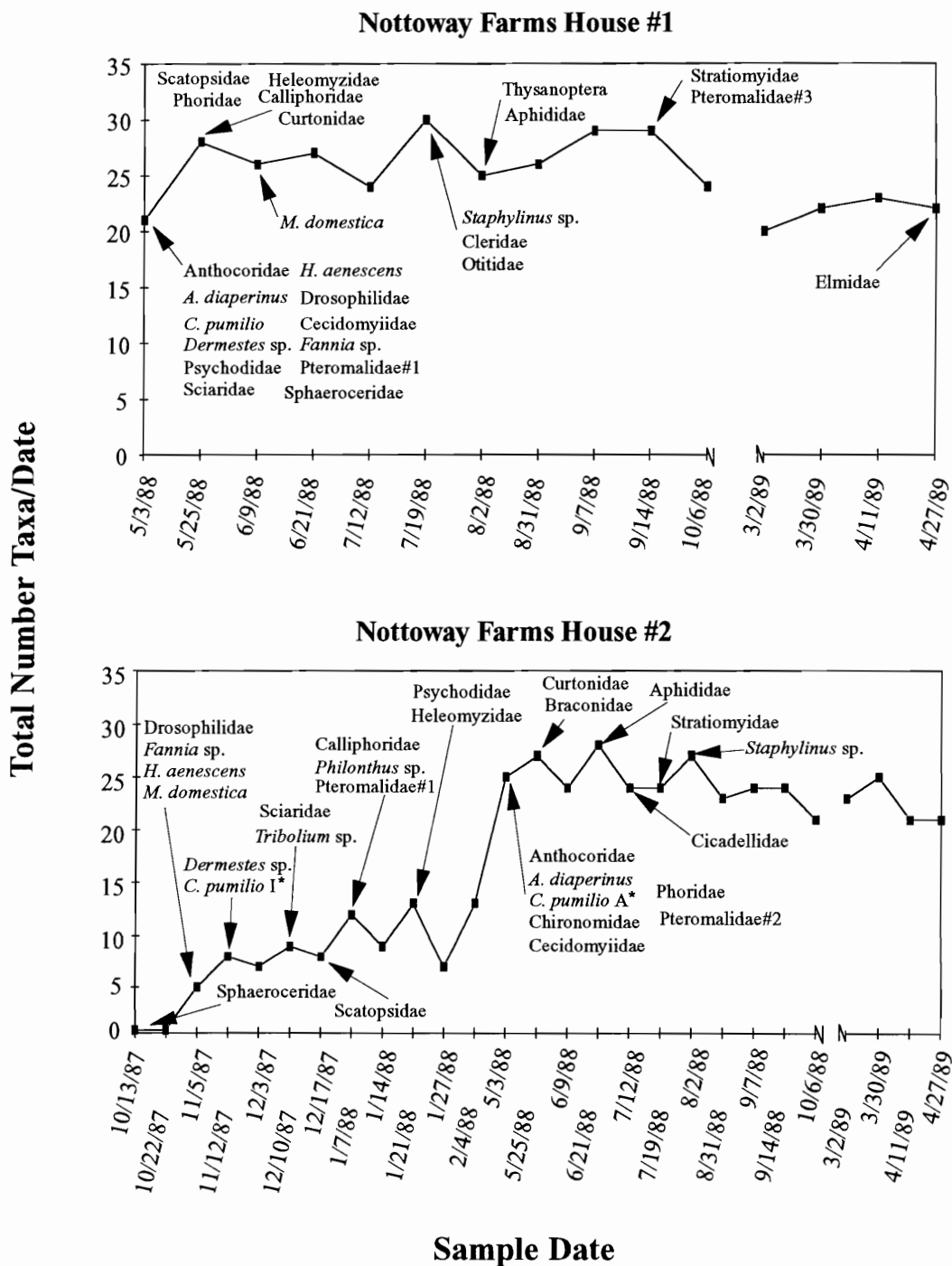


Fig. 4. Total number of taxa collected from manure on each sample date from high-rise, cage layer poultry houses at Nottoway Farms, Blackstone, Va. Arrows indicate the date when the given taxon was first encountered in the samples.

* A=adult, I=immature

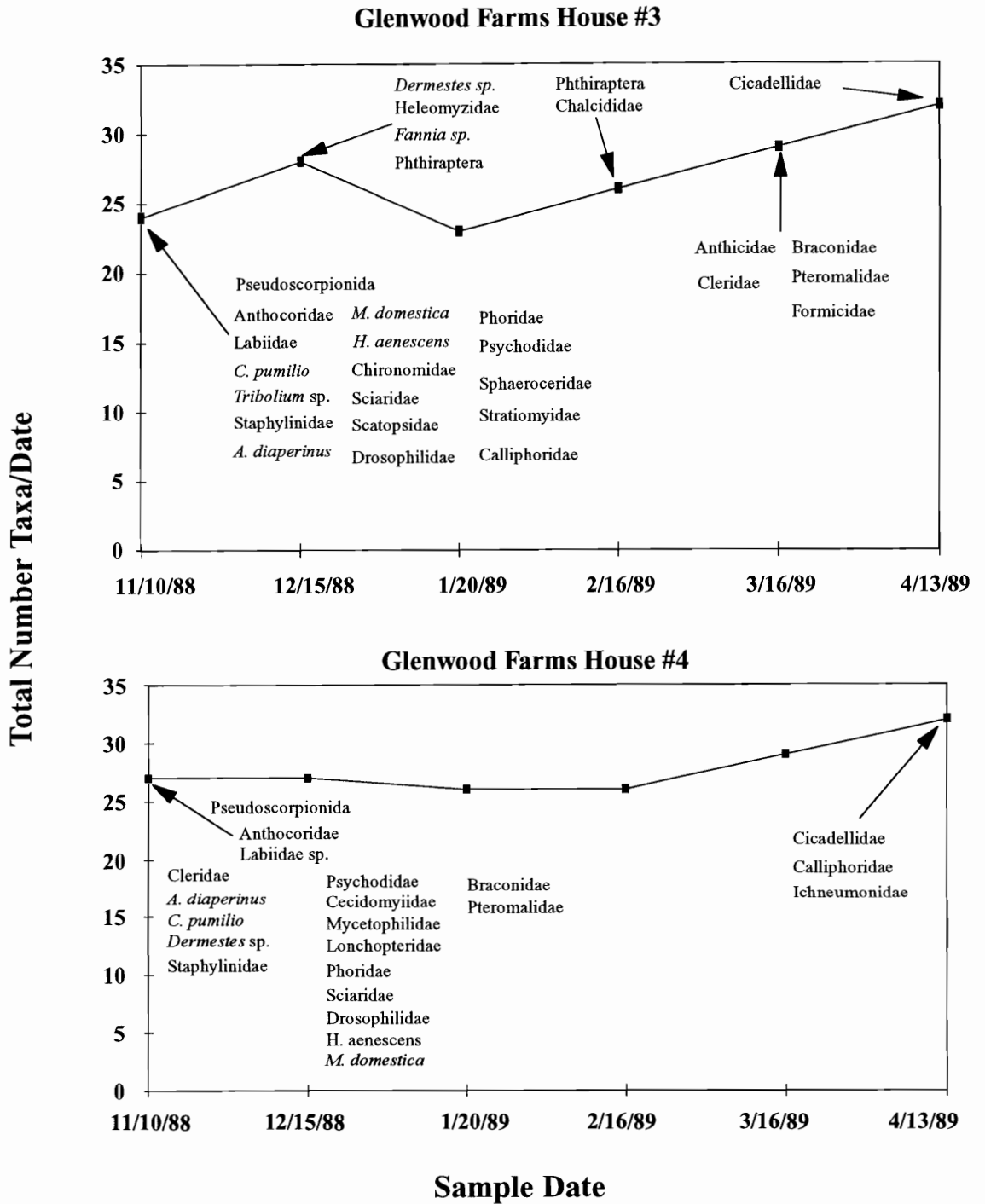


Fig. 5. Total number of taxa collected from manure on each sample date from high rise, cage layer poultry houses at Glenwood Farms, Jetersville, Va. Arrows indicate the date when the given taxon was first encountered in the samples.

Figs 6-13 show the mean Simpson diversity index, Heip's evenness index, the mean number of taxa per 100 g of manure, and the mean number of individuals per 100 g of manure; all plotted over time for the moisture categories 0%-50%, and 51%-90%. They were plotted this way because of the bimodal distribution of moistures that occurred, in order to clarify what was happening.

Simpson's diversity index (D_s) concerns the probability that two individuals are members of the same species if they have been selected at random from the community. If species diversity is high, then there is a low probability that both individuals will belong to the same species (Brower & Zar 1990). This index is among several indices that reflect two biologically meaningful characteristics of communities; i.e. species richness (number of species), and species evenness (distribution of individuals among the species). Simpson's diversity index was used because it is one which allows for calculation of diversity when samples are known to be a non-random representation of the community (Brower & Zar 1990), and this was the case with the sampling scheme used in the present study. In this study the Simpson's index does not reflect species diversity per se, since not all taxa were classed to species, but may be referred to as taxonomic diversity (Brower & Zar 1990).

Evenness indices, such as Heip's, are measures of the closeness between a set of observed species abundances and the abundances from a set of species having maximum possible diversity (Brower & Zar 1990). The main criticism of Heip's index (Ludwig & Reynolds 1988), is that it is sensitive to species richness, with its value being lowered by the addition of a rare species to the community. However, they do not feel that this prevents ecological interpretation of general patterns.

Diversity Estimates: Nottoway - House #2 (manure moisture 0%-50%)

Diversity (Fig. 6), like taxa (Fig. 7), and individuals (Fig 8) was actually zero for the first three sample periods, while evenness was at maximum (1.0). As the number of

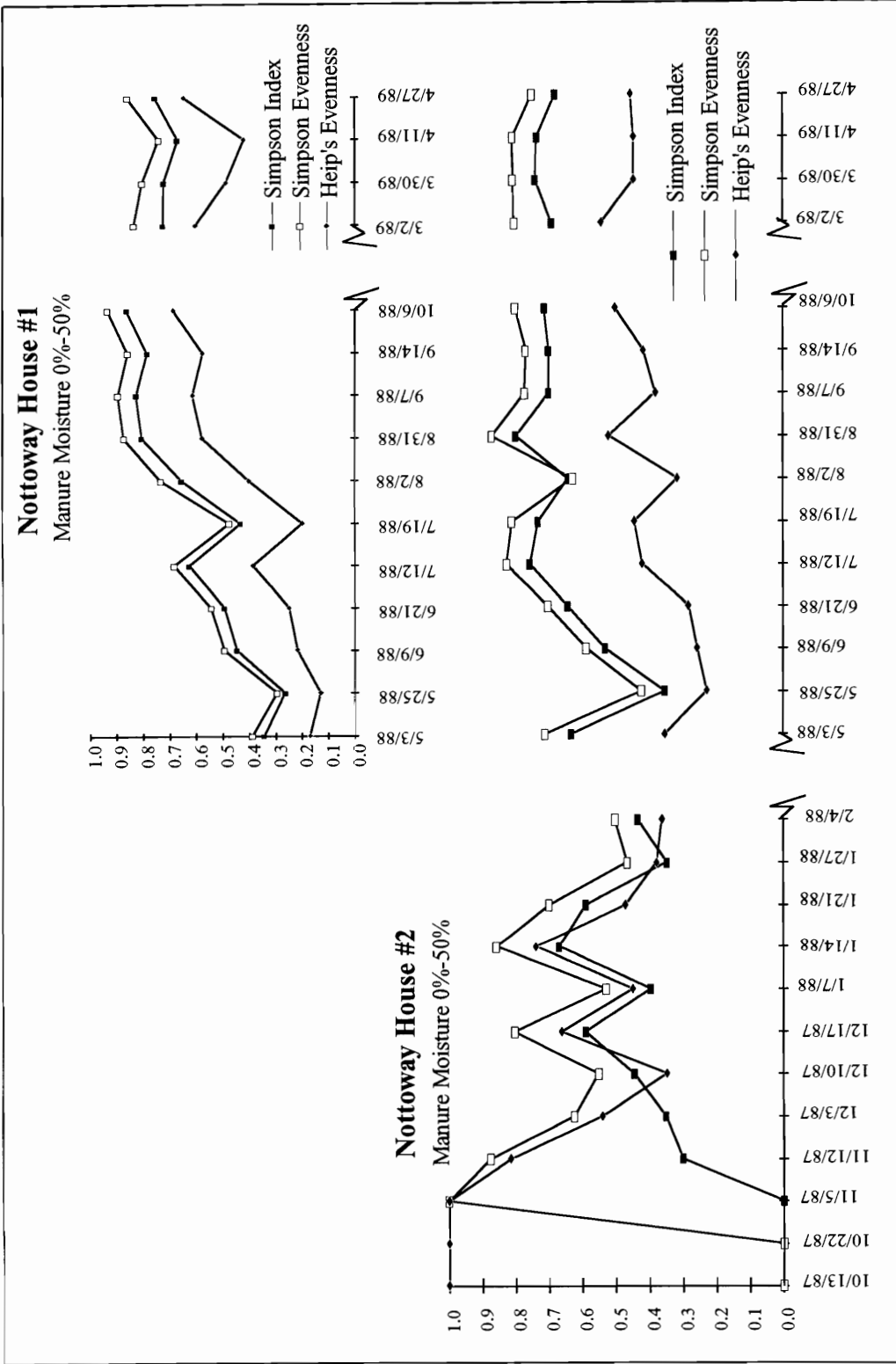


Fig. 6. Mean Simpson Diversity Index, mean Simpson Evenness, and mean Heip's Evenness Index of arthropod fauna per 100 g of manure in the manure moisture range 0%-50% from houses #1, and #2 at Nottoway Farms, Blackstone, Va.

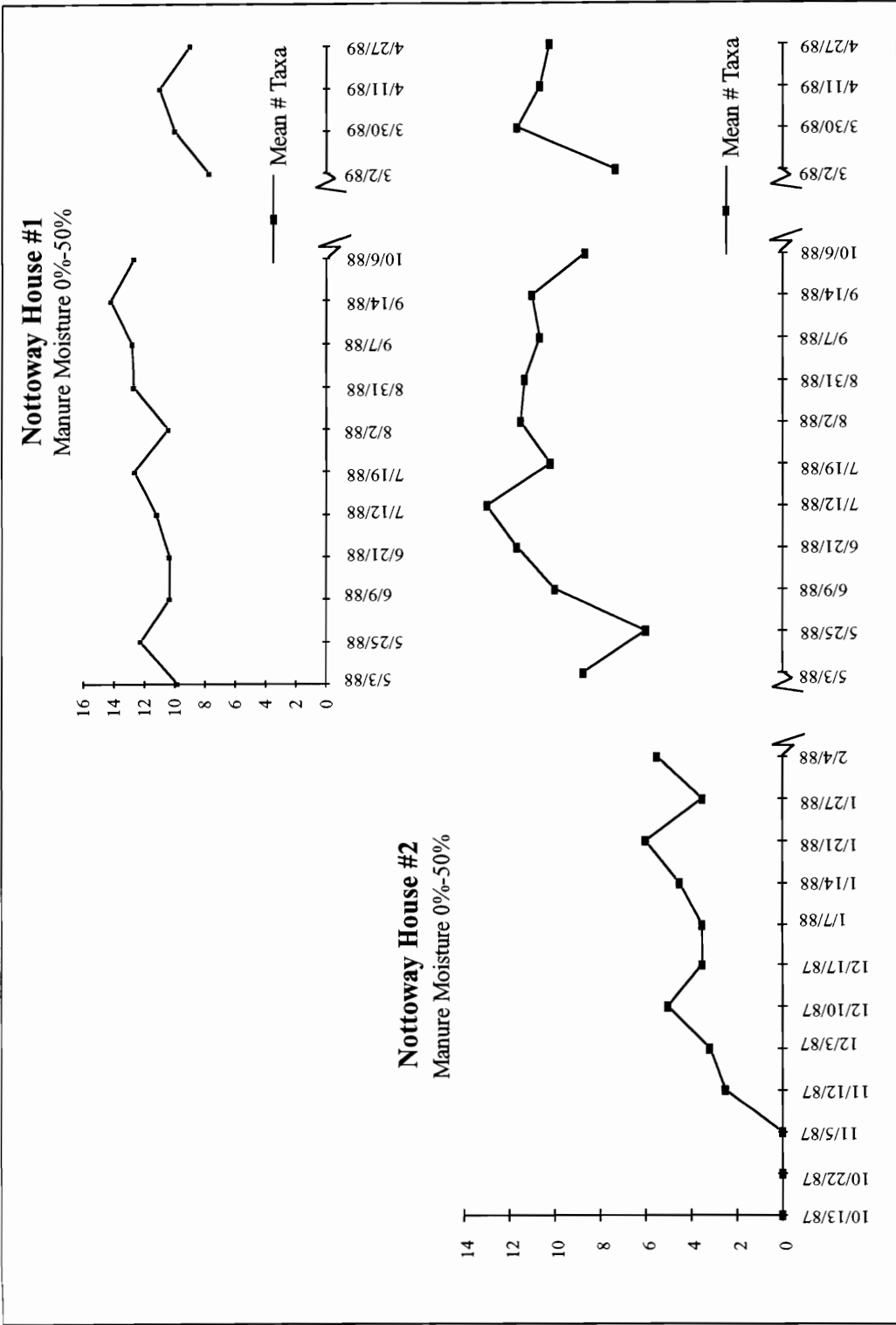


Fig. 7. Mean number of taxa per 100 g of manure in the manure moisture range 0%-50% from houses #1 and #2 at Nottoway Farms, Blackstone, Va..

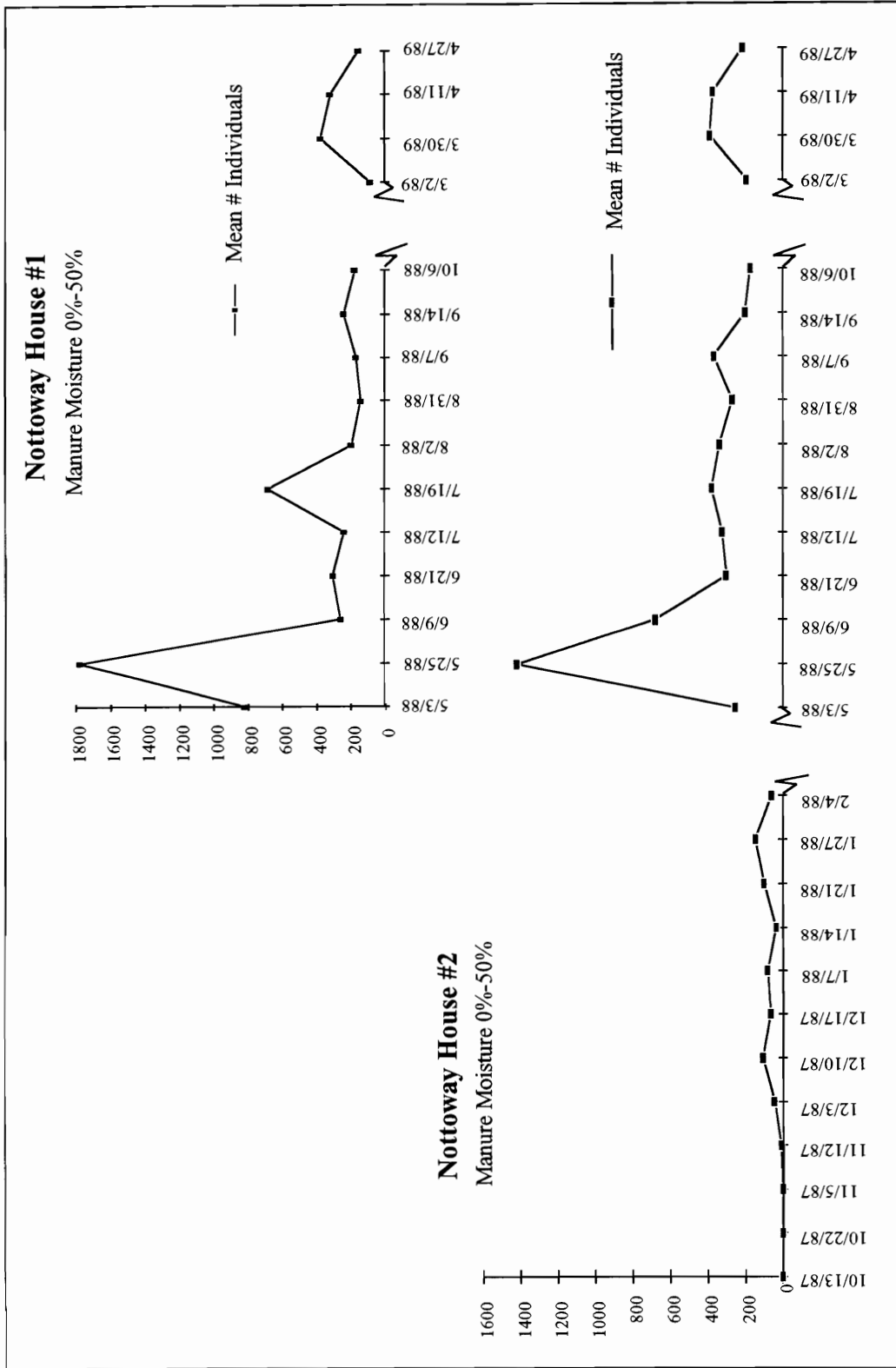


Fig. 8. Mean number of individuals per 100 g of manure in the manure moisture range 0%-50% from houses #1 and #2 at Nottoway Farms, Blackstone, Va.

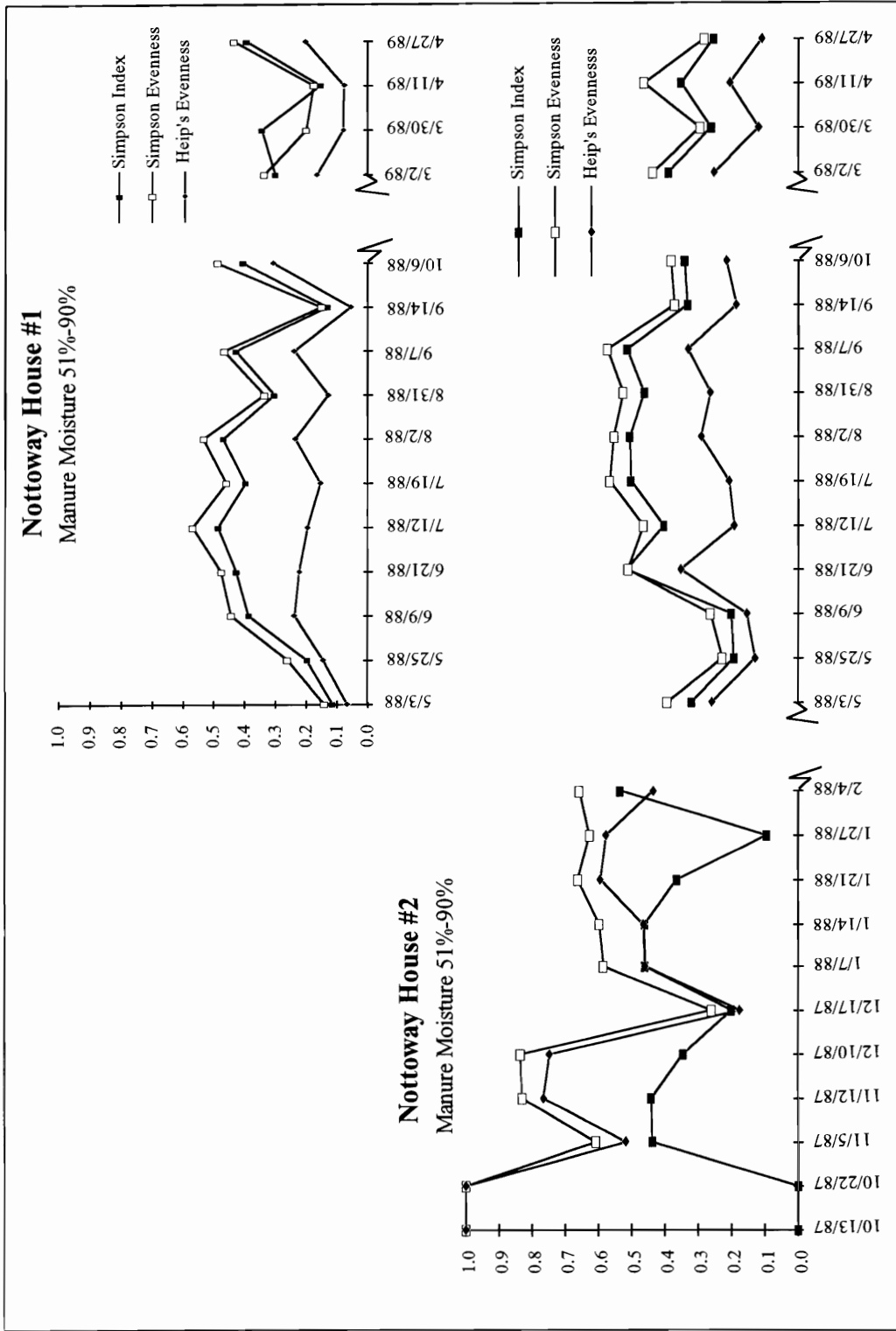


Fig. 9. Mean Simpson Diversity index, mean Simpson Evenness, and mean Heip Evenness index of arthropod fauna per 100 g of manure in the moisture range 51%-90% from houses #1 and #2 at Nottoway Farms, Blackstone, Va.

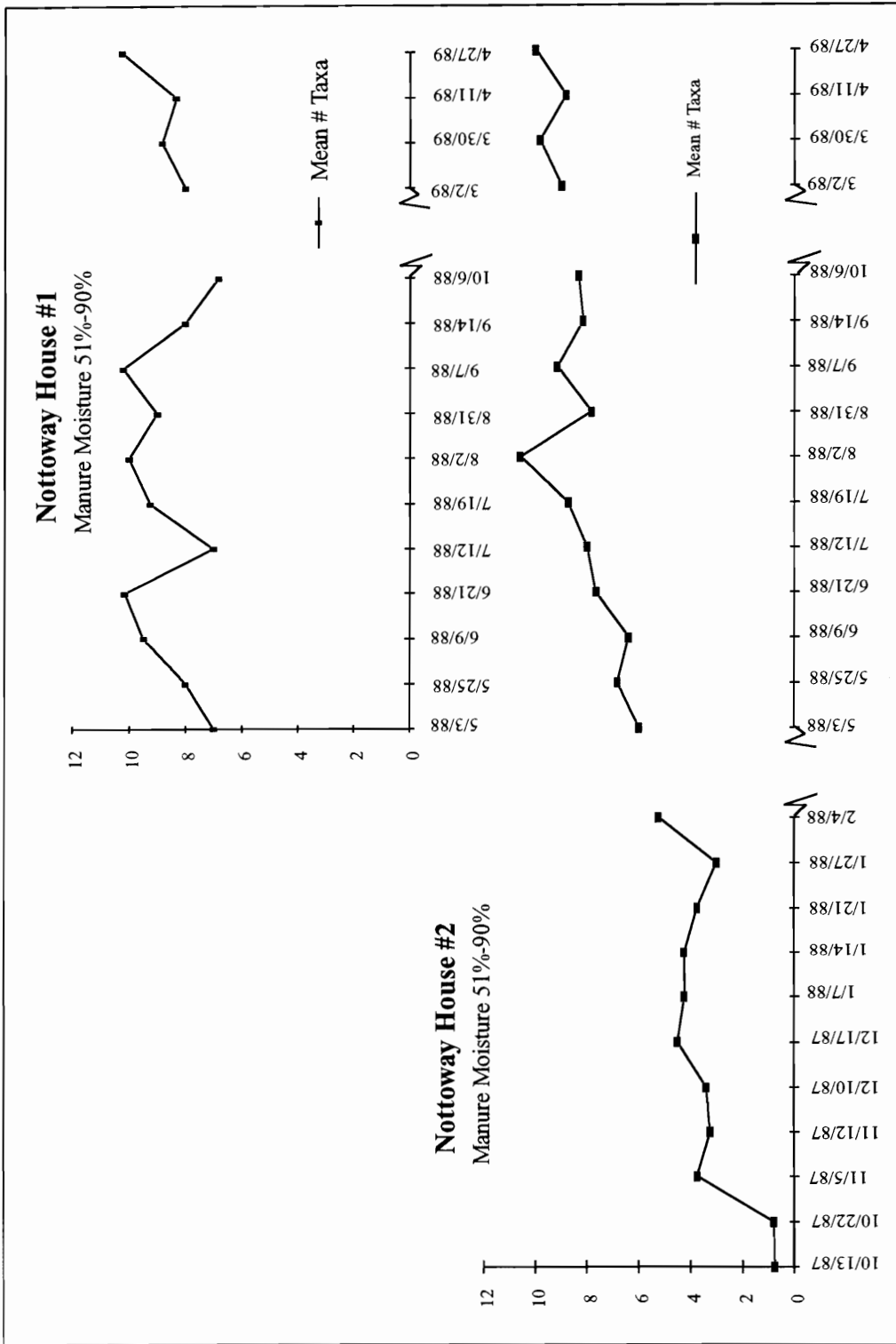


Fig. 10. Mean number of taxa per 100 g of manure in the manure moisture range 51%-90% from houses #1 and #2 at Nottoway Farms, Blackstone, Va.

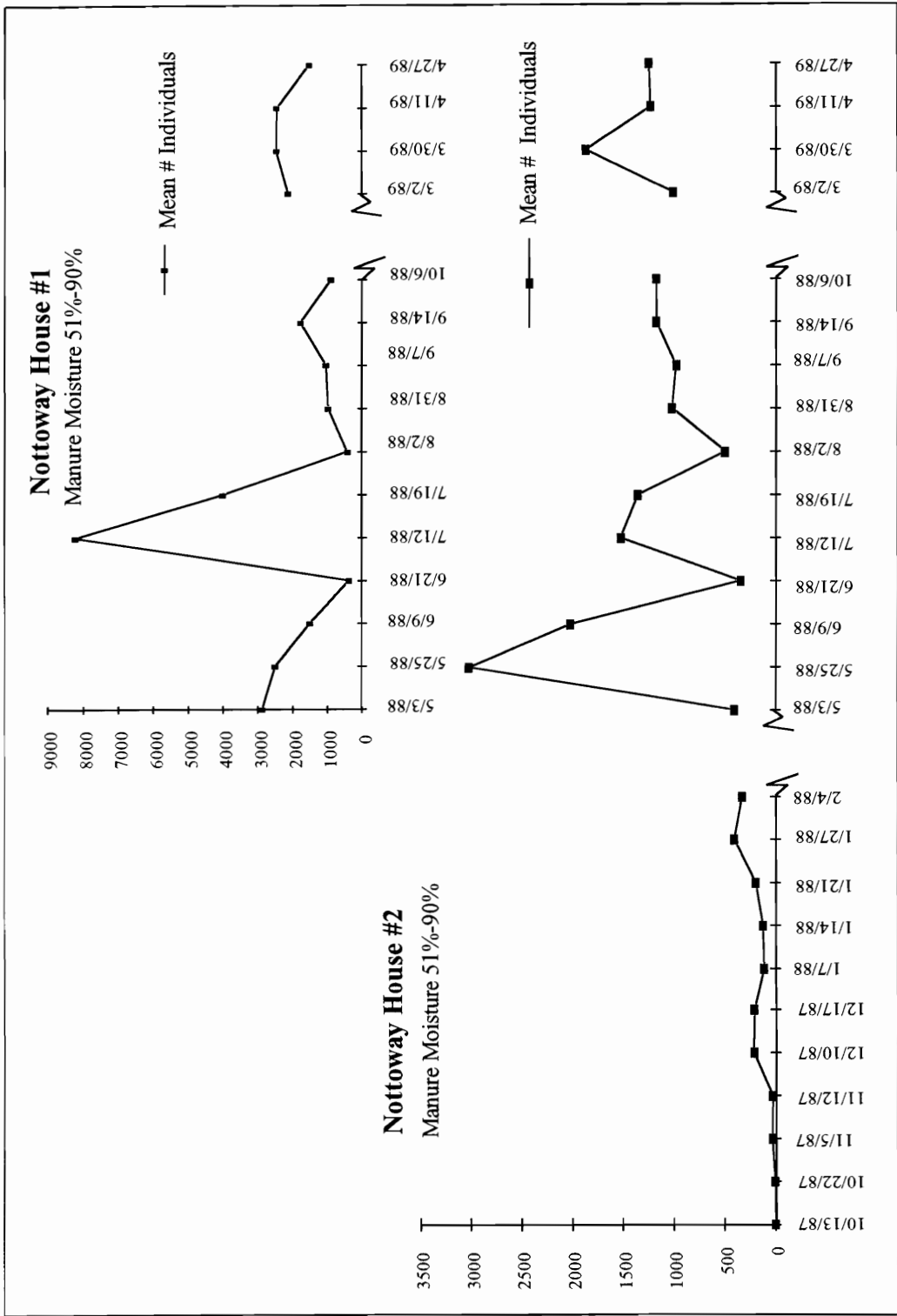


Fig. 11. Mean number of individuals per 100 g of manure in the manure moisture range 51%-90% from house #1 and #2 at Nottoway Farms, Blackstone, Va.

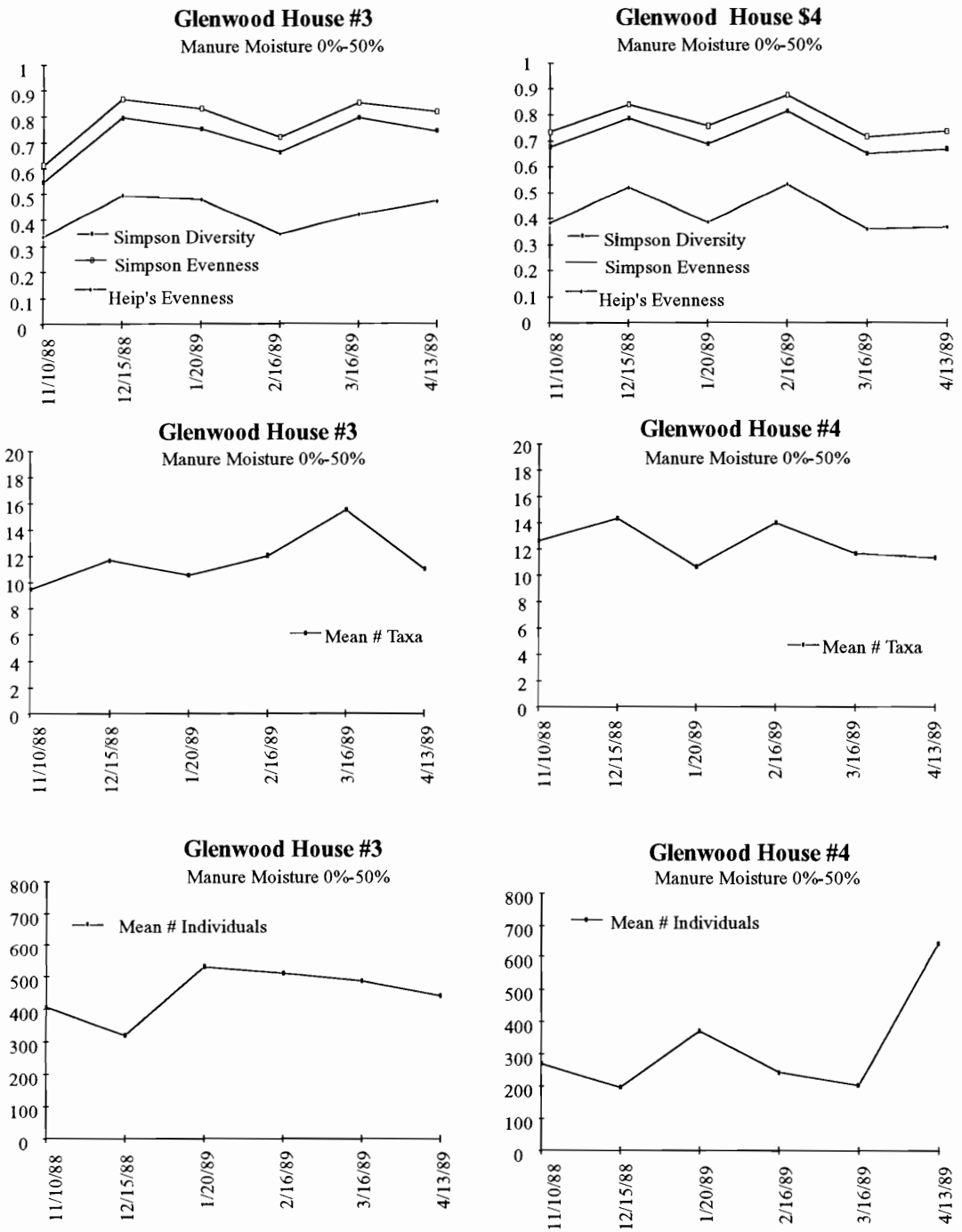


Fig. 12. Mean Simpson Diversity index, mean Simpson Evenness, mean Heip Evenness, mean number of taxa/100 g of manure, and mean numbers of individuals/100 g of manure in the manure moisture range 0%-50% from houses #3 and #4 at Glenwood Farms, Jetersville, Va.

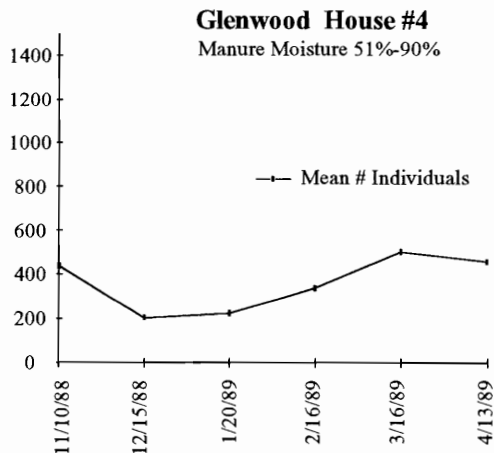
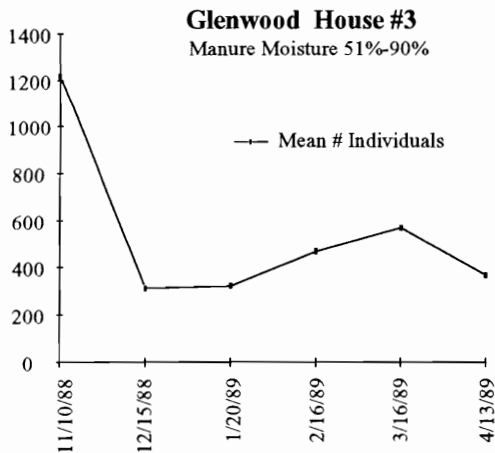
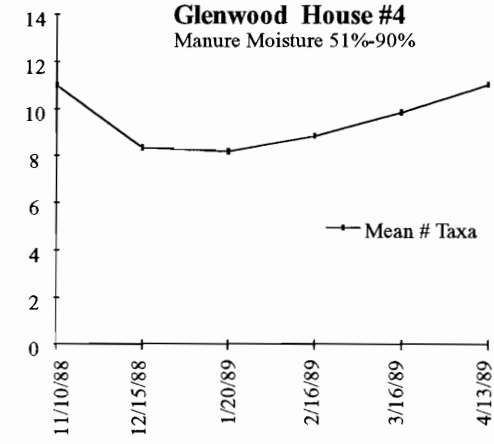
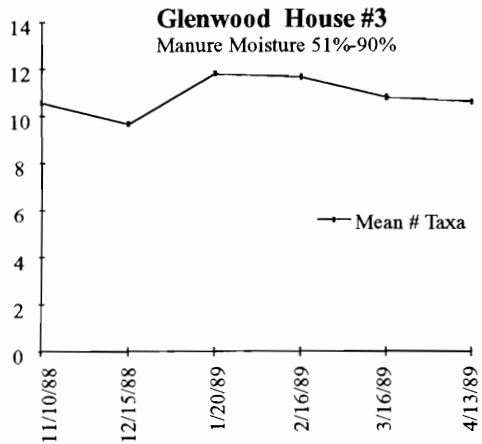
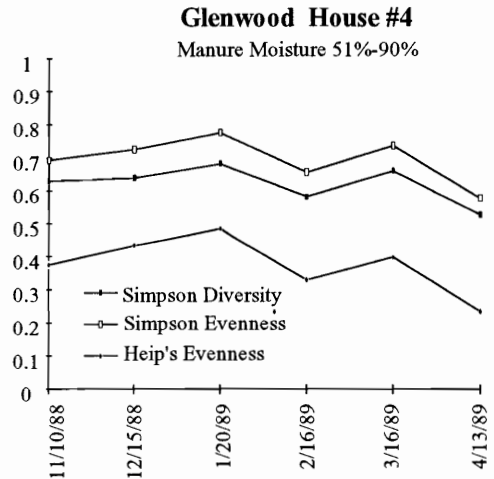
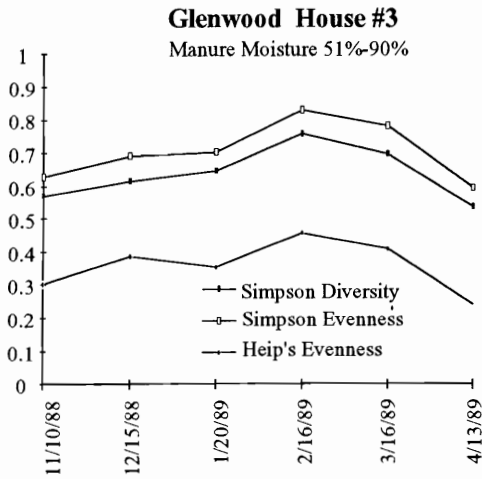


Fig. 13. Mean Simpson Diversity index, mean Simpson Evenness, mean Heip Evenness, mean number of taxa/100 g of manure, and mean number of individuals/100 g of manure in the manure moisture range 51%-90% from houses #3 and #4 at Glenwood Farms, Jetersville, Va.

individuals and number of taxa increased, so did diversity, to ca. 0.6 by 17 Dec. Evenness decreased to 0.4 by 10 Dec, indicating that the number of individuals were less evenly distributed over the number of taxa. On 7 Jan, both diversity and evenness declined. However, both traits increased again by the next sample date, and exceeded 0.6, and 0.7 for diversity and evenness respectively. Thereafter, diversity declined steadily to just above 0.4 when sampling was terminated. During the same period evenness also declined, an indication that perhaps rare species were turning up in the samples, since the number of taxa/100 g did not decline in a similar fashion (Fig 7). When sampling resumed in May 1988, diversity was higher (0.6) than when sampling was terminated in Feb., and evenness remained unchanged. When the number of taxa decreased (27 May 1988), diversity declined and evenness decreased as well, although the number of individuals increased dramatically. In spite of the mean number of individuals staying approximately the same (21 Jun 1988 and thereafter), the number of taxa increased and so did diversity (up to a maximum 0.8) in August. Evenness also increased steadily to the date when diversity was highest (31 Aug). This probably occurred because there were moderate numbers of individuals evenly distributed among several of the taxa, and also there were many taxa. Thereafter, diversity remained relatively constant (above 0.7) for the remainder of the study, and evenness also was similar before and after sampling was discontinued in Oct 1988, and resumed in March 1989. The arthropod community appears to have stabilized in terms of the diversity, evenness, and richness.

Diversity Estimates: Nottoway - House #2 (51%-90% manure moisture)

At the start of sampling from the higher moisture levels, diversity values were zero, and evenness was 1.0 (Fig. 9), as in the drier samples. The number of taxa increased by the third sample date of 5 Nov 1988 (Fig. 10), while the number of individuals was ca. 100 (Fig. 11), and diversity increased to ca. 0.45. Evenness

decreased to ca 0.5 at this time, because the number of taxa increased. When the number of individuals increased to ca. 300, the diversity and evenness decreased substantially to ca. 0.2, while the number of taxa remained the same. This indicates that more individuals occurred in fewer of the taxa, and that perhaps some rare taxa with one or two individuals were found. Diversity increased on 7 Jan, and after leveling briefly, dropped dramatically through Jan to ca. 0.1 (Fig.9). At this time, the number of individuals increased from the previous date to ca. 500 per 100 g of manure. Evenness however, stayed the same. By 4 Feb (the last sample date in 1988), diversity was up to ca. 0.55 due to the increase in number of taxa, and evenness was down to 0.45. When sampling resumed, diversity was ca. 0.3, and evenness about 0.25 (Fig. 9). During the next month, a decline in evenness caused a drop in diversity (Fig. 9), despite an increase in the number of taxa (Fig. 10). During this period, the number of individuals increased substantially to ca. 3000/100 g of manure. This indicates that a large number of individuals occurred in 1 or 2 taxa, while there were moderate numbers of individuals in the rest of the taxa. On 21 Jun, the mean number of individuals declined to below 500 and the diversity and evenness increased. Thereafter both density and evenness remained nearly constant (near 0.5 for diversity, and 0.3 for evenness) until 7 Sept (Fig. 9). The following spring, diversity and evenness were about the same levels as before. On 30 Mar, the number of individuals increased substantially (Fig. 11), and the number of taxa increased slightly (Fig. 10). However, diversity and evenness decreased (Fig. 9). This probably resulted from the occurrence of many individuals in one taxon. By the end of the sample period, the number of taxa was at its maximum and diversity and evenness had decreased slightly. Starting from the first summer, stabilization of the indices is apparent to the end of the sampling period.

Diversity Estimates: Nottoway - House #1 (0-50% manure moisture)

A dramatic increase in the number of individuals and the number of taxa occurred in both houses on 25 May (Fig. 8). A decrease in diversity and evenness occurred concurrently in both houses (Fig. 6). Diversity and evenness indices follow the same general pattern seen in house #2 (Fig. 6), except that the second significant decline in these indices occurred two weeks earlier in house #1. This resulted from an increase in the mean number of individuals in house #1 on 19 Jul (Fig. 8). On this date, the mean number of individuals in house #1 (ca. 800) was higher than that in house #2 (ca. 200).

Diversity Estimates: Nottoway - House #1 (51%-91% manure moisture)

The trends of the indices in the diversity between Nottoway #1 and #2 was less similar at this moisture level during 3 May to 9 Jun 1988 (Fig. 9). During this time diversity and evenness increased steadily in house #1, in contrast to a downward shift in house #2 which was caused by a dramatic rise in the number of individuals (Fig. 11). When the mean number of individuals increased dramatically on 12 Jul in house #1 (Fig. 11), there was a corresponding increase in diversity, and a slight decrease in evenness (Fig. 9). This could have been the result of a more even dispersal of the individuals among the taxa, or the inclusion of a few rare taxa. On 14 Sept 1988 diversity and evenness declined to low levels in house #1 and, to a lesser degree, in house #2 (Fig. 9). On the next sample date, diversity and evenness both increased in house #1, but remained constant in house #2. After resuming sampling in Mar 1989, diversity and evenness were ca. 0.3 and 0.25 and then dropped to ca. 0.2 and 0.1 on 11 Apr, but increased to the previous summer's levels by the next sample date. The mean number of individuals also increased on that date.

Diversity Estimates: Glenwood - Houses #3 and #4 (0%-50% manure moisture)

In these houses, diversity and evenness follow similar patterns, with diversity fluctuating between ca. 0.6 and 0.85 (Fig. 12). Prior to 20 Jan 1989, more than 120,000

H. aenescens were released in house #3. However, it was not until this date that the mean number of individuals/100 g of manure increased. The same thing occurred in house #4 (Fig. 12), where there was an increase in the number of individuals/100 g of manure after ca. 102,000 *H. aenescens* were released during Dec 1988 and Jan 1989. The highest number (ca. 15) of taxa/100 g of manure was collected from house #3 on 16 Mar 1989, while the highest mean number of individuals /100 g of manure (ca. 650), occurred on 14 Apr 1989. A decline in the number of individuals/100 g of manure occurred from 10 Nov to 15 Dec in both houses.

Diversity Estimates: Glenwood - Houses #3 and #4 (51%-90% manure moisture)

There was a much higher mean number of individuals collected from house #3 than from house #4 at the beginning of the sampling period (Fig. 13). This probably happened because ca. 62,900 adult *H. aenescens* were released prior to the first sampling date, whereas in house #4, only 30,600 *H. aenescens* were released. The mean number of individuals/100g of manure declined from ca. 1200 to ca. 300 in house #3, in spite of the release of 260,000 *H. aenescens* between the first and second sample dates (Fig. 12). As at the lower moisture levels, taxon numbers, diversity, and evenness, followed the same general pattern for both houses, with diversity fluctuating between ca. 0.5 and 0.8. In both houses, diversity and evenness declined to the lowest point on the last sample date.

Changes in Manure Moisture over Time

At Glenwood Farms, the mean percent manure moisture in the interval 0%-50% was ranged from ca. 27% to 45% in house #3, while the means from house #4 in the same interval fell between 12% and 27% (Fig. 14). The wetter samples in house #3 indicate there were few dry areas in the house. This would explain why the producer released high numbers of *H. aenescens* which were used to combat large numbers of

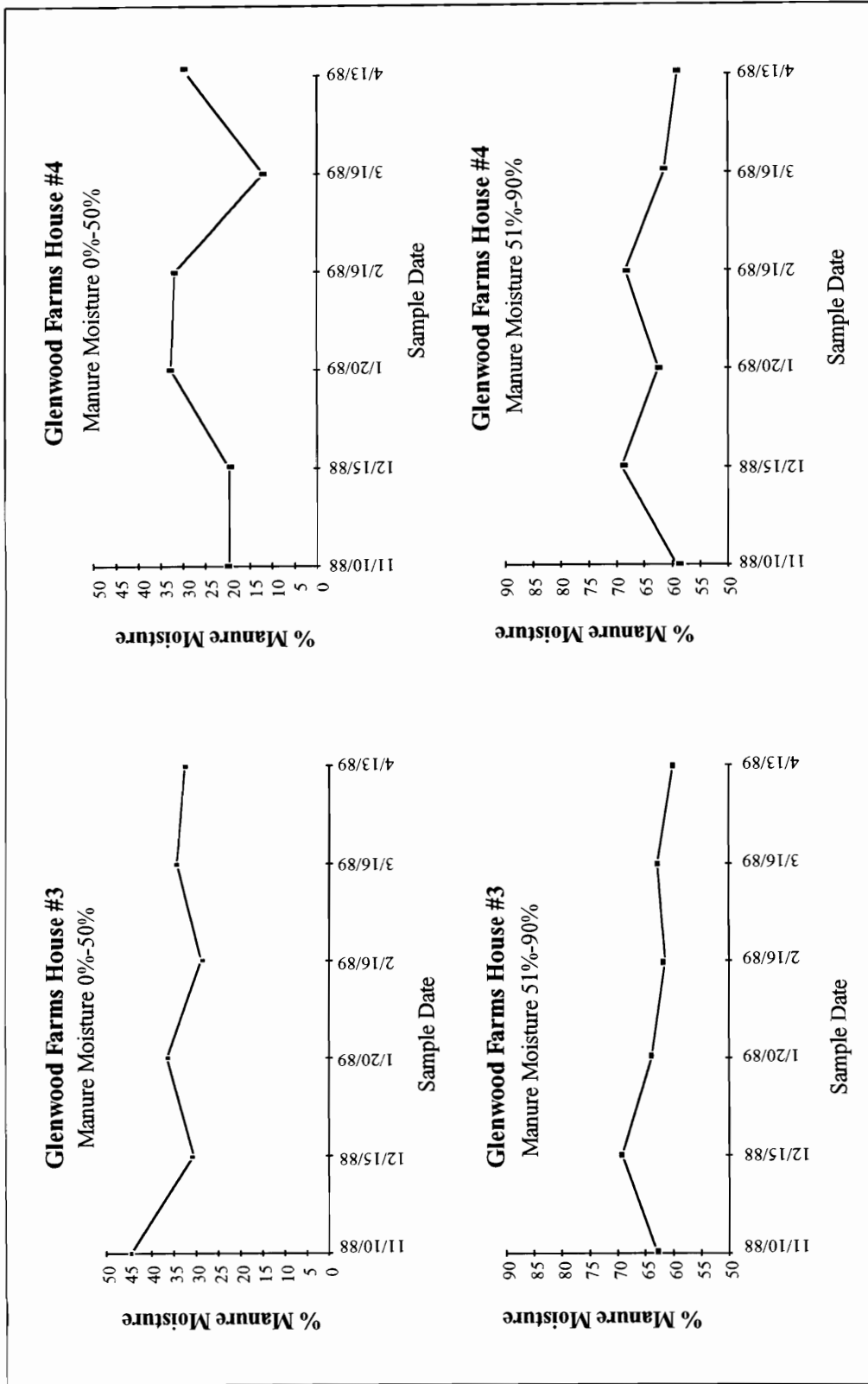


Fig. 14. Mean percent manure moisture in the ranges 0%-50%, and 51% to 90% from houses #3 and #4 at Glenwood Farms, Jetersville, Va.

house flies. In the 51%-90% interval, the values of mean percent manure moisture lay between 60% and 70% in both houses (Fig. 14).

In the 0%-50% interval at Nottoway Farms there were wide fluctuations in the mean percent moisture of the manure from house #2 throughout the sample period (between 20% and ca. 48%) (Fig. 15). However, it is important to note that, over time, the means and the magnitude of these fluctuations steadily decreased. This indicates that the habitat was stabilizing, with dry samples becoming more common. In house #1 (except for the first increase on 25 May), the means tended to fluctuate more narrowly than in house #2, with the range between 20% and 33%. The range of means in the 51%-90% moisture interval was much higher for house #1 (ca. 60%-82%) than for #2 (ca. 62%-72%) (Fig. 16). This probably reflects the wetter conditions overall in house #1, resulting from poor manure management practices.

Similarity Indices

Listed in Table 4 are the total number of individuals, the total taxa per house, (grouped primarily by order), and Morisita's similarity, and the percent similarity values. The group "Predators" includes pseudoscorpions, anthocorids, earwigs, *C. pumilio* larvae and adults, staphylinids, and *H. aenescens*. In order to maintain consistency, Morisita's index of similarity was used, as it is based on Simpson's diversity index. Morisita's index is an expression of the probability that a randomly selected individual from one community belongs to the same species as a randomly selected individual from the second community, relative to the probability that two randomly selected individuals will belong to the same species, if both are drawn from one of the communities. This principle can also be applied to the taxa level (Brower and Zar 1990).

The values of Morisita's index, and the percent similarity values indicate a high degree of similarity between the houses at Nottoway Farms for each of the taxonomic categories in Table 4. This is not the case for houses #3 and #4 at Glenwood Farms.

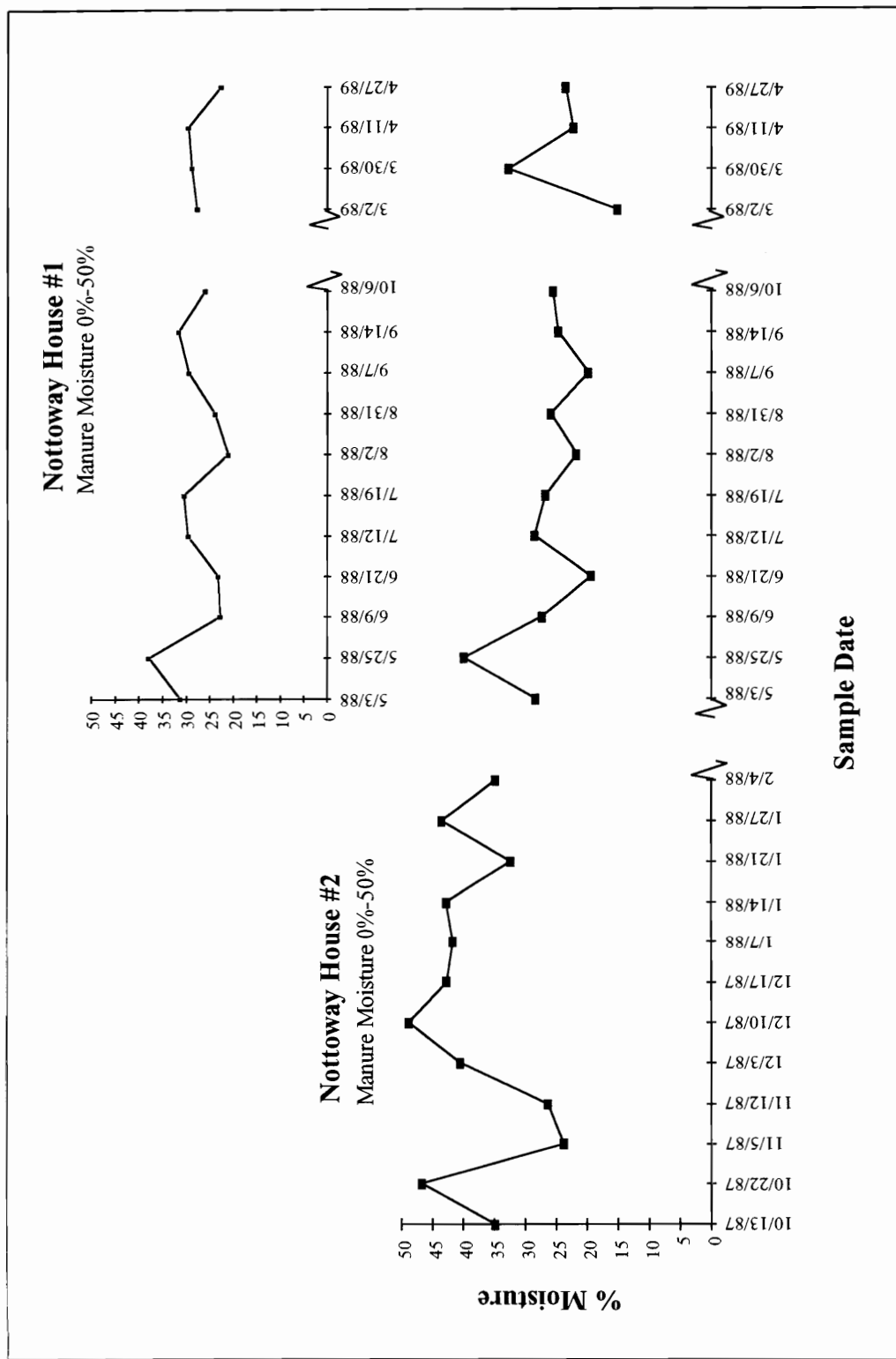


Fig. 15. Mean percent moisture in the ranges 0%-50% from houses #1 and #2 at Nottoway Farms, Blackstone, Va.

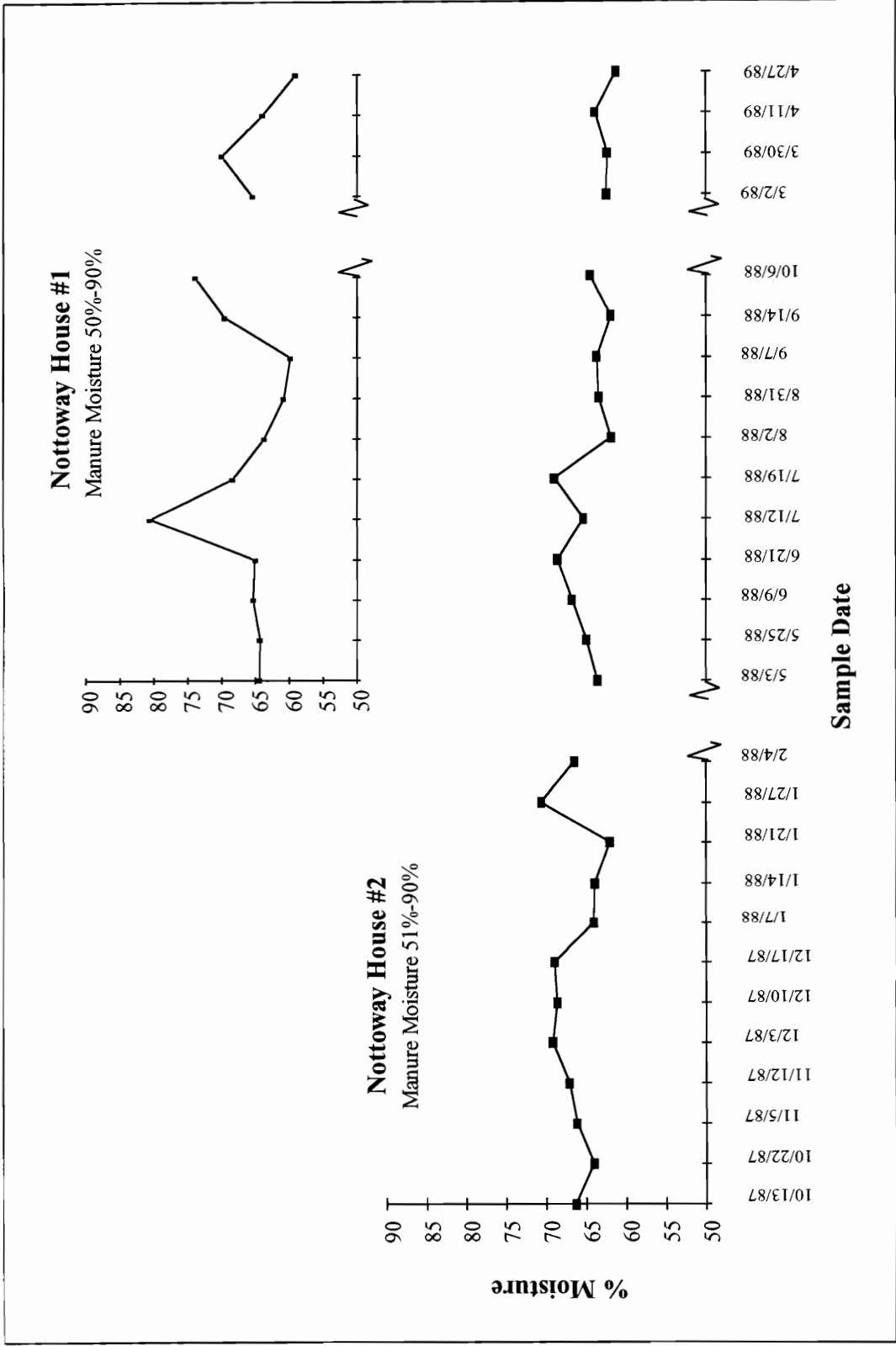


Fig. 16. Mean percent manure moisture in the ranges 51%-90% from houses #1 and #2 at Nottoway Farms, Blackstone, Va.

Table 4. Morisita's similarity indices, and percent similarity between houses #1 and #2 at Nottoway Farms, Blackstone, Va. between houses #3 and #4 at Glenwood Farms, Jetersville, Va., and between houses #3 and #4 compared with house #2 at Nottoway Farms.

Comparison	total #		total taxa		total #	total taxa		Morisita's Index	% Similarity
	total #	n-135/house	House 1	House 2		House 3	House 4		
Nottoway Farms									
House 1-vs-House 2		n=135/house	House 1	House 2					
All Taxa	164786		42	34	125855		All Taxa	0.996	92.49
Hymenoptera	265		6	6	117		Hymenoptera	0.891	73.869
Diptera	153566		23	16	109843		Diptera	0.997	94.243
Coleoptera	10525		10	9	15360		Coleoptera	0.946	82.375
Predators	13466		5	5	30024		Predators	0.999	97.53
Glenwood Farms									
House 3-vs-House 4		n=54/house	House 3	House 4					
All Taxa	28701		40	34	17988		All Taxa	0.869	76.306
Hymenoptera	34		6	4	120		Hymenoptera	0.486	41.74
Diptera	17824		16	15	8740		Diptera	0.874	71.963
Coleoptera	9286		13	12	8353		Coleoptera	0.996	94.888
Predators	14480		9	9	6086		Predators	0.929	78.59
Nottoway House 2**-vs-Glenwood House 3									
n=206		n=54	House 2	House 3					
All Taxa	28692		39	34	87024		All Taxa	0.559	49.59
Hymenoptera	54		4	8	68		Hymenoptera	0.447	27.39
Diptera	74586		15	16	17827		Diptera	0.619	51.00
Coleoptera	11946		9	13	92856		Coleoptera	0.934	79.88
Predators	15233		5	8	10311		Predators	0.976	82.61
Predators no HA*	5220		4	7	4123		Predators no HA*	0.785	57.89
Nottoway House 2**-vs-Glenwood House 4									
n=206		n=54	House 2	House 4					
All Taxa	17994		36	35	87016		Taxa	0.687	56.27
Hymenoptera	120		4	6	87		Hymenoptera	0.237	22.82
Diptera	74677		16	15	8740		Diptera	0.909	74.73
Coleoptera	11946		9	12	8352		Coleoptera	0.92	79.00
Predators	15233		5	8	6064		Predators	0.955	82.56
Predators no HA*	5220		4	7	3033		Predators no HA	0.867	67.02

* Similarity Indices calculated without *Hydrotaea aeneascens* included.

** indicates the entire sample period over which House #2 was sampled

Except for the Coleoptera, similarities of the different taxa between house #3 and #4 are lower than those from the houses at Nottoway Farms. The low value for Hymenoptera occurred because there were three individuals (two in different wasp genera, and one formicid), that were collected only from house #3. This was accentuated by the fact that there were no Hymenoptera collected from house #4 alone.

Morisita's similarity indices for all taxa between Nottoway #2 and Glenwood #3, and between Nottoway #2 and Glenwood #4 are lower than the similarity values between houses on the same farm (Table 4.) This verifies the results of the *t*-test where the diversity of taxa in house #2 collected over the entire sample period, was compared to the diversity of taxa collected from both houses at Glenwood Farms.

Discussion

The variety of Diptera collected during the present study, are associated with various types of wastes, and with damp, rotting material (McAlpine et al. 1987). In studies where Diptera were surveyed in poultry manure, researchers reported families that were also found in the present study, including Cecidomyiidae, Calliphoridae, Muscidae, and Drosophilidae (Green 1980, Hulley 1986).

Several researchers have reported high abundances of Sphaeroceridae. In fact, Hulley (1983) found that except for house flies, Sphaeroceridae were the second most abundant, accounting for 25% of the total number of flies collected. Armitage (1986) reported that Sphaeroceridae were among the most numerous families, and Geden & Stoffolano (1987) reported these flies to be the most numerous collected during a faunal succession study in shallow-pit houses. Bills (1973), in England, and Geden & Stoffolano (1987), in Massachusetts, reported that Sphaeroceridae were the first to become established, rapidly invading fresh manure. These populations were often associated with large numbers of predaceous mites.

The number of coleopteran taxa collected during the present study is low compared with the number collected in North Carolina high-rise poultry houses by Pfeiffer & Axtell (1980). They listed 52 species from 13 families as relatively common (10 individuals/species), and another 60 as rare species (<10 individuals/species). Of the beetle taxa collected during the present study, only the clerid and the elmid families fall into the "rare" category, the others being considered relatively common (Pfeiffer & Axtell 1980).

Other studies report a similar assemblage of Coleoptera from poultry manure. Green (1980) recorded the fauna of a deep-pit house in the U.K., and found 11 genera with several in common with the present study. These are the Histeridae, Dermestidae, Staphylinidae, Tenebrionidae, Cleridae, and Anthicidae. Hulley & Pfeleiderer (1988) conducted a survey of Coleoptera in poultry manure in S. Africa, and reported 12 families of beetles with only four families in common with the present study.

The most numerous arthropods collected during the present study are generally the same as those reported by other workers who examined taxa abundances and succession. These taxa are: *M. domestica*, *C. pumilio*, *A. diaperinus*, Sphaeroceridae (Legner & Olton 1970, Bills 1973, Pfeiffer & Axtell 1980, Hulley 1983, Armitage 1986, Hulley & Pfeleiderer 1988). There are also some reports of abundant populations of pseudoscorpions (Hulley 1983, Propp & Morgan 1985), earwigs (Legner & Olton 1970), anthocorids (Dunning et al. 1978, Propp & Morgan 1985, Rueda et al. 1990), and *H. aenescens* (Peck & Anderson 1969, Stafford et al. 1988).

It appears that there is a general guild of arthropods which commonly inhabits poultry manure regardless of characteristics such as geographic location and type of house, with several of the taxa probably being passively introduced into the houses with the chicken feed (e.g., *A. diaperinus*, *Tribolium* spp., *Dermestes* spp., and Cleridae). No doubt others migrate from the surrounding area (e.g., many of the Diptera, the Staphylinidae, Histeridae, Anthocoride, and parasitic Hymenoptera). Similarities

between accumulated poultry manure and nests have been reported (Green 1980), and the poultry manure habitat is described as comprising the characteristics of both 'wet' and 'dry' nests in the field. The long accumulation time of manure in most high-rise houses provides a permanent habitat for the variety of arthropod inhabitants, as opposed to the ephemeral nature of nests.

It is interesting to note that some of the taxa collected from the longer-established houses at Glenwood Farms were never collected from the houses at Nottoway Farms, even after 1 1/2 years of manure accumulation. These taxa include the predators, pseudoscorpions, and earwigs, which were often quite numerous in the samples.

The relative stability of the diversity and evenness indices over time reflect the decreasing availability of niches caused by the influx of newly colonizing arthropod taxa (Brower & Zar 1990). Diptera are fast colonizers and would be expected to be the first to arrive, with their parasites and predators following. Other workers have reported a delay in the occurrence of significant densities of *C. pumilio* of up to 10 weeks in a house from which manure was removed (Geden & Stoffolano 1987). The time required for significant colonization by this predator was considerably longer at house #2 at Nottoway Farms. This delay probably occurred because there were no other houses on the site to serve as reservoirs from which the arthropods could migrate.

Relationship of Selected Arthropods to Manure Moisture

The results of canonical correlation analysis indicate that manure moisture greatly affects overall taxon abundance. This is shown by the very high canonical correlation coefficients calculated from the taxon abundances at each farm. These were 0.925 for Glenwood Farms (Wilks' Lambda= 0.144, $F= 57.62$, $df=10$ $P<0.001$), and 0.924 for Nottoway Farms (Wilks' Lambda= 0.1445, $F= 192.34$, $df= 8$; $P<0.001$). The nearly identical canonical correlation coefficients indicate that the effect of moisture on taxon abundance was similar at both locations. Beyond indicating the obvious effect of

moisture, the results from canonical correlation analyses permit a grouping of taxa according to their relationships with each other, as well as with moisture. An ordination table can be produced from the results of the analysis (Appendix A). In the table, the taxa are ordered according to their canonical coefficients (most positive to most negative values), with the set of abundances of each taxon as well as the associated moisture values being sorted by the first canonical variable (Density1). The top row of the table contains the taxa names, Their position in this row indicates the degree of association with each other. In addition, the values of the first canonical variable, and the moisture values are tabled. In this case, dung gnats, house flies, and *H. aenescens* are closely associated with each other, and lesser mealworms, earwigs, and anthocorids are also closely associated. Pseudoscorpions, *C. pumilio* larvae and adults are in the center of the row, and this indicates that they are moderately associated with the taxa on either side, and are closely associated among themselves. When one examines the density 1 in relation to the manure moisture (moving down the table), it becomes clear that the first canonical variable (that which accounts for the most amount of variation among all of the arthropod densities) is, in fact, manure moisture. This is demonstrated by the transition in percent moisture values from low to high.

Correlation Among Taxa

Included in the results of the canonical correlation analysis are the correlation coefficients (r) among the selected taxa, and the coefficients for the correlation between each taxon density and manure moisture. These coefficients for between-taxa correlation, together with the associated correlation indices (r^2), for each farm, are presented in Tables 5 and 6. Correlation coefficients are a measure of the strength of the association between two variables (in this case, pairs of taxa), and r^2 values reflect the amount of variation that is explained by the correlation (Zar 1984). Therefore, negative coefficients indicate that as one variable increases the other variable decreases, while

Table 5. Correlations among selected taxa collected from manure samples from houses #3 and #4 at Glenwood Farms, Jetersville, Va.

Correlation Among Taxa Glenwood Farms (n=107)				
	<i>M. domestica</i>	<i>H. aenescens</i>	<i>C. pumilio</i> larvae	<i>C. pumilio</i> adults
$\bar{x} \pm (SD)\ddagger$	0.045 \pm (1.16)	16.64 \pm (7.85)	4.71 \pm (2.26)	14.63 \pm (2.56)
	r†	r	r	r
<i>M. domestica</i>	1.00	0.12	-0.21 *	-0.12
<i>H. aenescens</i>	0.12	1.00	0.02	-0.14
<i>C. pumilio</i> larvae	-0.21 *	0.01	1.00	0.41 **
<i>C. pumilio</i> adults	-0.12	0.14	0.41 *	1.00
<i>A. diaperinus</i> larvae	-0.12	0.51 *	0.17	0.43 **
<i>A. diaperinus</i> adults	-0.04	0.38 **	0.11	0.57 **
Pseudoscorpions	-0.09	0.01	0.21 *	0.45 **
Labiidae	-0.15	-0.48 **	-0.04	0.18
Sphaeroceridae	0.21 *	0.56 **	0.00	-0.02
Anthocoridae	-0.21 *	-0.47 **	-0.01	0.11
	<i>A. diaperinus</i> larvae	<i>A. diaperinus</i> adults	Pseudoscorpionida	Labiidae
$\bar{x} \pm (SD)\ddagger$	13.19 \pm (6.17)	11.63 \pm (4.99)	2.87 \pm (3.71)	2.12 \pm (3.03)
	r	r	r	r
<i>M. domestica</i>	-0.12	-0.04	-0.09	-0.15 **
<i>H. aenescens</i>	-0.51 **	-0.38 **	0.01	-0.48
<i>C. pumilio</i> larvae	0.17	0.11	0.21 *	-0.04 **
<i>C. pumilio</i> adults	0.43 **	0.57 **	0.45 **	0.18 **
<i>A. diaperinus</i> larvae	1.00	0.81 **	0.27 **	0.60 **
<i>A. diaperinus</i> adults	0.81 **	1.00	0.41 **	0.54 **
Pseudoscorpions	0.27 **	0.41 **	1.00	0.06 **
Labiidae	0.60 **	0.54 **	0.06	1.00
Sphaeroceridae	-0.60 **	-0.40 **	0.18	-0.57 **
Anthocoridae	0.59 **	0.45 **	-0.07	0.55 **
	Sphaeroceridae	Anthocoridae		
$\bar{x} \pm (SD)\ddagger$	41.33 \pm (4.41)	1.29 \pm (2.80)		
	r	r		
<i>M. domestica</i>	0.21 *	-0.21 *		
<i>H. aenescens</i>	0.56 **	-0.47 **		
<i>C. pumilio</i> larvae	0.00	-0.01		
<i>C. pumilio</i> adults	-0.01	0.02		
<i>A. diaperinus</i> larvae	-0.60 **	0.59 **		
<i>A. diaperinus</i> adults	-0.40 **	0.45 **		
Pseudoscorpions	0.18	-0.07		
Labiidae	-0.57 **	0.55 **		
Sphaeroceridae	1.00	-0.63 **		
Anthocoridae	-0.63 **	1.00		

† Pearson's Correlation Coefficient

‡ values in corresponding row are the mean number/100 g manure, \pm Std. Dev. of the mean

* significant at the 0.05 level [t -crit $\alpha=0.05$ (105)=1.98]

** significant at the 0.01 level [t -crit $\alpha=0.01$ (105)=2.62]

Table 6. Correlations among selected taxa in manure samples collected from houses #1 and #2 at Nottoway Farms, Blackstone, Va.

Correlations Among Taxa Nottoway Farm Houses #1 and #2 (n=269)				
	<i>M. domestica</i>	<i>H. aenescens</i>	<i>C. pumilio</i> larvae	<i>C. pumilio</i> adults
$\bar{x} \pm (SD)\ddagger$	3.68 \pm (4.48)	6.87 \pm (7.79)	3.35 \pm (3.43)	7.64 \pm (3.71)
	r†	r	r	r
<i>M. domestica</i>	1.00	0.24 **	-0.16 **	-0.30 **
<i>H. aenescens</i>	0.24 **	1.00	-0.10	-0.30 **
<i>C. pumilio</i> larvae	-0.16 **	-0.10	1.00	0.49 **
<i>C. pumilio</i> adults	-0.30 **	-0.30 **	0.49 **	1.00
<i>A. diaperinus</i> larvae	-0.38 **	-0.54 **	0.23 **	0.53 **
<i>A. diaperinus</i> adults	-0.37 **	-0.55 **	0.08	0.38 **
Sphaeroceridae	0.41 **	0.53 **	-0.08	-0.30 **
Anthocoridae	-0.25 **	-0.05	0.09	0.21 **
	<i>A. diaperinus</i> larvae	<i>A. diaperinus</i> adults	Sphaeroceridae	Anthocoridae
$\bar{x} \pm (SD)$	6.56 \pm (6.73)	2.43 \pm (3.84)	135.71 \pm (9.90)	0.93 \pm (1.76)
	r	r	r	r
<i>M. domestica</i>	-0.38 **	-0.37 **	0.41 **	-0.25 **
<i>H. aenescens</i>	-0.54 **	-0.55 **	0.53 **	-0.50 **
<i>C. pumilio</i> larvae	0.23 **	0.08	-0.08	0.09 **
<i>C. pumilio</i> adults	0.53 **	0.38 **	-0.30 **	0.21 **
<i>A. diaperinus</i> larvae	1.00	0.82	-0.65 **	0.59 **
<i>A. diaperinus</i> adults	0.82 **	1.00	-0.67 **	0.64 **
Sphaeroceridae	-0.65 **	-0.67 **	1.00	-0.65 **
Anthocoridae	-0.59 **	0.64 **	-0.65 **	1.00

† Pearson's Correlation Coefficient

‡ Values in corresponding row are mean number/100 g manure, \pm Std. Dev. of the mean.

* t -crit $\alpha=0.05(250)=1.97$, ** t -crit $\alpha=0.01(250)=2.59$

positive correlations indicate that, as one variable increases, the other also increases.

Although the majority of the correlation coefficients were moderate values, there were many highly significant ($\alpha=0.01$) correlations between taxa at both farms. The highest correlation at both farms occurred between lesser mealworm larvae and adults.

Otherwise the correlations, although significant, were generally weak. Examination of the r^2 values verifies this. Except for the non-significant correlation between house flies and *H. aenesceus* at Glenwood Farms, the Diptera were all significantly correlated with each other. The coleoptera, earwigs, pseudoscorpions, and Anthocoridae tended to be positively correlated with each other, and negatively correlated with the Diptera.

When considering the correlations among the taxa (Table 5), the most significant correlations occur where the taxa have a factor in common such as spatial distribution with respect to manure moisture. In fact, the taxa that require the more moist manure are positively correlated with each other, and those favoring drier manure are positively correlated with each other.

Correlation with Moisture

The coefficients for the correlation between taxa and moisture are presented in Table 7. Scatter plots of arthropod density versus percent manure moisture are shown in Figs. 17-19. There were highly significant ($\alpha=0.01$) correlations between taxon abundance and manure moisture for all of the taxa except *C. pumilio* adults and larvae at Glenwood Farms, and, although there were significant negative correlations for *C. pumilio* adults and larvae at Nottoway, the very low r^2 values indicate high variability of the data. These correlations should thus not be considered biologically significant. An examination of the scatter plots for other taxa with significant but loose correlations with moisture further illustrates this phenomenon where the r^2 negates the biological significance.

Table 7. Correlations between percent manure moisture and density of selected arthropod taxa collected from manure samples from houses #3 and #4 at Glenwood Farms, Jetersville, Va., and from houses #1 and #2 at Nottoway Farms, Blackstone, Va.

Correlations Between Moisture and Taxa Density (log n+1)					
Glenwood Farms (n=107)					
Variable	<i>M. domestica</i>	<i>H. aenescens</i>	<i>C. pumilio</i> larvae	<i>C. pumilio</i> adults	
	r†	r	r	r	r
Moisture	0.291**	0.608**	0.053		-0.026
	<i>A. diaperinus</i> larvae	<i>A. diaperinus</i> adults	Pseudoscorpions	Labiidae	
Moisture	-0.674**	-0.436**	0.075		-0.664
	Sphaeroceridae	Anthoceridae			
Moisture	0.848**	-0.746**			
Nottoway Farms (n=269)					
	<i>M. domestica</i>	<i>H. aenescens</i>	<i>C. pumilio</i> larvae	<i>C. pumilio</i> adults	
Moisture	0.487**	0.645**	-0.157**		-0.447**
	<i>A. diaperinus</i> larvae	<i>A. diaperinus</i> adults	Sphaeroceridae	Anthoceridae	
Moisture	-0.823**	-0.808**	0.810**		-0.688**

† Pearson's Correlation Coefficient

** Significant correlation $\alpha=0.01$

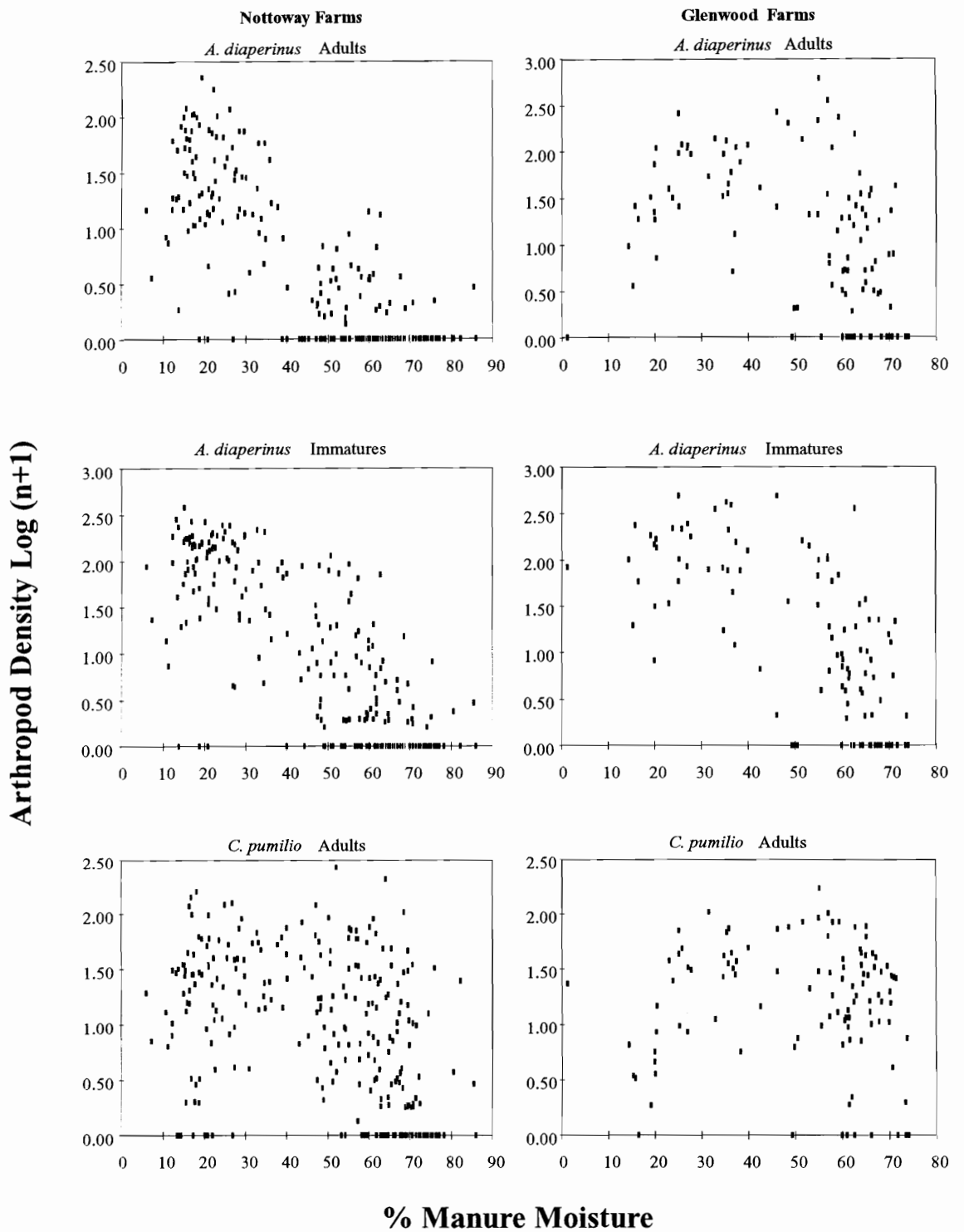


Fig. 17. Scatter-plots of arthropod density in relation to percent manure moisture at Nottoway Farms, and Glenwood Farms.

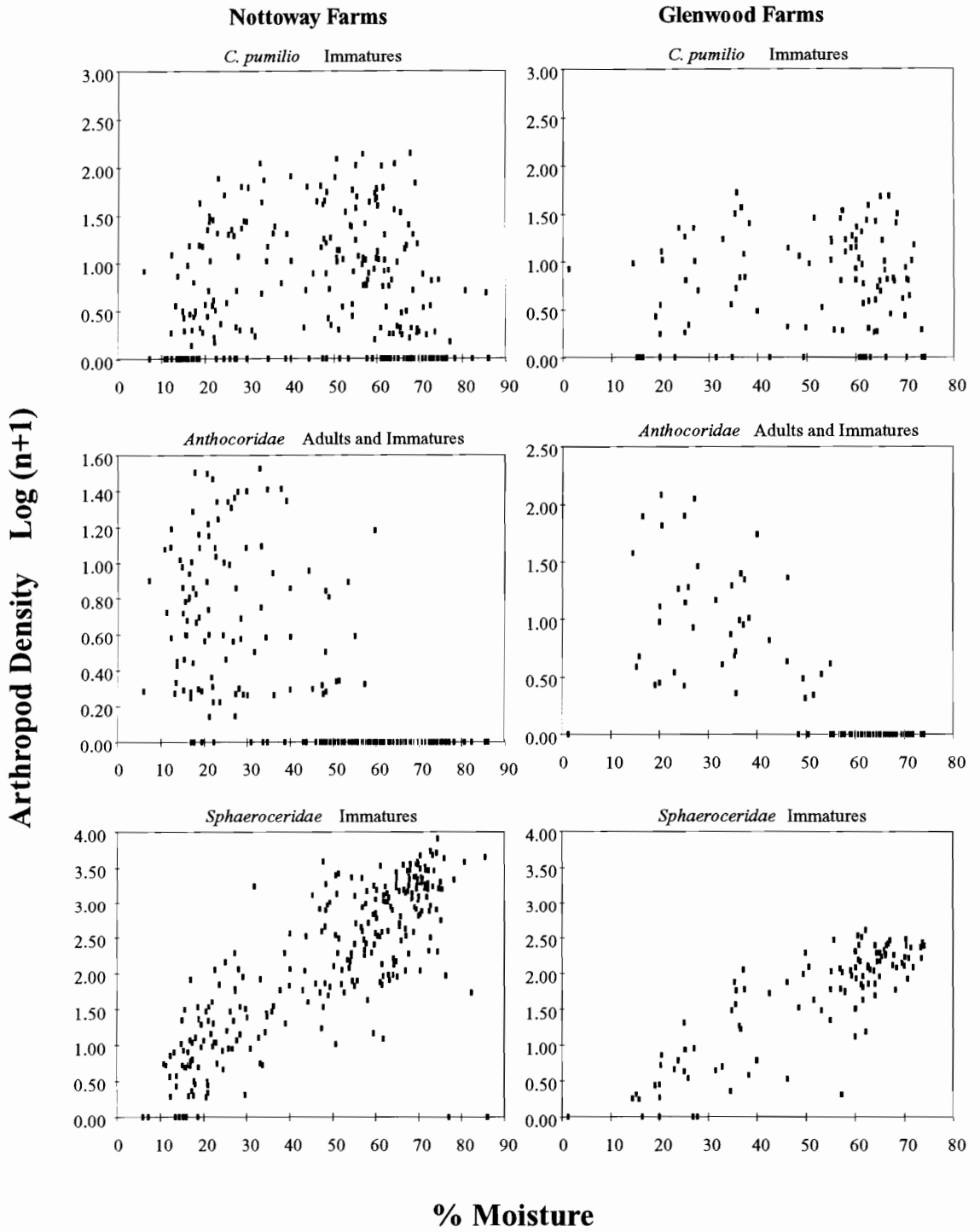


Fig. 18. Scatter-plots of arthropod density in relation to percent manure moisture at Nottoway Farms, and Glenwood Farms.

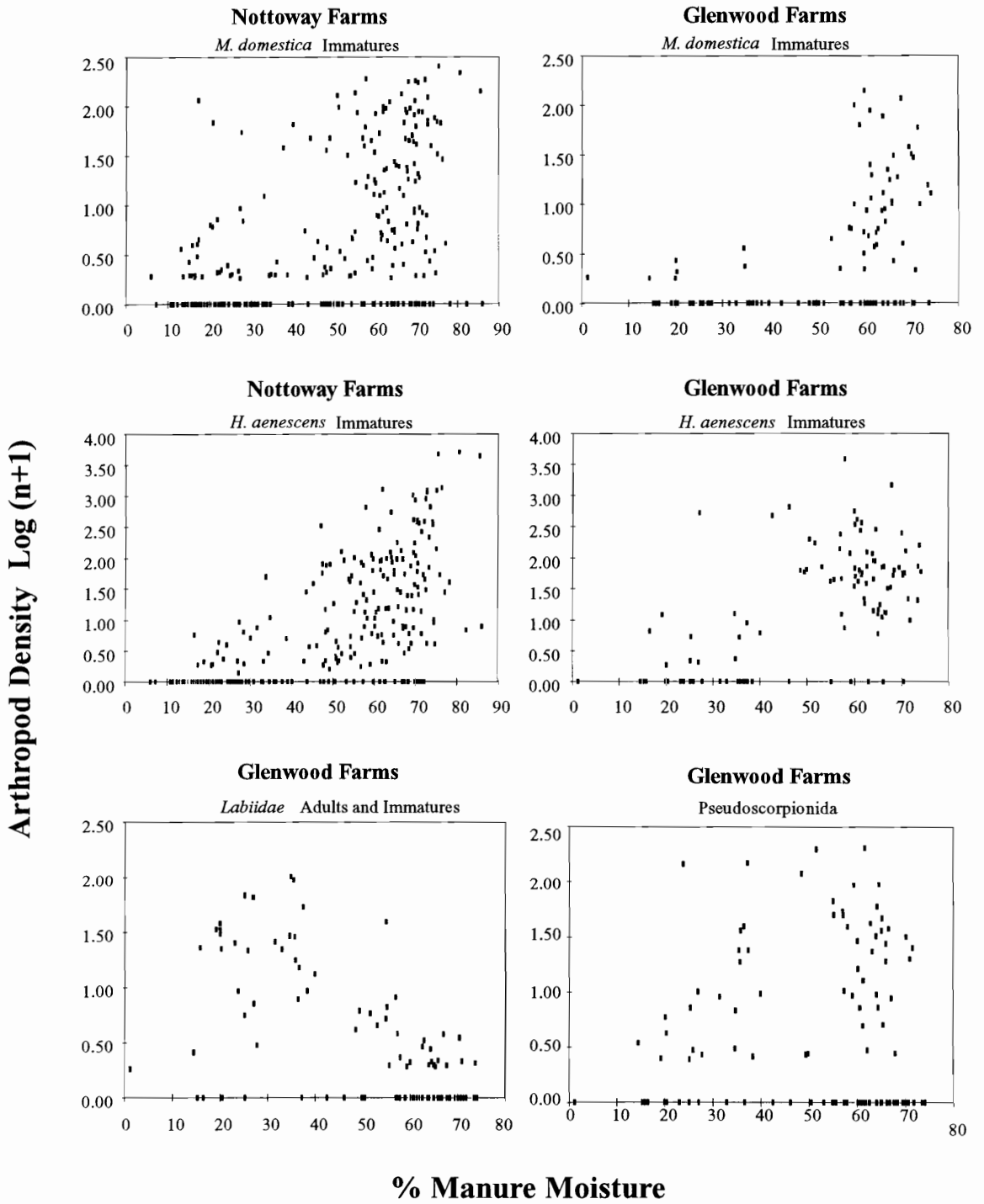


Fig. 19. Scatter-plots of arthropod density in relation to percent manure moisture at Nottoway Farms and Glenwood Farms.

The strongest correlations were found between the taxa which favor the more moist end of the spectrum - the dung gnats and *H. aenescens* - and between the taxa that favor the drier end - the lesser mealworms and Anthocoridae. Within each farm, *H. aenescens* showed a higher degree of correlation with moisture than did house flies. The difference is possibly the result of the mass releases which were conducted throughout the study, which elevated *H. aenescens* numbers overall. Another possible reason for this discrepancy is that house fly and *H. aenescens* larval stadia were not distinguished during sorting and counting of the specimens. Perhaps there were greater numbers of early instar *H. aenescens* larvae than early instar house fly larvae. Since house flies and other Diptera are known to migrate to drier habitats prior to pupation, if a greater proportion of house fly larvae collected were late instars, it is logical to assume that they would not be found in the moister manure.

It seems obvious that moisture significantly affects taxon density, however the results could have differed had different taxa been selected for enumeration - viz. taxa that are not as heavily affected by moisture. Although all organisms have certain physical requirements, the density of other arthropods might be more affected by densities of predators and/or prey in this system.

The loose correlation between density of *C. pumilio* adults and manure moisture observed during this study, agrees with the results obtained by other workers who reported that *C. pumilio* occurred across the range of manure moistures between 10% and 70% (Geden & Stoffolano 1988), and that they were not significantly correlated with manure moisture ($r = 0.226$). This loose correlation of *C. pumilio* adults with moisture is consistent with the behavior of a generalist foraging predator in this habitat. The lack of positive correlation of adult *C. pumilio* with moisture is important because while predation by *C. pumilio* on fly late instars may be minimal, house flies, *H. aenescens* and other Diptera have been shown by Fatchurochim et al. (1987) to prefer 70% manure for oviposition. Because fly eggs and first instar larvae are considered the preferred prey of

C. pumilio adults, the effectiveness of this predator in the field might not be equivalent to that observed in laboratory studies which attempt to simulate field conditions. Geden & Stoffolano (1988) also reported that *C. pumilio* larvae are abundant in the 50% to 70% range, but the findings from this study show that there was no effective correlation of larval densities with moisture.

Knowledge of the abundance of manure arthropods, especially in relation to manure moisture, is important in light of a recently available computer simulation management program for fly control on poultry farms (Axtell & Stinner 1990). This expert system operates on the basis of initial population estimates of certain predators and parasites. Therefore, it can be said that if one is interested in sampling for the predator *C. pumilio*, one should avoid wetter areas. Sampling other predators such as earwigs and anthocorids can also be made more efficient.

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Chapter 4

Temperature-dependent Development of Immature *Hydrotaea aenescens* (Weidemann) (Diptera: Muscidae)

Introduction

House flies (*Musca domestica* L.) continue to present problems in confined livestock facilities, particularly in high-rise, cage layer houses. Large amounts of accumulated manure that are infrequently removed offer an ideal habitat to support explosive house fly populations, causing nuisance and unsanitary conditions at the farm. These conditions occasionally spread to nearby residences, and have resulted in expensive legal proceedings for the producers, and temporary suspension of farm operations. Successful fly management programs for house flies at cage layer facilities rely on the combination of cultural, biological, and selective chemical control practices which constitute integrated pest management (IPM) (Axtell 1970, 1986a, Legner et al. 1975). Numerous studies have reported the importance of preserving and enhancing the manure-inhabiting natural enemy complex as a component of integrated fly control in these types of poultry houses (Anderson & Poorbaugh 1964, Peck & Anderson 1969a, Dunning et al. 1978, Hulley & Pfleiderer 1988).

It has been demonstrated that *Hydrotaea aenescens* (Weidemann) (Diptera: Muscidae) can be effective in controlling house flies in high-rise cage layer poultry houses, when these predators are mass-released with subsequent sustained releases (Nolan & Kissam 1985, Turner & Carter 1990, Turner et al. 1992). Earlier, many researchers were skeptical, but the number of poultry operators incorporating *H. aenescens* into practical IPM programs in the United States has increased annually since the mid-1980's (personal observation). By augmenting *H. aenescens* densities,

house fly populations have been successfully controlled at lower costs than previous programs that relied chiefly on chemicals (Turner et al. 1992). In addition to producers saving costs, decreased reliance on chemicals for house fly control should delay the development of resistance to insecticides by that species (Meyer & Georgiou 1987, Shen & Plapp 1990).

With IPM routinely practiced at today's cage layer facilities, and increasing interest in the use of *H. aenescens*, research efforts are now turning to ways that render IPM practices more practical and efficient. Recently Axtell & Stinner (1990) published a computer simulation model entitled *Fly Management Simulator (FMS)*, for house fly management in confined-livestock production systems. With particular application to confined-poultry systems, the model was developed by various researchers who have compiled data from many studies conducted during the past four decades. FMS includes components for the population dynamics of house flies and their natural enemies. These include a predatory mite, *Macrocheles muscaedomesticae* (Scopoli) (Acari: Macrochelidae), a predatory beetle, *Carcinops pumilio* (Erichson) (Coleoptera: Histeridae), and four species of parasites, *Spalangia endius* (Walker), *S. cameroni* (Walker), *Muscidifurax raptor* (Girault & Sanders), and *M. zaraptor* (Girault & Sanders) (Hymenoptera: Pteromalidae). It also includes a component to simulate the effects of various chemical, cultural, and biological pest management practices (Axtell & Stinner 1990).

The FMS model could easily be adapted to incorporate a sub-model of *H. aenescens* population dynamics and predation on house flies. Such a computer simulation model would lead to development of more effective *H. aenescens* release strategies when various conditions such as geographical location, livestock type, housing type, and specific pest management practices are considered. This information will also

help answer questions regarding the time it takes to achieve control, and the number of flies that should be released per time period.

However, to accomplish this specific information is required on the biology, ecology, and behavior of *H. aenescens*. Describing the relationship between temperature and immature development time is important base-line information that can be combined with information on predation to predict the impact of *H. aenescens* predation on house fly populations. The objective of this study was to investigate the effect of constant temperatures on the development rates of the egg, larval, and pupal stages of *H. aenescens*, and to estimate the parameters for both the temperature-dependent development model of Logan et al. (1976), and for the logistic distribution of development equation.

Materials and Methods

H. aenescens eggs and larvae used in the experiments were progeny of adults maintained in a laboratory colony at the Price's Fork Research Facility, VPI & SU, Blacksburg, Va. Adults were maintained on diet consisting of a 1:2:2 ratio of powdered meat product, powdered milk, and sugar (Youngman et al. 1991). Oviposition and larval medium for the colony consisted of a mixture of wheat bran, vermiculite, and powdered meat products in a ratio of 2:2:1, with the addition of sufficient water to moisten the medium, and a raw egg on the top of the medium to attract ovipositing females.

Experimental temperature used for both egg and larval development were 22.2^o C, 25.6^o C, 28.9^o C, 32.2^o C, and 35^o C. Eggs and larvae were maintained in 0.61 x 0.61 x 0.61 m wooden cabinets with one cabinet used for each temperature. Each cabinet contained a temperature adjustment apparatus and a thermostat. In preliminary studies, a hygrothermograph was used to verify the constancy at which the cabinets

maintained the desired temperature. It was found that temperatures varied by $\pm 1.1^{\circ}$ C. During the present study a maximum/minimum thermometer and a hygrometer were used in each cabinet to monitor temperature and relative humidity, respectively. A pan of water was placed in each cabinet to maintain the relative humidity at ca. 50%.

Egg development

The rates of development from oviposition to larval eclosion were determined using eggs less than 1 h old. Moistened ovipositional medium in a small plastic ice-cream container was placed on the floor of the *H. aenescens* insectary. After 0.5 h the containers were examined for the presence of eggs, and if insufficient eggs were present, the containers were returned to the insectary floor for up to an additional 0.5 h. The eggs were gathered and counted into small plastic petri dishes 5.8 cm diameter by 1.4 cm deep, and then fitted with water-moistened construction paper to cover the bottom of each dish. Throughout these experiments, care was taken to ensure that the eggs were positioned so that they were contacting each other, so as to simulate natural oviposition as nearly as possible. Care was taken also to ensure that manipulation times did not exceed 10% of eclosion time at each temperature.

Four petri dishes, each containing 100 eggs, were placed in the dark at the experimental temperatures. Preliminary studies had been performed to determine the approximate period required for the first larval eclosion at each temperature. As the time for eclosion of the first instar approached, the petri dishes were removed from the chamber and examined under a dissecting microscope (500 X) with a fiber-optic illuminator. Eclosed larvae were counted and removed, and the dishes were returned to the cabinet. The paper was remoistened if necessary with water at the appropriate temperature. The time of day the dishes were removed, and the time that the dishes were

returned to the cabinet were recorded. The experiment was replicated three times for a total of 1,200 eggs tested at each temperature.

Larval development

Eggs were harvested and transferred to a dish containing ovipositional medium and were incubated at 28.9^o C until larval eclosion. Newly emerged larvae were picked out with forceps and placed into plastic jars containing 23 g of larval medium. This consisted of 3.5 g vermiculite, 5 g wheat bran, 3 g dried meat product, and 11.5 g dried poultry manure which had been collected from a cage layer house, frozen to kill arthropod fauna, and oven-dried. 53 g of water were added to yield a 50% moisture content. Four replicates, each containing medium and 100 larvae, were placed in each of the temperature cabinets. The experimental temperatures were the same as those used for the egg development test. A container of water was placed in each cabinet to maintain relative humidity and the cabinets were kept dark. After 3 days, the cups were examined twice daily for the presence of pupae and these were counted.

Pupal development

For the pupal development experiment, a cohort of newly formed puparia (untanned and unsclerotized) was removed from the laboratory colony. Individual puparia were placed into gelatin capsules using fine forceps. 500 capsules were placed in each temperature cabinet and were examined daily for adult emergence. Only the pupae held at 32.2^o C developed into adults. This probably occurred due to the selection of very new pupae for this treatment. Pupae were selected for the entire range of temperatures, and the last ones to be placed in the capsules (the ones at 32.2^o C), were appreciably more tanned and sclerotized than those at the other temperature treatments. These newly-formed pupae therefore probably suffered desiccation. This could be avoided by the use of a randomized complete block design using temperature and date as

blocks. Consequently data on mean pupal development time at four laboratory experimental temperatures 18.0° C, 20° C, 24° C, and 30° C, were obtained from a study by Ellewanger (Neudorff Beneficial Insects Co., Hamlin Germany) (unpublished). Pupal development time at 32.2° C, from the present study, were included with these four temperatures.

Data Analysis

The mean proportion survival at each temperature was calculated for both the eggs and larvae by dividing the number completing that stage by the number of individuals per replicate at the beginning of the stage. Analysis of variance (ANOVA) (True Epistat®, Version 3.1) (Gustafson 1991) was performed on these proportions to determine whether there were differences in survival between the temperature treatments. The proportions were transformed using the arcsine-square root transformation before analysis.

The observations on egg and larval development time at each temperature were transformed to rates by simply using the ratio of 1/time. The median developmental rate at which 50% of the population developed for the egg and larval stages at each temperature was estimated by Spline interpolation on True Epistat® Version 3.1. Median rates (one for each temperature) were fitted to the model of temperature-dependent development rate in arthropods by Logan et al. (1976). This was used to describe the effect of constant temperature on median development rate. The model has the form:

$$RT(T) = PHI*(EXP (RHO*T)-EXP\{RHO*TM - [(TM-T) / DELTAT]\}) \quad (1)$$

Where $RT(T)$ = the rate of development (1/time) at temperature T. PHI = the developmental rate at the lowest temperature examined. RHO, TM, and DELTAT are parameters estimated by the nonlinear regression using PROC NLIN on SAS (SAS Institute 1985). RHO = the rate of increase to an optimum temperature. TM = the thermal maximum at which life processes can no longer be maintained for prolonged periods of time. DELTAT = the temperature range over which "thermal breakdown" becomes the over-riding influence. The goodness of fit was determined using the REG procedure on SAS.

The predicted median rates were calculated by the Logan et al. (1976) model hereafter called model 1. The distribution of development surrounding the median rate of the individuals in each immature stage at the different temperatures was described using a logistic equation, since development rates of insects exposed to constant temperature cannot be assumed to be normally distributed. The model has the form of:

$$y = (1 / (1 + e^{-\alpha(x-\beta)})) \lambda^{-1} \quad (2)$$

where y = the cumulative frequency, x = the relative development rate (rate/median rate), and α , β , and λ are parameters estimated by nonlinear regression on SAS (Régnière 1984). The distribution of development is therefore expressed as a function of the median development rate and this relationship is constant over all temperatures.

For describing temperature-dependent development of the pupal stage, estimates of mean development rates provided by Ellewanger (unpublished) and the mean rate at 32.2° C, observed from the present study (i.e. 1/mean development time) were fitted to model 1 of Logan et al. (1976). Predicted median rates were calculated, and model parameters were estimated by model 1 (Logan et al. 1976).

Results and Discussion

The observed mean and median development times, and the predicted median times calculated from Logan's model for *H. aenescens* eggs and larvae are given in Table 1. This also shows the mean pupal development times obtained from Ellewanger (unpublished), and the observed mean development time at 32.2° C. Wilhoit et al. (1991) stated that "mean rates [are] good estimates of the median rates because development rate distributions are generally close to symmetric", therefore, the mean rate is considered to be acceptable here.

The longest developmental times for eggs and larvae occurred at 22.2° C with development times decreasing as temperature increased. Pupal development [based on Ellewanger's data] followed the same pattern. This is the expected picture for poikilothermic development.

For stage-specific development of the immature stages, each distribution of the cumulative proportion of individuals completing development at each temperature is different (Figs. 1, 2). The cumulative proportion of flies completing a developmental stage can be described by a single equation (Eq. 2), with similar distributions for temperatures (Figs. 3, 4). This is done by transforming age in days, to rates (1/days), and then to relative developmental rates (rate/median rate) (Figs. 5,6).

Table 2 presents the mean survival for eggs and larvae at the five experimental temperatures. The highest survival rate was 96%, observed at 22.2° C. There was no significant difference in the survival of eggs at any temperature ($F= 0.63$, $df= 59$; $P= 0.64$). These results differ from the work of Stein et al. (1977b). Although those workers also reported decreased mean survival of *H. aenescens* eggs with increasing temperature, they found lower rates than the present study, i.e. 70% at 28° C, 47% at 32° C, and 22% at 36° C. This difference may be because those workers used a total of

Table 1. Mean (\pm SD), and median (observed and predicted) number of days for egg, larval, and pupal¹ development of *Hydrotaea aenescens* at five constant temperatures.

Stage	Temp $\pm 1.1^\circ \text{C}$	Mean \pm SD		Median		R ² †
				Observed	Predicted	
Egg	22.2	1.26	0.05	1.25	1.27	0.983
	25.6	0.97	0.05	0.97	0.94	
	28.9	0.68	0.06	0.65	0.71	
	32.2	0.63	0.06	0.6	0.57	
	35.0	0.49	0.04	0.49	0.49	
Larva	22.2	13.35	1.55	12.87	12.81	0.986
	25.6	11.86	1.82	10.42	10.19	
	28.9	8.54	1.80	8.00	8.93	
	32.2	8.08	1.97	7.35	7.11	
	35.0	7.84	2.09	6.41	6.46	
Pupa	22.2	15.00	-	-	15.91	0.994
	25.6	14.00	-	-	13.42	
	28.9	10.00	-	-	9.86	
	32.2	7.00	-	-	7.10	
	35.0	6.84	-	-	6.80	

¹ Pupal data provided by Ellewanger (unpublished).

† R² of predicted versus observed number of days to completion of development. For each stadium, the slope and intercept were not significantly different from 1, and 0, respectively (P=0.05).

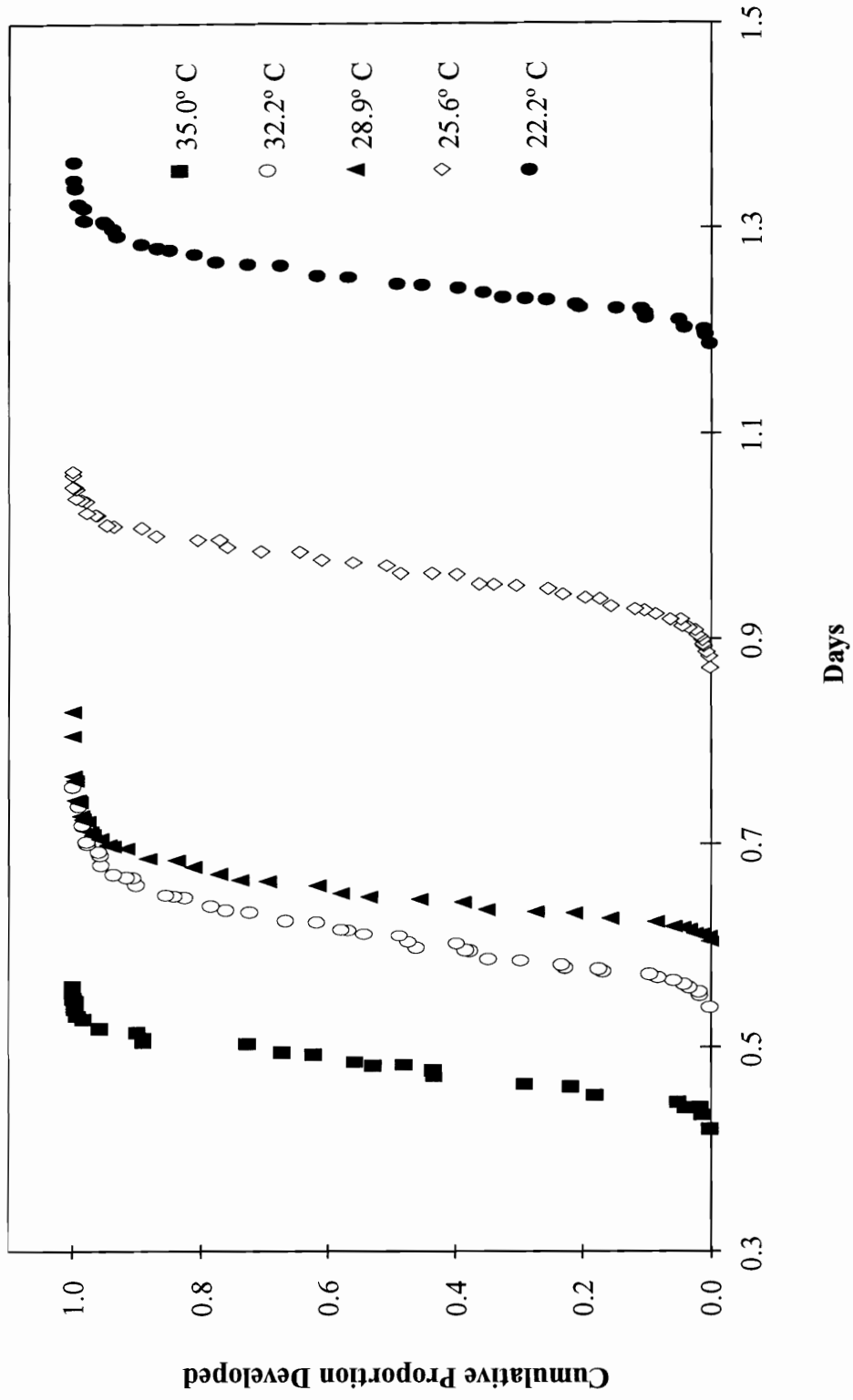


Fig. 1. Cumulative proportion of *Hydrataea aenescens* emerged from eggs held at five temperatures (Temperatures $\pm 1.1^\circ\text{C}$).

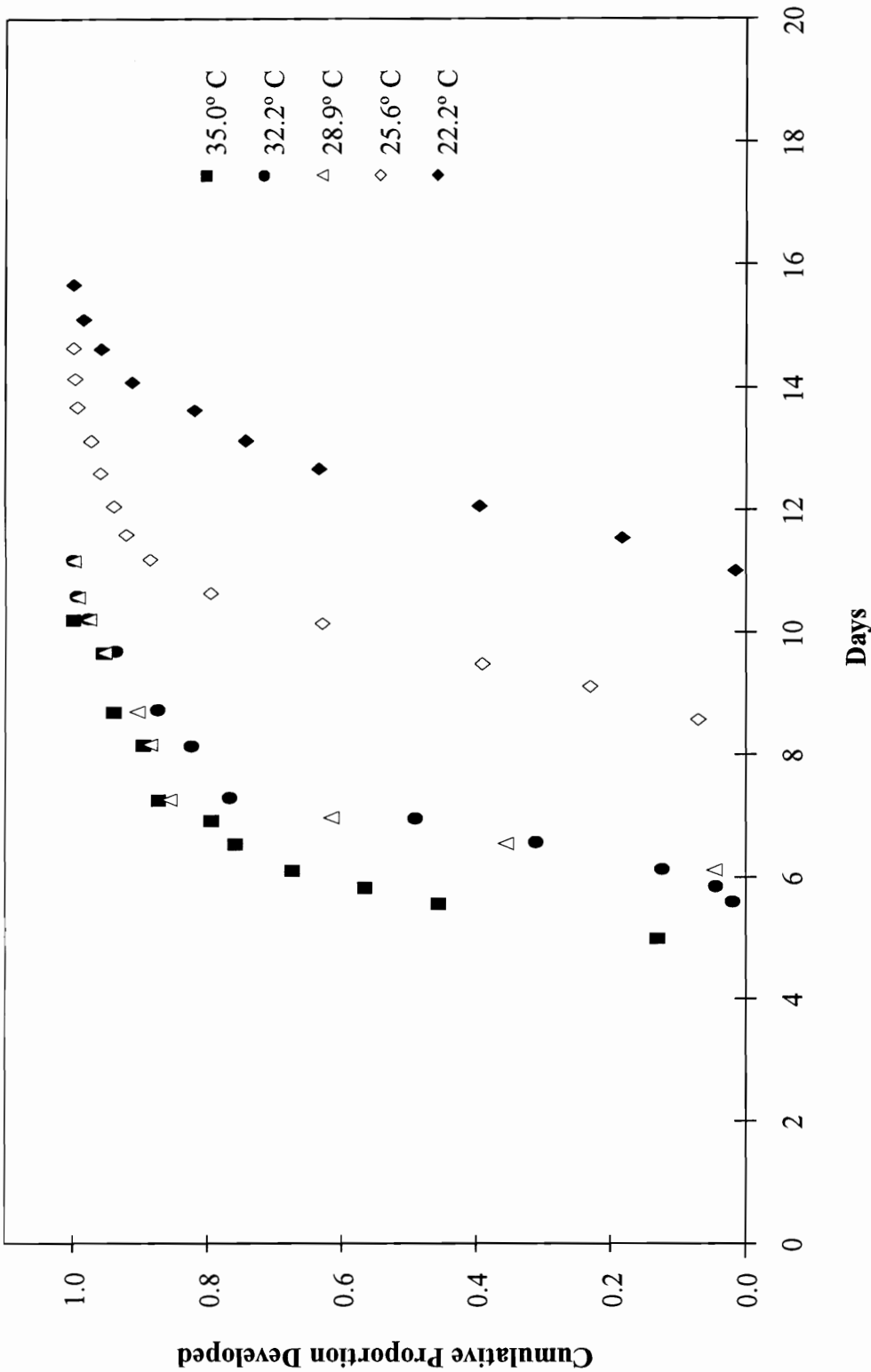


Fig. 2. Cumulative proportion of *Hydrotaea aeneascens* larvae developed to the pupal stage at five different temperatures. (Temperature \pm 1.1°C)

H. aeneascens Eggs

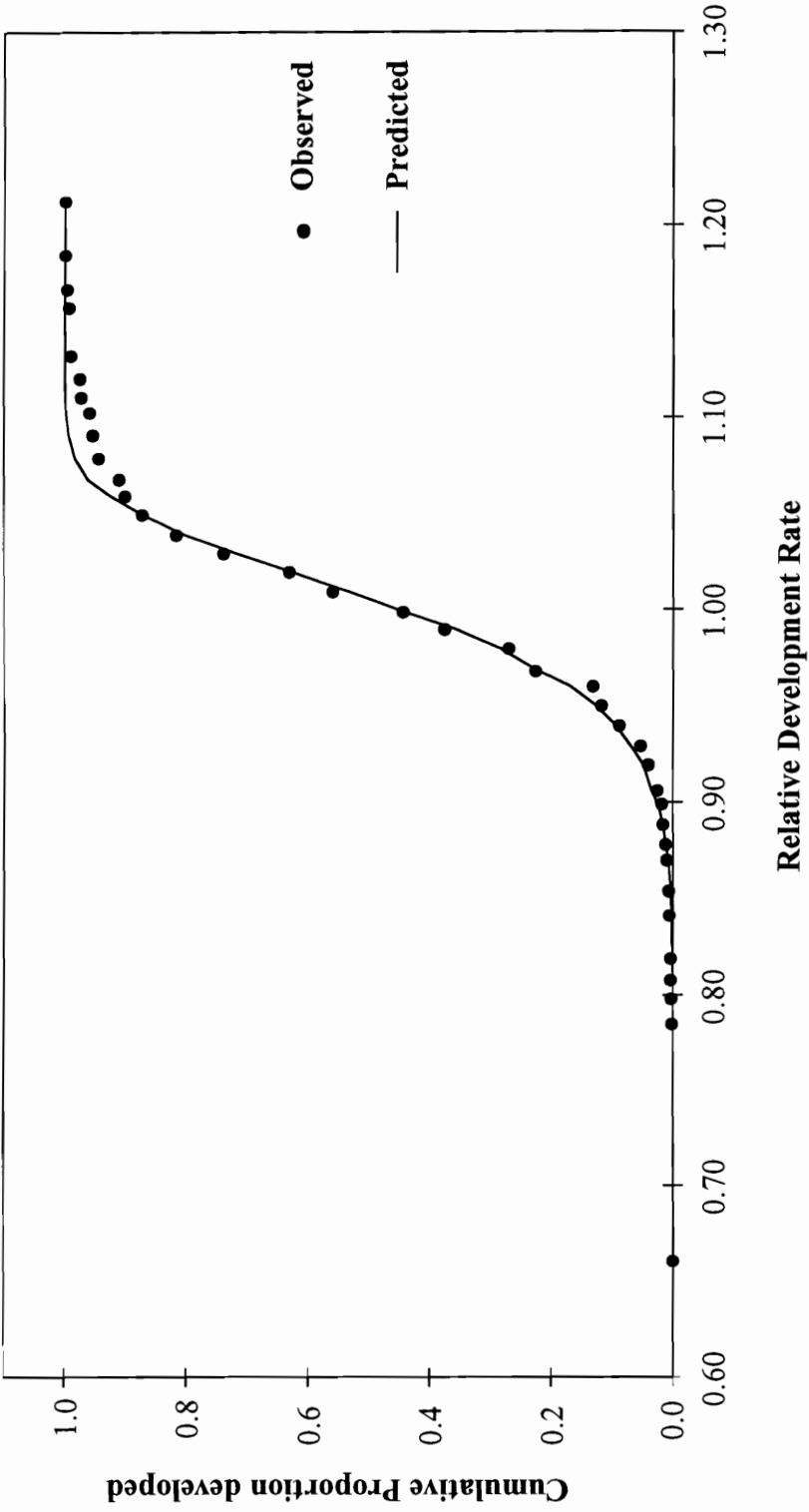


Fig. 3. Cumulative proportion of *Hydrotaea aeneascens* eggs developed to the first larval stadium at five constant temperatures, compared with the distribution predicted by Eq. 2. $[y = (1 / (1 + e^{-39.216 (x - 1.006)})) 0.0966^{-1}]$ based on the median development rate (1.0 rate = 0.5 proportion of individuals developed).

H. aenesceus Larvae

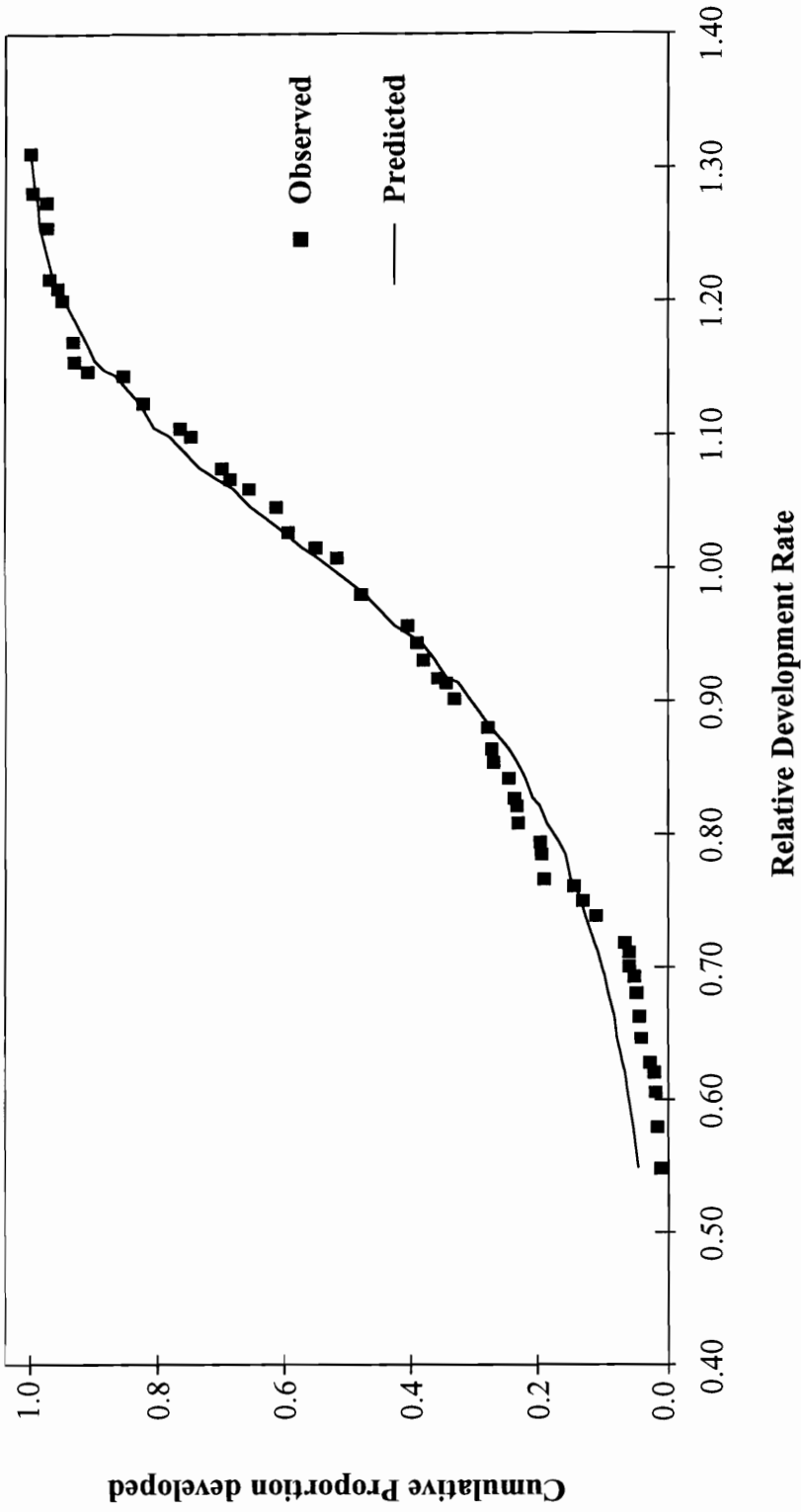


Fig. 4. Cumulative proportion of *Hydrotaea aenesceus* larval population developed to the pupal stadium at five constant temperatures, compared with the distribution predicted by Eq. 2. $[y = \frac{1}{1 + e^{-16.557(x - 1.105)}}]$ based on the median development rate (1.0 rate = 0.5 proportion of individuals developed).

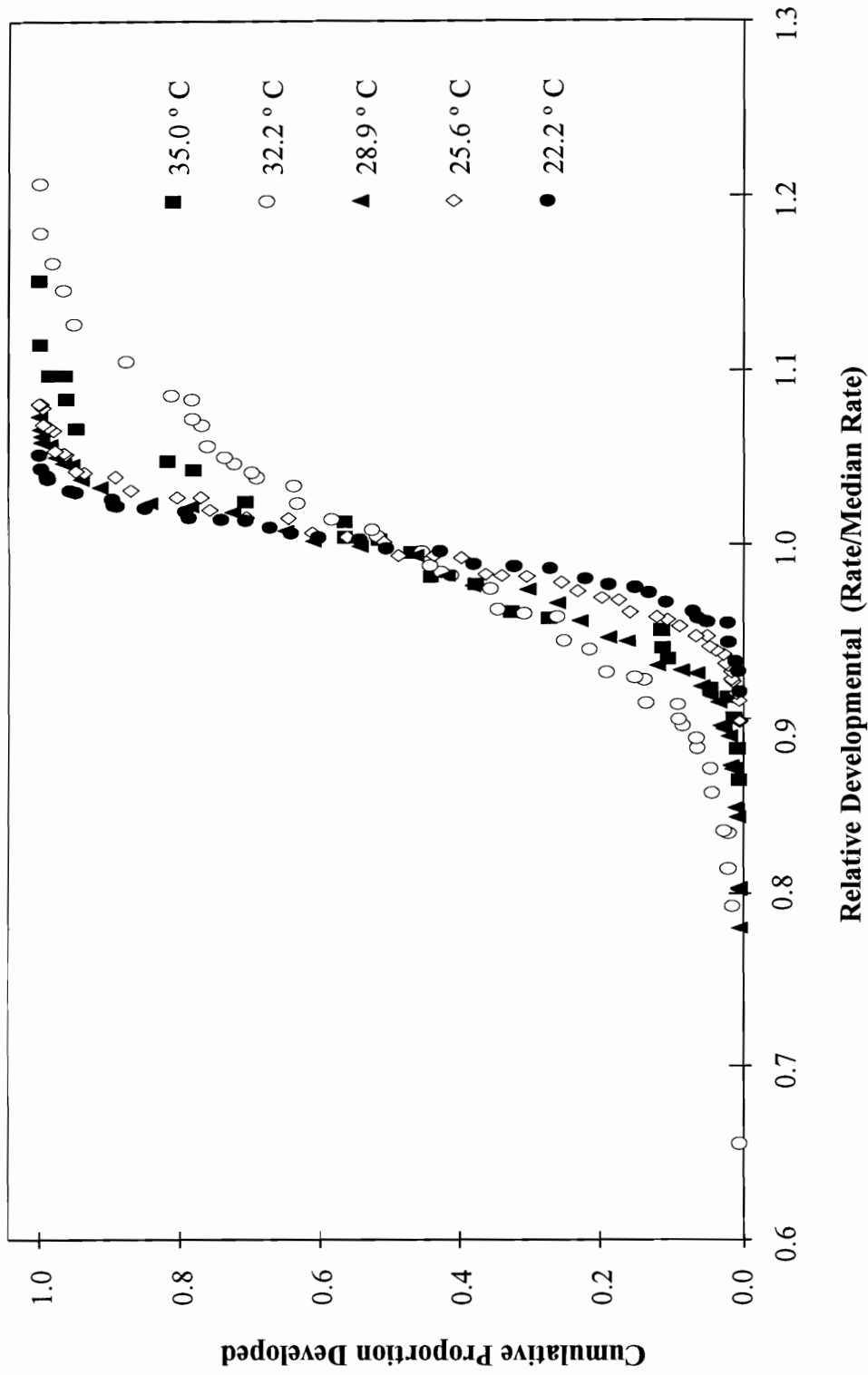


Fig. 5. Relative development rate of *Hydrataea aeneascens* eggs, where median rate = the rate at which 50% of the eggs developed to the larval stage. (Temperature $\pm 0.1^\circ\text{C}$)

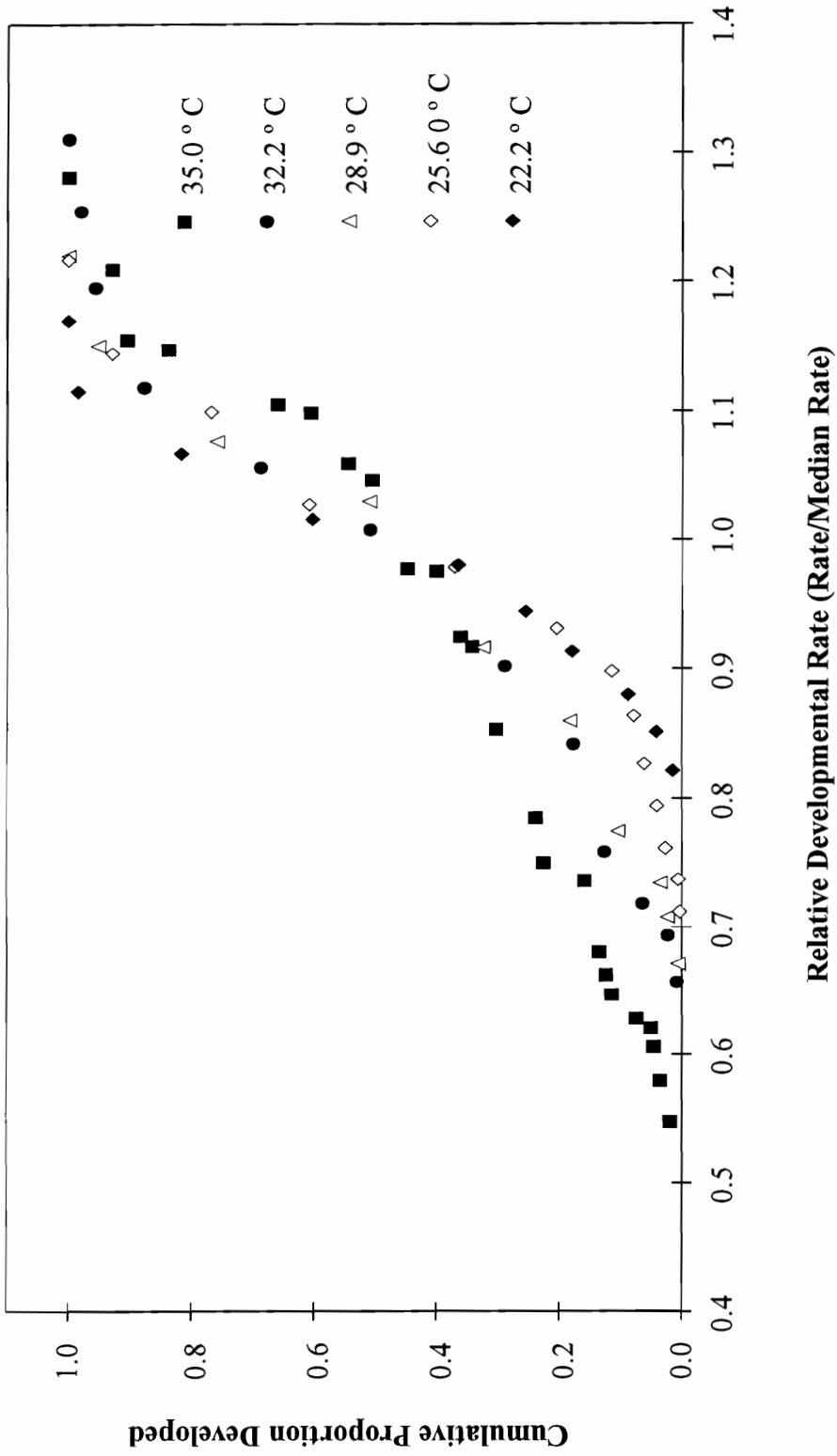


Fig. 6. Relative development rate of *Hydrataea aeneascens* larvae, where median rate = rate at which 50% of the larvae developed to the pupal stage. (Temperature \pm 1.1 °C)

Table 2. Mean (\pm SD) proportion survival of eggs, and larvae of *Hydrotaea aenescens* at five constant temperatures.

Temperature $\pm 1.1^\circ \text{C}$	Mean Proportion Survival \pm SD			
	Eggs n=1200 [†]		Larvae n=400	
22.2	0.96	0.03	0.66	0.03
25.6	0.94	0.05	0.86	0.03
28.9	0.94	0.05	0.87	0.09
32.2	0.95	0.03	0.78	0.04
35.0	0.95	0.04	0.73	0.04

[†] n= number individuals tested per temperature

300 eggs compared with the 1200 used in the present study. Another factor could be that Stein et al. (1977b) "placed the eggs in a 0.5% NaCl solution" for incubation, which could have impeded respiration if the eggs were submersed.

A Kruskal-Wallis ANOVA, a test for independent samples that evaluates differences between the mean ranks, was employed for the larval data due to large variance differences which rendered a parametric ANOVA unsuitable (True Epistat® Version 3.1, Gustafson 1991). Significant differences in larval survival were observed ($H= 13.70$, $df= 4$; $P= 0.01$). Tukey's tests ($\alpha=0.05$) were performed on the mean rank assigned to each sample. The results indicate that the lowest survival rate occurred at 22.2° C, however, this rate was not significantly different from that at 35° C or 32.2° C. The highest survival rate occurred at 25.6° C, but this was only significantly different from the rate at 22.2° C, and not from the others. It is expected that larval survival would increase from lower to higher temperature, although it would then decrease with further temperature increase (Table 2). Larvae exposed at extreme temperatures would produce the lowest survival.

Table 3 shows the parameter estimates derived from Logan's model for temperature-dependent development, and from the logistic model describing distribution of development. The predicted cumulative distributions of emergence (Eq. 2), for eggs and larvae, were not significantly different from the observed distributions as determined by linear regression ($R^2= 0.999$, and 0.993 , respectively). The values for the slopes for both distributions were not significantly different from 1, and intercepts were not different from 0 ($P= 0.05$).

Very little information is available on temperature-dependent development of *H. aenescens*, making comparison difficult. A few researchers have reported decreased development times for *H. aenescens*, with temperature increases up to an optimum point,

Table 3. Parameter estimates (\pm SE) for Logan's model of temperature dependent immature *Hydrotaea aenescens* developmental rate (Eq. 1), and parameter estimates for the distribution of development (Eq. 2).

	Parameter Estimates					
	Eggs		Larvae		Pupae	
(A) Rate						
RHO	0.094	(0.024)	0.099	(0.020)	0.121	(0.013)
TM	19.662	(6.225)	24.337	(5.819)	21.925	(2.066)
DELTAT	3.030	(4.885)	7.858	(1.553)	6.803	(0.646)
(B) Distribution						
α	39.216	(1.328)	16.557	(1.760)	—	—
β	1.006	(0.003)	1.105	(0.012)	—	—
λ	0.097	(0.086)	0.347	(0.052)	—	—
R²	0.999		0.993		—	—

and no further development beyond some thermal maximum. Of these, Johnson & Venard (1957) reported that development times at 28^o C ranged from 0.50-0.67 d for eggs, with larval and pupal development of at least 5, and 4 d, respectively. The closest comparisons observed during the present study were at 28.9^o C, where the median development times were 0.65 d for eggs, and 8 d for larvae (Table 1). Johnson & Venard (1957) also showed that the complete cycle required a minimum of 14 d at 28^o C, and they reported that at temperatures exceeding 42^oC, larvae emerged, but died after a few hours. On the other hand, Stein et al. (1977) reported that temperatures exceeding 28^o C caused decreased egg hatch, and at 40^o C no larvae hatched. The developmental times observed in the present study (Table 1) differ from data of Johnson & Venard (1957), and Stein et al. (1977), but the experimental conditions were so different that it is not possible to draw comparisons.

Figure 7 is adapted from Axtell & Stinner (1990), and illustrates where the *H. aenescens* component would fit into the existing model of Axtell & Stinner (1990). A connection could be made on this diagram to indicate that both *M. muscaedomesticae*, and *C. pumilio* are predaceous on *H. aenescens*, and another to indicate parasitization of *H. aenescens* by the parasite complex. Fig. 8 (also from Axtell & Stinner 1990) represents this inserted *H. aenescens* component modified as a submodel. This chart diagrams the information that is necessary for an *H. aenescens* module to function.

The model by Wilhoit et al. (1991) predicts populations of house flies over a specified time and optionally provides predictions of populations of the predators *C. pumilio* and *M. muscaedomesticae*, as well as the parasites *Spalangia* sp. and *Muscidifurax* sp. The predictions are based upon initial population estimates specified by the user, who is also prompted for species-specific information on survival, immigration

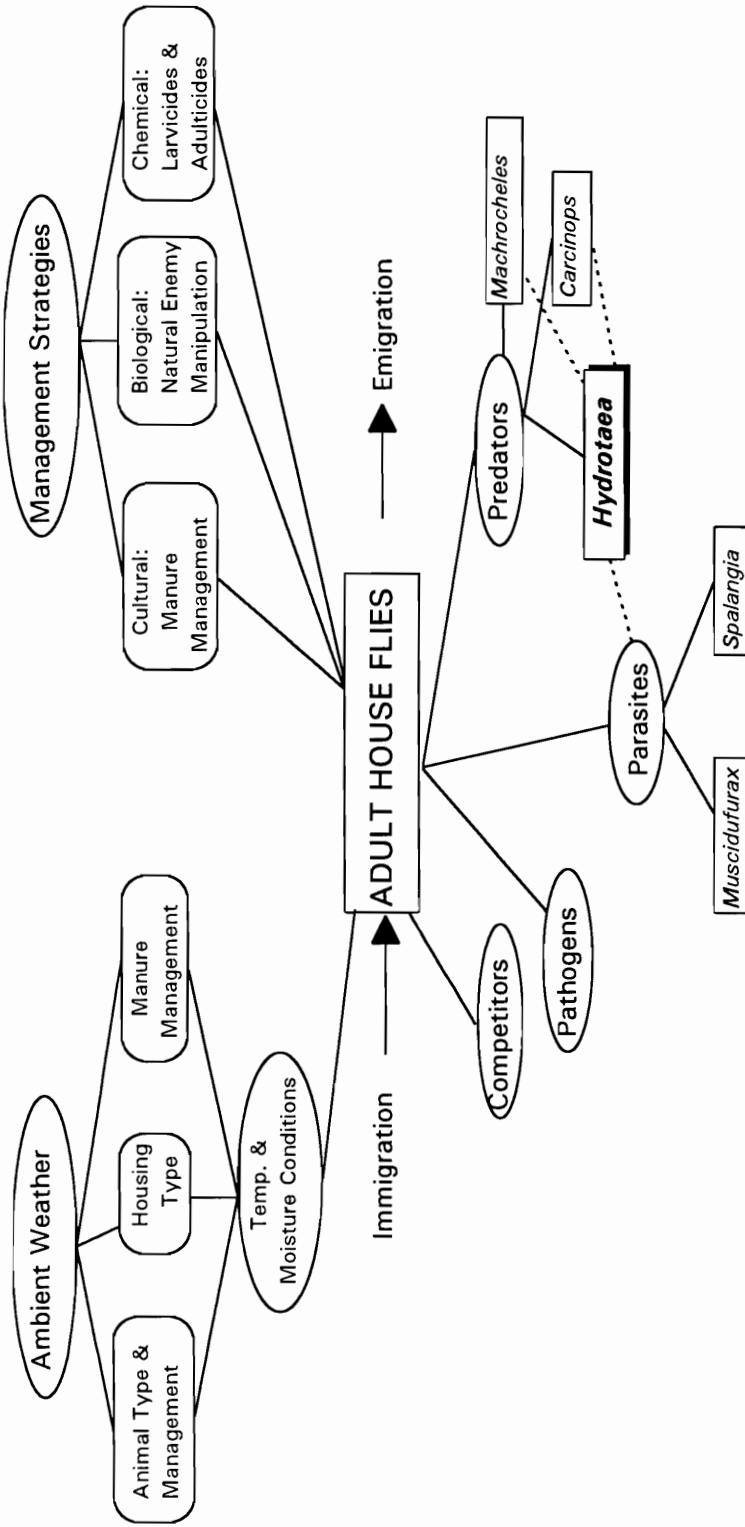


Fig. 7. Diagram (adapted from Axtell & Stinner 1990), of their house fly management simulation model with the addition of a hypothetical *Hydrotaea aenescens* component.

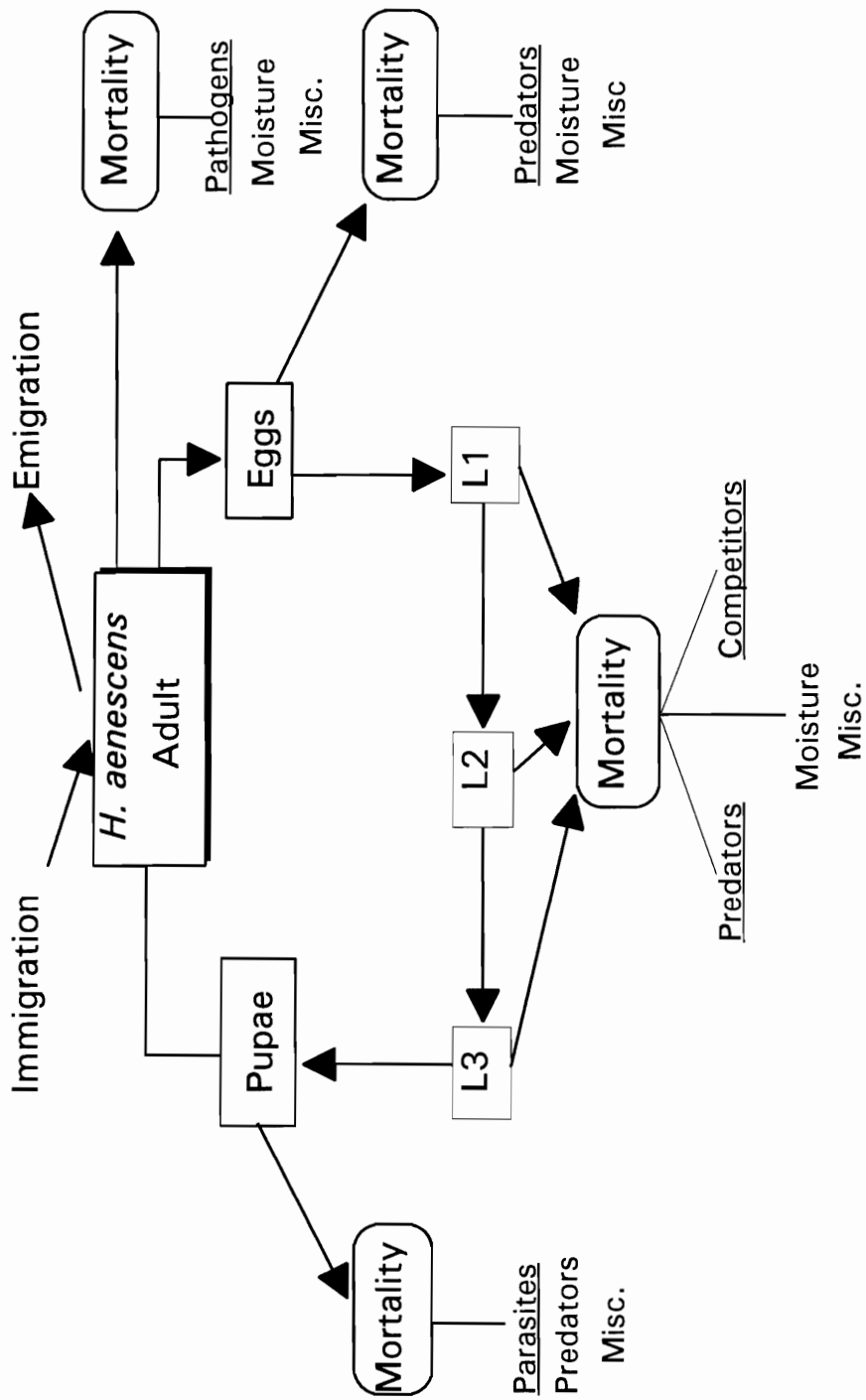


Fig. 8. Diagram (adapted from Axtell & Stinner 1990), of a hypothetical *Hydrotaea aeneascens* submodel.

and emigration, presence of alternative prey, and competition. Other options include chemical control measures, and allowance for excessive manure moisture.

The results of the present study constitute a large portion of the total information needed to develop an *H. aenescens* model which could be an option available to the user as above. Further data are required on adult longevity and survival as a function of temperature before the temperature dependent development and distribution models can be validated under variable temperature conditions. Data on fecundity as a function of temperature will complete the inner loop of the *H. aenescens* model (Fig. 8). Additional information needed to complete the submodel includes: 1) functional response of *H. aenescens* and the effects of manure moisture on this response, 2) prey per parasite effects on survival of *H. aenescens*, since *H. aenescens* functions as both predator and prey in the manure habitat, 3) preferences by other predators for *H. aenescens* or *M. domestica*, and 4) effects of pathogens and competitors on *H. aenescens* survival. When all of these data are collected and incorporated, the resulting model could be a powerful addition to the growing area of computer-aided integrated pest management.

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Chapter 5

A Comparison of the Effects of the Predators *Hydrotaea aenescens* and *Carcinops pumilio* on *Musca domestica* at Two Moisture Levels and Two Densities

Introduction

There are numerous field and laboratory studies which concentrate on the role of manure-inhabiting arthropods in the control of filth flies which breed in accumulated poultry manure (Legner & Olton 1970, Pfeiffer & Axtell 1980, Geden & Stoffolano 1987, Geden et al. 1988, Stafford et al. 1988). Throughout these studies, the predator *Carcinops pumilio* (Erichson) (Coleoptera: Histeridae) is often mentioned as the most significant coleopteran predator of fly eggs and first instar larvae. This beetle has also been included in a computer simulation model of house fly management in confined-animal production systems (Axtell & Stinner 1990).

Carcinops spp. are known to be some of the most abundant predators in the manure-inhabiting arthropod complex, occurring across a wide range of manure moisture conditions in confined-poultry manure pits (Peck 1969, Legner & Olton 1971, Bills 1973, Pfeiffer & Axtell 1980, Green 1982, Propp & Morgan 1985, Geden & Stoffolano 1987, Rueda et al. 1990). Their abundance, combined with their high predation rates, are the reasons why *Carcinops* spp. are regarded as key predators of house flies. However, reports from previous workers show that predation rates of *C. pumilio* on house fly, *Musca domestica* (L.) (Diptera: Muscidae), eggs and larvae are extremely variable, ranging from 13 to 104 house fly immatures destroyed per predator per day (Morgan et al. 1983, Geden et al. 1988). This variability can be attributed to widely differing conditions in the experiments from which these data are derived, however, these beetles are still regarded as highly effective predators under field conditions.

Another known predator of house flies is *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae), which exhibits predation rates on house flies ranging from 10-25 prey per predator per day (Geden et al. 1988), thus demonstrating that it also can be very effective. In fact, it has been used successfully in integrated pest management (IPM) programs in high-rise cage layer poultry houses, resulting in economic advantage to the poultry producer by lowering pesticide costs (Turner et al. 1992). However, *H. aenescens* are seldom found naturally in high numbers (Nolan & Kissam 1987) making mass releases of larvae and pupae necessary to achieve house fly control (Nolan & Kissam 1985).

Interest in, and application of *H. aenescens* is increasing among both researchers and poultry operators, and *H. aenescens* is now being sold by a commercial beneficial insectary in the midwestern U.S. (Daar et al. 1992). It is likely that this interest will continue. Therefore basic biological and ecological information on *H. aenescens* and its predators (e.g. *C. pumilio*) is needed. Such information is needed in making adjustments to the IPM program in order to render it the most labor and cost efficient. For example an issue needing attention was the impact of inundative releases of *H. aenescens* on other predators, such as *C. pumilio*, which under desirable manure conditions would be present at the time of release. Another question is whether interspecific interaction occurs between *C. pumilio* and *H. aenescens*. Although *C. pumilio* preys primarily on fly eggs and first instar larvae, the beetle has been observed attacking second and third instar house fly larvae when alternative food was not available (personal observation). Thus, *C. pumilio* may further reduce predation by *H. aenescens* in addition to reducing *H. aenescens* numbers. The preferred situation would be to have *C. pumilio* and *H. aenescens* working in a complimentary fashion, as each has advantages as a house fly predator. While *C. pumilio* is an agile predator which also preys upon sphaerocerid

larvae and acarid mites (Geden et al 1987, Geden & Stoffolano 1987, 1988, Geden & Axtell 1988), *H. aenescens* life stages overlap more closely with house flies, and they are closely associated in the same habitat. *C. pumilio* is more abundant at lower manure moisture levels (Peck & Anderson 1969, Bills 1973, Armitage 1986), while *H. aenescens* survives well at higher moisture levels (Fatchurochim 1989) where most outbreaks of house flies occur. This study was conducted to facilitate understanding of the interactions between these predators to achieve ideal conditions for this system. Consequently, experiments were performed to 1) clarify the predation rate of *C. pumilio* on house flies under conditions similar to those in the field, such as variable manure moisture, prey density, and prey species, 2) determine the predation rate of *C. pumilio* on *H. aenescens*, and 3) clarify interspecific interactions between *C. pumilio* and *H. aenescens* in a accumulated poultry manure system.

Materials and Methods

Poultry manure was collected from the manure pit of a high-rise cage layer house in Jetersville, Va., Amelia Co., placed into plastic trash bags, and transported to the laboratory where it was frozen to kill any arthropods present. Upon freezing, the manure was transferred onto the upper and lower mesh racks of a metal cart which was placed into a wooden drying chamber measuring 1.2 x 0.6 x 1.1 m. The chamber was constructed with 10.2 cm diameter screened air-intake and exhaust openings. A plastic laundry dryer hose was attached to the exhaust opening, and the other end of the hose was connected to the main exhaust fan of the insectary building, so that a continuous air current was drawn in and blown across the manure. A small electric heater-fan was placed at the intake end of the box to heat the air current. Typically, the manure dried

for a period of three weeks. The door of the chamber was constructed to be tightly fitting to prevent entry by insects.

To ascertain the moisture remaining in the air-dried manure, 10 replicates of 100g samples of air-dried manure were dried further at 90°C for 24 h in an oven. After oven-drying, the samples were weighed and the weight of water removed was calculated and recorded. One hundred grams of the air-dried manure were placed into plastic ice cream containers of 600 ml capacity (11.4 cm top diameter, 8.9 cm bottom diameter, and 8.25 cm height). Sufficient water was added to each container to yield a moisture content of either 50% or 70%, according to the methods of Fatchurochim et al. (1989). Moisture levels of 50% and 70% were selected because I wanted to bracket the moisture range over which house fly, and *H. aenescens* larval development occurs (Peck & Anderson 1969, Fatchurochim et al. 1989). The following formula was employed:

$$X = ((Y) (D) / 1 - Y) - C$$

where X= grams of water added to the air dried manure; Y= desired moisture content of the manure; C= grams of water in 100g of oven-dried manure, and D= the oven dried weight of 100 g of the air dried manure. From this, it was determined that to prepare manure containing 50% moisture content, X= 100g, and for manure at 70% moisture, X= 233g.

C. pumilio were collected from manure obtained from the same high-rise poultry house in Jetersville. The adults were removed from the manure (using forceps), and were placed in an 18.9 l aquarium containing a mixture of vermiculite, wheat bran, and dried meat powder. After 48 h, 10 adults of undetermined age or gender were removed from the colony and placed into cups containing manure of either 50% or 70% moisture.

These cups were held in a rearing chamber at room temperature for 6 h to allow the beetles to acclimate.

Experimental Design

Eggs less than 2 h old, from laboratory colonies of *H. aenescens* and house flies, were separated into containers according to the following treatments with or without *C. pumilio* adults. Five replicates were used for each treatment at both 50% and 70% moisture level, and were coded as follows:

Control: 250 house flies or *H. aenescens* alone (Md250C or Ha250C)

250 house flies + 250 *H. aenescens* (Md250Ha)

250 house flies + 10 *C. pumilio* (Md250Cp)

250 *H. aenescens* + 10 *C. pumilio* (Ha250Cp)

250 house flies + 250 *H. aenescens* + 10 *C. pumilio* (Md250HaCp)

Control: 500 house flies or *H. aenescens* alone (Md500C or Ha500C)

500 house flies + 500 *H. aenescens* (Md500Ha)

500 house flies + 10 *C. pumilio* (Md500Cp)

500 *H. aenescens* + 10 *C. pumilio* (Ha500Cp)

500 house flies + 500 *H. aenescens* + 10 *C. pumilio* (Md500HaCp)

To simulate oviposition by female flies, clumps of eggs were deposited in small depressions in the manure and partially covered. These were clumps of 25 eggs for the 250 density, and 50 for the 500 density treatments. The cups were held in rearing chambers (0.6 x 0.6 x 0.6 m) at 70-75% relative humidity (RH), and 27°C in darkness. Each cup was weighed daily, and manure moisture was maintained by adding water to

each container to re-establish its original weight. The number of adult flies that emerged was recorded for each species.

This experiment was repeated, with pupae being counted instead of adults. Pupae were used as it had been shown in previous trials that they were less likely to be damaged than adults.

Calculations

Percent mortality from each treatment container was calculated. *t*-tests were performed on the results of the two test trials, and indicated that there was no difference between the treatments. Data from adult and pupal counts were pooled to make ten replicates per treatment for subsequent analysis.

Mortality data were transformed using an arcsine square-root transformation prior to analysis (Zar 1984). Values reported in tables however, are original values.

Control Mortality

To examine the effect of density and moisture on each fly species alone, a three-way analysis of variance (ANOVA) was performed using the Number Cruncher Statistical System, Ver. 5.03 (NCSS) (Hintze 1990), with the main effects: type of fly (house fly, or *H. aenescens*), fly density (250 or 500 flies/ container), and manure moisture (50% and 70%). The analysis was performed using percent mortality observed in the controls. *t*-tests were used to determine whether significant differences in mortality occurred between house flies and *H. aenescens* at each combination of density and moisture level in the controls

House Fly and *H. aenescens* Mortality

The effects of the factors *H. aenescens*, *C. pumilio*, fly density, and manure moisture, on house fly mortality were analyzed by GLM analysis of variance (ANOVA) using NCSS (Hintze 1990). Interactions between treatment effects were examined using Fisher's protected Least Significant Difference procedure (LSD) (Lentner & Bishop 1986). *H. aenescens* mortality was also analyzed using GLM ANOVA to test for the effects of house flies, *C. pumilio*, fly density, and manure moisture.

Predation rate of *C. pumilio*

House fly mortality data were corrected for mortality in the controls (Abbott 1925) for each cup containing the predators (*H. aenescens* or *C. pumilio*). These data were multiplied by either 250 or 500 to yield the number of flies destroyed/cup.

Interspecific Interference

The resulting values were used to test for interspecific interference between *H. aenescens* and *C. pumilio* (Geden et al. 1988). The expected number of eggs destroyed was calculated by adding the number of eggs destroyed by each predator alone. *t*-tests (Gustafson 1991) were used to compare this sum to the observed number of eggs destroyed in the treatments containing both predators. Total destruction of house flies was used for statistical analysis because predation rates (# house flies/predator/day) depend on the relative proportion of each predator when both species were present.

Results

Control Mortality

Mortality of house flies in the treatments containing no predators ranged from 21% to 33%, and that of *H. aenescens* alone ranged from 56% to 70% (Table 1). The only significant main effect on overall fly mortality detected by ANOVA was the type of fly (either house fly or *H. aenescens*), ($F=258.29$, $df=1,72$; $P<0.01$). The main effects of Density and Moisture were not significant ($F=0.39$, $df=1,72$; $P=0.54$, $F=0.01$, $df=1,72$; $P=0.95$, respectively). There was, however, a significant interaction between Fly and Moisture ($F=28.50$, $df=1,72$; $P<0.01$). These results indicate that for both species there was no significant difference in mortality between density levels, but there was a difference in mortality between moisture levels, and the effect was different for each fly species. To clarify, mortality data of each fly species were separated, and density removed as a factor. Fisher's LSD tests ($\alpha=0.05$) were performed, where the mean percent mortality from the 50% moisture level was compared to the 70% level, for each fly.

House fly mortality was significantly greater at the 70% moisture level (31.51%) than at the 50% level (20.67%), while mortality of *H. aenescens* was significantly higher at the 50% level (68.25%) than at the 70% level (56.12%).

Results of *t*-tests comparing mortality between house flies and *H. aenescens* at each density and moisture treatment are presented in Table 1. *H. aenescens* experienced significantly greater mortality than house flies at all treatment levels.

Mortality of House Flies

Mean percent mortality of house flies in the various treatments ranged from 21% to 97% (Table 2). The lowest mean percent mortality in each density-moisture group of treatments occurred in the control treatments. Results from ANOVA on these means is

Table 1. Mean % mortality of *Musca domestica* and *Hydrotaea aenescens* in the controls, at two density and two moisture levels, and results of *t*-tests ($\alpha=0.05$) between these means.

<i>M. domestica</i> (n=10)				<i>H. aenescens</i> (n=10)			
Density	Moisture %	\bar{X} Mortality	SE(\bar{x})	\bar{X} Mortality	SE(\bar{x})	<i>t</i>	<i>P</i>
250	50	20.72	(1.79)	66.66	(2.52)	13.98	<0.01
250	70	29.66	(2.46)	56.32	(1.65)	8.68	<0.01
500	50	20.62	(2.95)	69.84	(3.00)	10.38	<0.01
500	70	33.37	(3.22)	55.92	(5.52)	3.63	<0.01

Table 2. Mean % mortality (n=10) of *Musca domestica* at two density levels, and two moisture levels, with the presence or absence of the predators *Hydrotaea aenescens* larvae, and/or *Carcinops pumilio* adults.

Density 250		50% Moisture		70% Moisture	
Treatment	\bar{X}	$\pm(\text{SE } \bar{x})$	\bar{X}	$\pm(\text{SE } \bar{x})$	
Control	20.72	1.79	29.66	2.46	
HA^a	96.84	0.98	51.76	2.78	
CP^b	73.32	5.12	62.60	2.74	
HA+CP^c	83.40	1.87	72.84	6.49	
Density 500		50% Moisture		70% Moisture	
Control	20.62	2.95	33.37	3.22	
HA	89.38	2.04	66.92	5.40	
CP	76.26	2.06	55.34	4.70	
HA+CP	90.88	4.51	63.82	7.58	

^a 500 *H. aenescens* larvae/cup.

^b 10 *C. pumilio* adults/cup.

^c 500 *H. aenescens* + 10 *C. pumilio* adults/cup

presented in Table 3. It is not possible to interpret the separate effects of the experimental factors on house fly mortality because of the occurrence of significant interactions ($P \leq 0.05$) between the factors. To clarify, Fisher's Least Significant Difference (LSD) multiple comparisons were used to examine mortality at all of the combinations of treatment level. The significant interactions examined for house fly mortality are: 1) *Hydrotaea aenescens* x *C. pumilio* x manure moisture, 2) *Hydrotaea aenescens* x fly density x manure moisture, and 3) *C. pumilio* x fly density. Although additional significant two-factor interactions were found, and appear in Table 3, they have not been examined because of their inclusion in significantly higher order interactions that were statistically examined.

Table 4 shows the results of LSD tests on the interaction *Hydrotaea aenescens* x *C. pumilio* x manure moisture. The highest mortality of house flies was observed at the 50% moisture level, with either *H. aenescens* alone or *H. aenescens* with *C. pumilio*. In fact, mortality of nearly 90% occurred with these treatments. This was significantly different from mortality in the controls, also from mortality at the higher moisture level whether *H. aenescens* was present or not, and from mortality of house flies at 50% moisture when *H. aenescens* was not present. Mortality of house flies in the latter treatment was higher than the corresponding treatment at 70%.

Table 5 shows the results of LSD tests on the interaction *Hydrotaea aenescens* x fly density x manure moisture. Significantly greater mortality was observed in the treatments with *H. aenescens* present across both levels of density and moisture. Again however, the highest mortality (ca. 90%) occurred in the treatments with 50% moisture in the presence of *H. aenescens*. Of these the lower density produced the highest. Mean mortality of ca. 46% was observed in the treatments without *H. aenescens* at both density and both moisture levels.

Table 3. Results of Analysis of variance on mean percent mortality of *Musca domestica* larvae at two density levels, and two moisture levels with, or without the predators *Hydrotaea aenesens* and/or *Carcinops pumilio*.

Factor	df	Mean Square	F ratio	P
<i>H. aenesens</i> (Ha)	1	18456.89	227.07	<0.01 **
<i>C. pumilio</i> (Cp)	1	6882.63	84.86	<0.01 **
Ha x Cp	1	5761.50	70.88	<0.01 **
Density	1	297.13	3.66	0.058 NS
Ha x Density	1	125.87	1.55	0.215 NS
Cp x Density	1	376.60	4.63	0.033 *
Ha x Cp x Density	1	71.42	0.88	0.350 NS
Moisture	1	4723.11	58.11	<0.01 **
Ha x Moisture	1	3584.31	44.10	<0.01 **
Cp x Moisture	1	93.34	1.15	0.286
Ha x Cp x Moisture	1	2004.43	24.66	<0.01 *
Moisture x Density	1	387.10	4.76	0.031 *
Ha x Density x Moisture	1	406.01	5.00	0.027 *
Cp x Density x Moisture	1	215.49	2.65	0.106 NS
Error	145	81.28		
Total (adj)	159			

Table 4. Results of LSD comparisons of mean percent mortality of *Musca domestica* from combinations of the factors *Hydrotaea aenescens*, *Carcinops pumilio*, and Moisture.

<i>H. aenescens</i>	Factor		% Moisture	\bar{x} % Mortality **	
	<i>C. pumilio</i>	<i>M. domestica</i>		(n=20)	±(SE \bar{x})
-*	-	-	50	20.67 a	1.68
-	-	-	70	31.29 a	1.87
-	+	-	70	58.97 b	2.78
+	-	-	70	59.34 b	3.43
+	+	-	70	68.33 c	4.97
-	+	+	50	74.79 c	2.70
+	+	+	50	87.14 d	3.50
+	-	+	50	93.11 d	1.39

* - = not present in treatment, + = present in treatment

** Comparisons were conducted using arcsine square transformed values.

Means followed by the same letter within the column are not significantly different, (t -crit= 7.897; df=1, 145; P<0.05, Fisher's Protected LSD).

Table 5. Results of LSD comparisons of mean percent mortality of *Musca domestica* from combinations of the factors *Hydrotaea aeneascens*, Density, and Moisture.

		Factor			\bar{x} % Mortality **
<i>H. aeneascens</i>	Density	Moisture	<i>M. domestica</i>		
			(n=20)	\pm (SE \bar{x})	
-*	500	70	44.13 a	3.71	
-	250	70	46.13 a	4.18	
-	500	50	46.97 a	6.69	
-	250	50	48.49 a	6.51	
+	250	70	62.30 b	4.20	
+	500	70	65.37 b	4.54	
+	500	50	86.39 c	1.51	
+	250	50	93.85 d	2.35	

* - = not present in treatment, + = present in treatment

** Comparisons were conducted using arcsine square transformed values.

Means followed by the same letter within the column are not significantly different (t -crit= 7.897; df= 1, 145; $P < 0.05$, Fisher's Protected LSD).

The results of the LSD comparisons of the interaction *C. pumilio* x fly density are presented in Table 6. When these two factors were isolated, the presence of *C. pumilio* produced significantly higher mortality regardless of density.

Mortality of *H. aenescens*

Mean percent mortality of *H. aenescens* is presented in Table 7. Again, significant interaction between the treatment factors was detected by ANOVA (Table 8). LSD comparisons ($\alpha=0.05$) were performed on the means from the interactions *M. domestica* x *C. pumilio* x manure moisture, and *M. domestica* x fly density.

Table 9 presents the results of the examination of the interaction *M. domestica* x *C. pumilio* x manure moisture. There was considerable overlap between the treatment means, but in general, mortality of *H. aenescens* was higher in the treatments with *C. pumilio* present with, or without house flies. The highest level of mortality was observed where house flies were absent, and *C. pumilio* was present at 70% moisture. However, this was not significantly different from the same treatment at 50%. When house flies were present without *C. pumilio*, mortality of *H. aenescens* was significantly lower at the 50% moisture level from the same treatment at 70%, and from its control.

Results from the examination of the *M. domestica* x fly density interaction are presented in Table 10. Mortality of *H. aenescens* was greater in the absence of house flies at the low density, than in the presence of house flies at either density; mortality was intermediate in the absence of house flies at the high density. Generally speaking, mortality was lower when house flies were present regardless of density.

Predation Rate of *C. pumilio* on House Flies and *H. aenescens*.

Mean predation rate of *C. pumilio* on house flies and *H. aenescens* at two densities and two moisture levels is presented in Table 11. Results from ANOVA on

Table 6. Results of LSD comparisons on mean percent mortality of *Musca domestica* from combinations of the factors *Carcinops pumilio*, and Density.

Factor		\bar{x} % Mortality **	
<i>C. pumilio</i>	Density	<i>M. domestica</i> (n=40)	$\pm(\text{SE } \bar{x})$
-*	250	49.75 a	
-	500	52.46 a	
+	500	68.97 b	
+	250	75.67 c	

* - = not present in treatment, + = present in treatment

** Comparisons were conducted using arcsine square transformed values. Means followed by the same letter within the column are not significantly different (t -crit= 7.897; df= 1, 145; $P < 0.05$, Fisher's Protected LSD).

Table 7. Mean percent mortality (n=10) of *Hydrotaea aenescens* at two density levels and two moisture levels with the presence or absence of *Musca domestica* larvae, and the predator *Carcinops pumilio* adults.

Density 250		50% Moisture		70% Moisture	
Treatment	\bar{X}	$\pm(\text{SE})$	\bar{X}	$\pm(\text{SE})$	
Control	66.66	2.52	56.32	1.65	
MD ^a	33.48	4.28	38.88	3.56	
CP ^b	85.84	2.21	85.76	2.32	
MD+CP ^c	67.44	8.41	67.72	4.88	
Density 500		50% Moisture		70% Moisture	
Control	69.84	3.00	55.92	5.52	
MD	32.62	3.98	59.72	3.47	
CP	75.64	7.18	87.72	2.62	
MD+CP	76.52	2.40	75.63	4.50	

^a 500 *M. domestica* larvae/cup.

^b 10 *C. pumilio* adults/ cup.

^c 500 *M. domestica*+ 10 *C. pumilio* adults/cup

Table 8. Results of Analysis of variance on mean percent mortality of *Hydrotaea aenescens* larvae at two density levels and two moisture levels with or without the presence of *Musca domestica* larvae and *Carcinops pumilio* adults.

Factor	df	Mean Square	F ratio	P
<i>M. domestica</i> (Md)	1	4342.11	49.93	<0.01 **
<i>C. pumilio</i> (Cp)	1	11994.53	137.94	<0.01 **
Md x Cp	1	189.81	2.18	0.142 NS
Density	1	192.05	2.21	0.139 NS
Md x Density	1	340.64	3.92	0.050 *
Cp x Density	1	59.50	0.68	0.500 NS
Md x Cp x Density	1	7.19	0.08	0.774 NS
Moisture	1	105.41	2.21	0.273 NS
Md x Moisture	1	306.23	3.52	0.063 NS
Cp x Moisture	1	5.97	0.07	0.794 NS
Md x Cp x Moisture	1	1371.81	15.78	<0.01 **
Density x Moisture	1	328.99	3.78	0.054 NS
Md x Density x Moisture	1	28.31	0.33	0.056 NS
Cp x Density x Moisture	1	2.47	0.03	0.866 NS
Error	145	86.96		
Total (adj)	159			

Table 9. Results of LSD comparisons on mean percent mortality of *Hydrotaea aenescens* from combinations of the factors *Musca domestica*, *Carcinops pumilio*, and Moisture.

		Factor			\bar{x} % Mortality	
<i>M. domestica</i>	<i>C. pumilio</i>	Moisture	<i>H. aenescens</i>	\pm (SE \bar{x})		
+	-	50%	(n=20)	33.05 a	2.85	
+	-	70%		49.30 b	3.40	
-	-	70%		56.12 bc	2.80	
-	-	50%		68.25 cd	1.94	
+	+	70%		71.67 cde	3.35	
+	+	50%		71.98 de	4.38	
-	+	50%		80.74 ef	3.84	
-	+	70%		86.83 f	1.72	

* - = not present in treatment, + = present in treatment

** Comparisons were conducted using arcsine-square root transformed values.

Means followed by the same letter within the column are not significantly

different (t crit= 8.1682; df= 1, 145; $P < 0.05$; Fisher's Protected LSD).

Table 10. Results of LSD comparisons on mean percent mortality of *Hydrotaea aenescens* from combinations of the factors *Musca domestica* and fly density.

Factor		\bar{x} % Mortality **	
<i>M. domestica</i>	Density	<i>H. aenescens</i> (n=40)	$\pm(\text{SE } \bar{x})$
+*	250	51.88 a	3.69
+	500	61.12 a	3.35
-*	500	72.28 ab	3.00
-	250	73.65 b	2.29

*- = not present in treatment, + = present in treatment

** Comparisons were conducted using arcsine-square root transformed values. Means followed by the same letter within the column are not significantly different (t crit= 8.1682; df= 1, 145; $P < 0.05$; Fisher's Protected LSD).

Table 11. Predation rates of *Carcinops pumilio* on *Musca domestica* and *Hydrotaea aenescens*, and results of LSD comparisons on these rates at two fly densities, and two manure moisture levels.

Factor			Mean # fly immatures destroyed/ beetle/day**	
Fly Species	Density	Moisture	(n=10)	±(SE \bar{x})
Ha*	250	50	7.51 a	0.78
Md	250	70	8.05 a	0.63
Ha	250	70	9.50 a	0.77
Md	500	70	11.19 a	2.33
Ha	500	50	11.29 a	1.19
Md	250	50	11.68 a	0.44
Ha	500	70	20.04 b	1.65
Md	500	50	22.13 b	2.15

*Ha= *H. aenescens*, Md= *M. domestica*

** Means followed by the same letter within the column are not significantly different (t crit= 4.288; df=1, 72; $P < 0.05$, Fisher's Protected LSD).

these means are given in Table 12. Because of a highly significant three-factor interaction (Fly x Density x Moisture) Fisher's LSD tests were used to investigate further. These results are presented in Table 11.

Mean predation rate by *C. pumilio* ranged from 7.5 to 22.1 immature flies per beetle per day (Table 11). The predation rate was significantly higher in the treatments with either *H. aenescens* or house flies at the 500 density level, but with moisture at 70% for *H. aenescens*, and 50% for house flies.

Interspecific Interference

Results of *t*-tests to determine whether predator interaction occurred between *C. pumilio* and *H. aenescens* are presented in Table 13. At 50% moisture, and either density level, highly significant interference was observed. In both cases fewer fly eggs or larvae were destroyed than were expected based on the sum of the number of house flies destroyed by the predators when tested separately. There was also significant interference between the two predators at the 500 density level at 70% moisture, although P-value is close to non-significance.

Discussion

Effects of Density and Moisture on House flies and *H. aenescens*.

The values of ca. 20-30% mortality of house flies (in the controls) obtained from the present study generally agree with those obtained from a study conducted under similar experimental conditions by Koller (1991). She reported that mortality increased from manure moisture of 50% to 70% at density levels of 25, 50 and 75 larvae per cup. Her results, however become more difficult to interpret when the intermediate moisture of 60% used in her study is considered. Mortality of larvae in the 25 density treatment was higher in the 50% moisture level than in the 60% level, but mortality from the 60%

Table 12. Results of Analysis of Variance on the mean predation rate of *Carcinops pumilio* on *Musca domestica* and *Hydrotaea aenescens* at density levels of 250 and 500 fly eggs/cup, and manure moisture levels of 50% and 70%.

Factor	df	Mean Square	F ratio	P
Fly	1	27.77	1.39	0.243 NS
Density	1	953.78	48.68	<0.01 **
Fly x Density	1	0.66	0.03	0.856 NS
Moisture	1	18.38	0.92	0.341 NS
Fly x Moisture	1	800.80	40.03	<0.01 **
Density x Moisture	1	0.37	0.02	0.893 NS
Fly x Density x Moisture	1	246.99	12.35	<0.01 **
Error	72	20.01		
Total (adj)	79			

Table 13. Predation by *Carcinops pumilio* and *Hydrotaea aenescens* on *Musca domestica* when the predators were tested separately and together in different combinations of fly density and manure moisture. Values reported are mean \pm (SE) number *Musca domestica* destroyed. ^a

Density	Moisture	<i>C. pumilio</i>		<i>H. aenescens</i>		<i>C. pumilio</i> tested with		<i>t</i>	df	<i>P</i>
		tested seperately (A)	tested seperately (B)	tested seperately (B)	A + B (expected)	<i>H. aenescens</i> (C) (actual)	(A + B) vs C			
500	70	167.91 (34.97)	254.02 (40.18)	421.92 (54.97)	273.35 (41.57)	2.156	18	0.0449		
500	50	433.11 (12.85)	331.95 (32.32)	765.05 (39.70)	395.44 (11.79)	8.431	11	<0.01 ^b		
250	70	78.55 (9.87)	117.08 (9.74)	195.62 (15.00)	153.47 (23.06)	1.710	18	0.1045		
250	50	240.04 (3.08)	175.14 (6.48)	415.17 (7.92)	221.24 (14.23)	11.850	14	<0.01 ^b		

^a Calculated by multiplying corrected mortality (Abbot 1925) by the number of eggs used in the test.

^b Satterthwaite's Method for Unequal Variances.

level lower than that in the 70% moisture level. At the middle density level, increased from 14% to 49% mortality between the 50% and 60% levels, but then decreased to 25% at the 70% moisture level. At the highest density of 75 larvae per cup, mortality increased slightly from the 50% to the 60% moisture level, and remained about the same at the 70% level. When mortality data from all densities were pooled, mortality of 25%, 19%, and 21% occurred at manure moisture levels of 50%, 60%, and 70%, respectively. However, Fatchurochim et al. (1988) observed lower mortality in a study of filth fly oviposition and larval development in poultry manure of various moisture levels. They reported mortality of house flies of ca. 14%, with no significant differences in mortality between the 50%, 60% and 70% moisture levels. They did find significantly higher mortality (66%) in manure of 40% moisture. Both studies above used fewer densities of eggs per container, and neither examined the effect of density. Although there was no significant effect of density in the present study the levels of density used were 5-20 times greater than those used by Fatchurochim et al. (1988) and by Koller (1991). This may partly explain the higher mortality observed in the present study.

Mortality of *H. aenescens* in the various density-moisture treatments ranged from 56% to 70%, and these values are higher than values reported by Fatchurochim et al. (1988). These researchers observed an average of 37% mortality at manure moisture levels of 50%, 60%, and 70%, and reported no significant difference in mortality between these levels. They also found that unlike house flies, mortality of *H. aenescens* at these levels was not significantly different from mortality at 40% moisture. Koller (1991) observed 58% mortality of *H. aenescens* in manure of 50% moisture, however she observed only 10% mortality in the containers held at 70% moisture. Although *H. aenescens* mortality in the present study also declined significantly at the higher moisture, the effect was not dramatic.

It is important to consider the nature of the manure that was used in this study as well as in others. Beard & Sands (1973) reported that stored, as opposed to freshly deposited manure is highly anaerobic and that it fails to support house flies and other fly species, but *H. aenescens* was not included in their studies. However, autoclaved anaerobic manure that was aerated did support house fly larvae. It is doubtful that oven drying as conducted in the present study, altered the nutritional quality of the manure, and Fatchurochim et al. (1988) also used oven-dried manure. In the present study particular attention was not given to the physical nature of the manure which was collected. Although the collector was instructed not to sample from the inner aspects of the manure cone, the manure was collected from a farm which had a substantial accumulation of manure that was well coned so that some of the anaerobic manure inevitably was collected.

Effects of *H. aenescens*, *C. pumilio*, Density, and Moisture on House Flies.

The high levels of mortality of house flies observed in the treatments containing manure of 50% moisture, with *H. aenescens*, and both with, or without *C. pumilio* raise the question whether *H. aenescens* is a more successful predator at 50% moisture than at 70%. From the control mortality results, it seems that *H. aenescens* actually survives better at 70% in the absence of prey. Fatchurochim et al. (1988) also found that the development time of *H. aenescens* from egg to adult decreased significantly as moisture increased, from 42 d at 50% to 29 d at 70%. This effect was not observed for house flies, which required ca. 13 d to emerge as adults at either moisture. In addition, those workers found that adult dry weight and adult head-width of both *H. aenescens* and house flies were significantly greater in manure of 70% than in 50%. Together with the results from this study it might be concluded that *H. aenescens* does in fact function better at 70% moisture than at 50% however, those tests were conducted without prey.

Manure of 50% moisture, because of its drier state may be less suitable nutritionally to *H. aenescens*. When an alternative nutritional source such as house fly larvae is available at this moisture level, predation and hence survival by *H. aenescens* increases.

At 50% manure moisture, mortality of house flies in the treatments containing both *H. aenescens* and *C. pumilio* was not different from mortality in those with *C. pumilio* alone. This suggests that there could be differential predation by *C. pumilio*, perhaps preying upon more house flies than *H. aenescens*. At 70% moisture, mortality of house flies was higher when the two predators were together than from either predator acting alone. Considering density, the highest mortality of house flies occurred at the combination of lower moisture, and lower density. With a 1:1 ratio of *H. aenescens* to house flies, more house flies would more likely be encountered by *H. aenescens* larvae when the initial house fly density was high. The difference between moisture levels probably has a greater effect than that caused by the difference between density treatments. The effect of density might have been more pronounced had additional higher and lower treatment levels been used in the study.

Higher mortality of house flies was observed when *C. pumilio* was present at either density which was expected since these include the treatments with and without *H. aenescens* at both moisture levels. Since the house fly eggs used in this study were placed in clutches in order to simulate field conditions, and the first instar larvae usually remain clustered for a brief time before dispersing (personal observation), upon location by a predator, in this case *C. pumilio*, a high number might be destroyed during a single encounter.

Lower mortality of *H. aenescens* in the treatments with house flies present, and without *C. pumilio* was expected. However the reason that greater mortality of *H. aenescens* occurred at under moisture levels regardless of density is not known.

Regardless of density there was higher mortality of *H. aenescens* when *C. pumilio* was alone than when *C. pumilio* has the opportunity to prey on either or both fly species. This is obviously because *C. pumilio* perhaps did not exhibit any preference for either species and merely preyed upon that which it first contacted.

Predation Rates of *C. pumilio*

The rate of 22 house fly eggs and larvae per beetle per day observed in the present study is consistent with the rate of 21 per beetle per day reported by Geden et al. (1988), although the latter rate was based on a prey:predator ratio of 20-25:1. The highest ratio used during the present study occurred at a prey-predator ratio of 50:1. Higher rates are reported by other researchers and probably resulted from experimental conditions different from the present study. For example, Rueda et al. (1990) reported a rate of 37 eggs per adult *C. pumilio* per day when the beetles were isolated in containers devoid of manure or manure-like medium. Geden et al. (1988) observed a rate of 49 fly eggs and first instar larvae per beetle per day at a prey-predator ratio of ca. 500:1 when the fly eggs were pipetted directly onto the manure surface. They also reported a high rate of 104 house fly eggs and first instar larvae per beetle per day when the beetles were starved for 5 days prior to testing. In another study, Geden & Axtell (1988) used prey-predator ratios similar to those used in the present study, however like the aforementioned study, fly eggs were again pipetted onto the medium surface. They observed rates of 4 and 9 house fly eggs and first instar larvae per beetle per day at prey-ratios of 25:1 and 50:1, respectively. Rates lower than those obtained from the present study (ca. 13 eggs per beetle per day) are reported by Morgan et al. (1983), and are likely the result of using frozen rather than fresh house fly eggs.

The rates of predation of *C. pumilio* on *H. aenescens* were similar to those observed for *C. pumilio* on *M. domestica*. Therefore, under the conditions of this study

is appears that *C. pumilio* preys equally on both fly species although this study was not designed specifically to investigate prey preference by the *C. pumilio*.

Interspecific Interference

At the lower moisture level at both densities, the number of house fly immatures destroyed when *H. aenescens* and *C. pumilio* were tested together was ca. 50% lower than the rate expected by summing the rates of the predators tested separately. This seems to indicate highly significant interspecific interference between *C. pumilio* and *H. aenescens*. But whether this was true is another question. Predation by both *H. aenescens* and by *C. pumilio* separately was greater at 50% moisture than 70% at both densities. It would therefore be expected that when the two predators are combined greater predation would occur again at the lower moisture. I think that the effect is really on the survival of *H. aenescens* eggs and first instars rather than on the later instars as suggested above. *C. pumilio* may be capable differential predation on house flies and *H. aenescens*, because decreasing moisture causes increased development time of *H. aenescens*, and it is likely that some of this increase occurs in the duration of the egg stage as well as the first instar larva). The effect would be an increase in the prey vulnerability time of *H. aenescens* and a subsequent decrease *H. aenescens* predation ability. Since the effect at 70 % moisture was not as great as that observed at 50%, manure moisture is probably disrupting foraging/searching by *C. pumilio*. Both flies have been shown to oviposit greater percentage of eggs in medium of 70% moisture in the laboratory (ca. 87% and 77% for *H. aenescens* and house flies respectively, and they will oviposit very few eggs at moisture of 50%) (Fatchurochim et al. 1988), and from this study greater interference occurs at 50% than at 70%. This has implications for release strategies of *H. aenescens*. Under current recommendations for optimum house fly control, manure management is considered crucial, and maintenance of the manure in

the driest state is ideal. When the manure is not being wetted by leaking waterers there should be limited optimum oviposition area available, and we would do better to conduct releases of second instar *H. aenescens* onto areas of house fly breeding. This would allow for the *H. aenescens* cohorts to prey upon house fly larvae. If we released late instar larvae, the females from these would be forced to oviposit in lower moisture areas and the eggs and first instars would suffer losses to *C. pumilio*. However, there will inevitably be leaking waterers, and other factors contributing to the formation of wet areas in the manure. If an abundance of wet manure areas occurs, we might consider releasing greater numbers of pupae, which would allow the *H. aenescens* females to oviposit in moister manure.

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Chapter 6

Laboratory investigation of the effect of density, and instar synchrony between *Musca domestica* and *Hydrotaea aenescens*, on survival of *Musca domestica* larvae

Introduction

The house fly is the most important filth fly species breeding in high-rise cage layer poultry houses. Because these controlled-environment facilities allow massive accumulations of poultry manure, house fly control is a primary concern to egg producers at such operations. Control is often very difficult for the producer. Failure can result in explosive fly populations causing nuisance to livestock and workers, potential disease transmission, and legal action by annoyed neighbors, often culminating in temporary suspension of the farm operations. Traditional reliance on insecticides for fly control has also proven costly and increasingly ineffective, producing widespread resistance in house flies (Keiding 1977, Meyer & Georghiou 1989, Geden 1992). For several decades, integrated pest management (IPM) for house fly control involving cultural, biological, and chemical methods has been promoted as part of the total poultry management system (Anderson & Poorbaugh 1964, Peck & Anderson 1970, Axtell 1970, 1990). The biological control component of poultry IPM has received much attention during these years. Numerous studies have demonstrated the importance of both proper manure management to sustain numerous fly parasites and predators, as well as house fly control using mass-releases of laboratory-reared fly parasites (Legner & Brydon 1966, Axtell 1970, Peck & Anderson 1970, Legner et al. 1975, Rutz & Axtell 1979, Morgan et al. 1981).

The incorporation of inundative releases of a mass-reared house fly predator, *Hydrotaea aenescens* (Weidemann) (Diptera: Muscidae), into existing IPM programs in

high-rise cage-layer houses has received increased attention recently from researchers and egg producers (Turner & Carter 1990, Turner et al. 1992). Previous studies on *Hydrotaea* spp. demonstrated their natural occurrence, and non-synanthropic habits (Stein et al. 1977b, Nolan & Kissam 1985, 1987, Schultka et al. 1986) as well as the predaceous nature of the larvae (Anderson & Poorbaugh 1964, Schumann, 1982, Olkers & Hulley 1984). Youngman et al. (1991), and Turner et al. (1992), demonstrated the ease of mass-rearing and releasing *H. aenescens* with substantial cost-saving to the egg producer as well as decreased insecticide application to the poultry houses. Despite these findings, some researchers have been reluctant to consider *H. aenescens* an acceptable control agent (Axtell 1985, Moon & Meyer 1985, Axtell & Rutz 1986), even when these studies report predation rates for *H. aenescens* to be comparable to other principal predators such as *Carcinops pumilio* (Erichson) (Coleoptera: Histeridae), and *Macrocheles muscaedomesticae* (Scopoli) (Acari: Macrochelidae) (Geden et al. 1988).

In studies comparing the predation rates of *H. aenescens*, *C. pumilio*, and *M. muscaedomesticae* in the laboratory, Geden et al. (1988) reported the number of immature house flies destroyed per predator per day as varying from 25-104 for *C. pumilio* adults, depending on feeding history, 10-21 for *M. muscaedomesticae* adults, and 7-18 for third instar *H. aenescens* larvae. Estimates of field predation rates were 37 house fly eggs and larvae per predator per day for *C. pumilio* adults, 7.5 for *M. muscaedomesticae*, and 25 for *H. aenescens* third instars.

The *M. domestica*:*H. aenescens* (prey:predator) ratios from which Geden et al. (1988) derived laboratory predation rates, varied from 50:1 to 180:1. However, in these laboratory assays the density of *H. aenescens* third instars per assay container was held constant at 10 per container. Mass releases conducted in high-rise cage layer houses typically involve the release of several hundred to several thousand *H. aenescens* larvae

per patch of wet manure (personal observation). It was felt that increasing the density of *H. aenescens* in laboratory studies on predation would more accurately represent field conditions under mass-release situations.

Also, regarding predation rates reported in the literature, a question arose concerning the period over which the prey are vulnerable, i.e. whether studies were conducted in a manner which allowed *H. aenescens* third instars to exploit the entire period during which they are predaceous. Although there are variable reports regarding predation by first or second instar *H. aenescens* (Skidmore 1985, Muller 1982), only the third instars are reported to possess the cephalopharyngeal apparatus indicative of predatory behavior (Hogsette 1979, Schumann, 1982). Therefore the use of first or second instars in predation studies would introduce a misleading bias toward lower predatory potential. However, the use of second instar *H. aenescens* would allow predation from the instant the behavior is initiated (i.e. from the absolute beginning of the third stadium). If third instars are applied, their predaceous period would be short because as *H. aenescens* mature they become less active, cease feeding, and seek drier areas in which to pupate, therefore lowering the predation rate.

The laboratory study presented here was designed to provide further information concerning the ability of *H. aenescens* to effectively reduce house fly larval numbers using densities of *H. aenescens* similar to those produced by mass releases in the field. Based on preliminary experiments which showed mortality that was less than expected when prey:predator larval stages were equal, a series of experiments was set up to vary prey density and the prey:predator ratio.

Materials and Methods

Newly-emerged *H. aenescens* and house fly larvae were obtained from laboratory colonies maintained at the Price's Fork Research Center, VPI & SU, Blacksburg, Va. Adults in these colonies are fed a mixture of powdered milk, sugar, and powdered meat product. Larvae were obtained from cohorts of eggs gathered from the laboratory colony, and were reared on a medium consisting of a 2:2:1 volumetric ratio of wheat bran, vermiculite, and powdered meat products. The appropriate instars used in the experiments were selected from these cohorts and reared in a medium consisting of 30 g of wheat bran, 24 g of dried meat powder, and 28 g of vermiculite (the same 2:2:1 ratio). About 500 g of the medium were measured into 600 ml capacity plastic ice-cream containers to a depth of 6 cm, and 190 g of water were added to each cup to yield a 70% moisture content. The cups were weighed before, and after filling, with the weights recorded for subsequent gravimetric maintenance of moisture content. Their gross mass ranged from 306-310 g.

Experimental Design

Three replicates of each of the following 30 treatments were established, using one cup per replicate for a total of 90 experimental units.

- (1) 100 *H. aenescens* first instars vs 100, 200, 300, 400, or 500 first instar house fly.
- (2) 100 *H. aenescens* second instars vs 100, 200, 300, 400, or 500 first instar house fly .
- (3) 100 *H. aenescens* third instars vs 100, 200, 300, 400, or 500 first instar house.

- (4) 100 *H. aenescens* second instars vs 100, 200, 300, 400, or 500 second instar house fly.
- (5) 100 *H. aenescens* third instars vs 100, 200, 300, 400, or 500 second instar house fly.
- (6) 100 *H. aenescens* third instars vs 100, 200, 300, 400, or 500 third instar house fly.

Treatments 1, 4, and 6 are termed "equal-instar" treatments, and treatments 2, 3, and 5 are termed "unequal-instar" treatments, either "+1" or "+2" depending on whether *H. aenescens* was one stadium or two stadia higher than the house flies. All combinations involving predators of equal, or greater instar than prey were used; it was considered unnecessary to use predators smaller than prey. Three replicates of each of the following control treatments were established, using one cup per replicate.

- (1) 100, 200, 300, 400, or 500 house fly first instar.
- (2) 100, 200, 300, 400, or 500 house fly second instar.
- (3) 100, 200, 300, 400, or 500 house fly third instar.

Each container was covered with a fine mesh screen, placed into a rearing chamber, and held in continuous darkness at 27° C and ca. 50% relative humidity. Humidity was maintained by placing a pan of water inside the chamber. This was determined by frequent inspection of a thermohygrometer located inside the chamber. Moisture content was maintained by weighing the cups daily, and adding water to obtain the original recorded weight. After adding water, the contents of each container was gently stirred to distribute the water uniformly.

After several days the treatments were examined for pupae which were removed and the number recorded. The cups were returned to the chamber to allow the remaining larvae to pupate. The cups were examined daily and additional pupae were removed until there were no remaining pupae or viable larvae.

Calculations and Analyses

Survival of house fly larvae was calculated for each treatment by dividing the number of recovered pupae by the initial larval density. Abbott's formula was used to correct for control mortality (Abbott 1925). The resulting survival data were transformed using an arcsine-square root transformation (Zar 1984). These values were subject to analysis of variance (ANOVA) using Number Cruncher Version 5.03 (Hintze 1990). However, data presented both in tabular and graphic form are the original non-transformed values.

Factors used in the analyses were house fly instar, *H. aenescens* instar, and the density of house flies. Where significant main effects occurred, Tukey's HSD multiple comparisons ($\alpha=0.05$) were used to determine differences between the treatment means.

The experimental design of this study was inherently unbalanced because all combinations of treatment factors were not utilizable e.g. the "+1 instar" treatment for third instar house flies is the pupal stage, etc. (see Fig. 1 below).

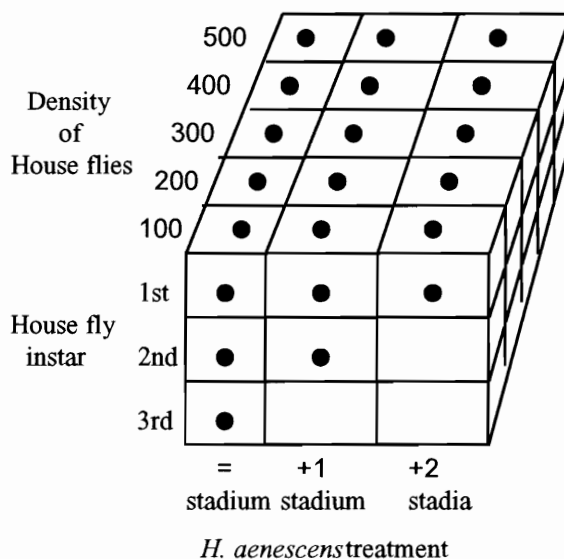


Fig. 1. Diagram of the experimental design. Blank cells indicate non-existent treatments.

A two-way ANOVA (not diagrammed) was used to analyze the effect of density on survival of house flies of each instar cohort in the controls, with density (5 levels) and house fly instar (3 levels) as the main effects. A three-way ANOVA (Fig. 2) was used to examine survival of house flies in treatments where the two fly species were the same larval stadium ("equal-instars"), and in the treatments where the *H. aenescent* larvae were one stadium higher than the house flies ("unequal-instars"). Again density was included as a main effect in the ANOVA.

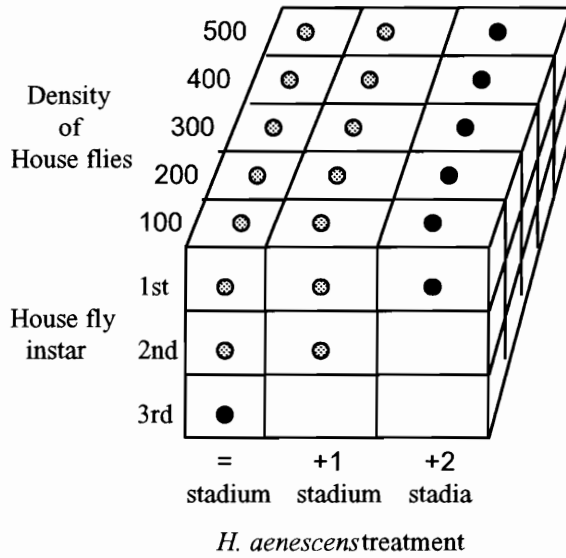


Fig. 2. ANOVA #2. Shaded circles represent treatments analyzed.

A third ANOVA (Fig. 3) was used to test the effect of density of house flies and *H. aenescens* instar on only first instar house flies. This enabled testing of the effect of the *H. aenescens* larvae which were two larval stadia higher than the house flies.

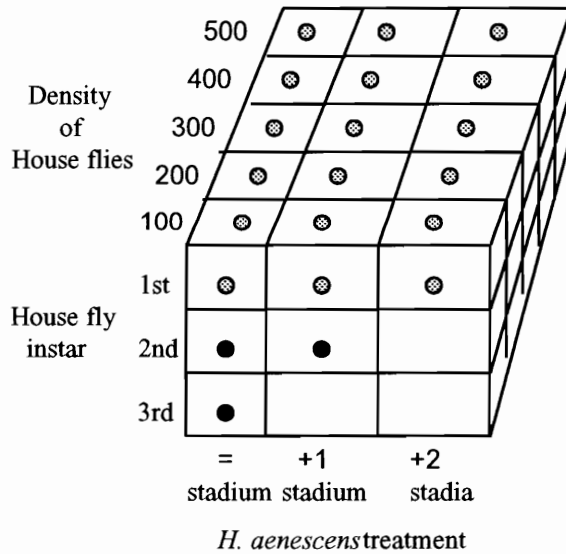


Fig. 3. ANOVA #3. Shaded circles represent treatments analyzed.

A fourth ANOVA (Fig. 4) was performed to test the effect of density on all three house fly instars in the equal-instar treatments with *H. aenescens*.

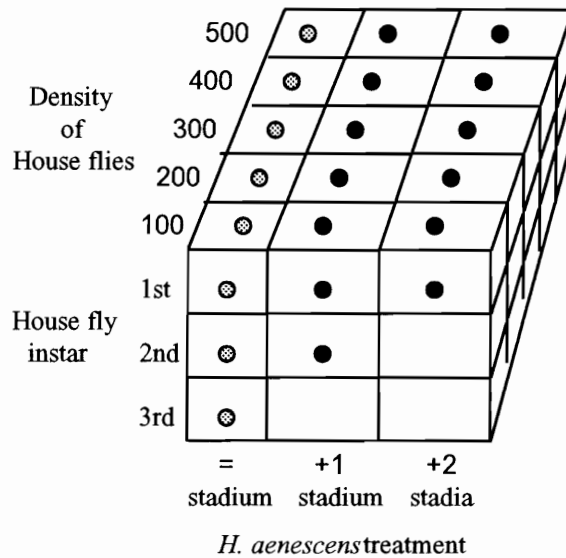


Fig. 4. ANOVA #4. Shaded circles represent treatments analyzed.

Results

Survival of *H. aenescens*

In general, high survival of *H. aenescens* was observed. Survival from all but two treatments ranged from a mean of 91.7% to 99.7%. The treatment containing 200 first instar house flies, and the one with 500 house fly larvae, both with 100 *H. aenescens* larvae, produced 81.7% and 87.7% survival of *H. aenescens*, respectively.

Survival of House flies in Controls

Mean percent survival of house fly larvae in the controls is shown in Table 1 and Fig. 5. Survival ranged from 81.67%-96.83%, and tended to decrease as density increased for each house fly instar.

Table 1. Uncorrected and corrected percent survival of five densities of *Musca domestica* larvae exposed to *Hydrotaea aenescens* larvae of equal, and advanced stadia, and controls.

Treatment			Uncorrected		Corrected	
<i>M. domestica</i>		<i>H. aenescens</i>	\bar{x} % Survival (\pm SE \bar{x})		\bar{x} % Survival (\pm SE \bar{x})	
Instar	Density	Density Instar	of <i>M. domestica</i>		of <i>M. domestica</i>	
First	100	control	95.00	3.61 a ¹	-	-
	200	control	90.33	1.36 ab	-	-
	300	control	91.00	1.65 ab	-	-
	400	control	82.17	2.10 b	-	-
	500	control	84.13	1.77 ab	-	-
	100	100 first	67.33	6.44	70.88	6.78
	200	100 first	73.67	3.19	81.55	3.35
	300	100 first	67.44	1.53	74.11	1.7
	400	100 first	65.58	1.83	79.81	2.22
	500	100 first	58.80	3.22	69.89	3.83
	100	100 second	0.00	0.00	0.00	0.00
	200	100 second	0.50	0.29	0.55	0.32
	300	100 second	1.67	1.04	1.83	1.12
	400	100 second	2.33	0.74	2.84	0.90
	500	100 second	2.20	0.61	2.62	0.73
	100	100 third	1.67	0.88	1.75	0.93
	200	100 third	1.33	0.60	1.48	0.67
	300	100 third	0.78	0.39	0.85	0.44
	400	100 third	1.50	0.76	1.83	0.93
	500	100 third	2.40	0.76	2.85	0.90
Second	100	control	95.67	1.86 a	-	-
	200	control	96.83	2.19 a	-	-
	300	control	84.11	2.25 b	-	-
	400	control	81.67	3.21 b	-	-
	500	control	79.40	2.44 b	-	-
	100	100 second	54.33	7.31	56.79	7.64
	200	100 second	51.00	4.65	52.67	4.80
	300	100 second	56.11	6.31	66.71	7.47
	400	100 second	49.83	2.12	61.02	2.58
	500	100 second	45.27	2.26	57.01	2.84
	100	100 third	1.67	1.20	1.74	1.26
	200	100 third	0.67	0.44	0.69	0.46
	300	100 third	0.44	0.13	0.53	0.13
	400	100 third	1.50	0.54	1.84	0.64
	500	100 third	2.20	0.31	2.77	0.38
Third	100	control	96.67	1.20 a	-	-
	200	control	95.50	0.58 a	-	-
	300	control	94.44	2.76 ab	-	-
	400	control	83.33	1.16 b	-	-
	500	control	85.80	2.00 b	-	-
	100	100 third	38.00	6.24	39.19	6.44
	200	100 third	52.50	2.02	54.97	2.12
	300	100 third	50.44	1.47	53.41	1.56
	400	100 third	56.00	5.00	67.20	5.99
	500	100 third	51.93	4.02	60.53	4.69

¹ Means within each house fly instar, followed by letters were used to test for differences in control mortality among density treatments. Means followed by the same letter are not significantly different ($P > 0.05$; Tukey's HSD test).

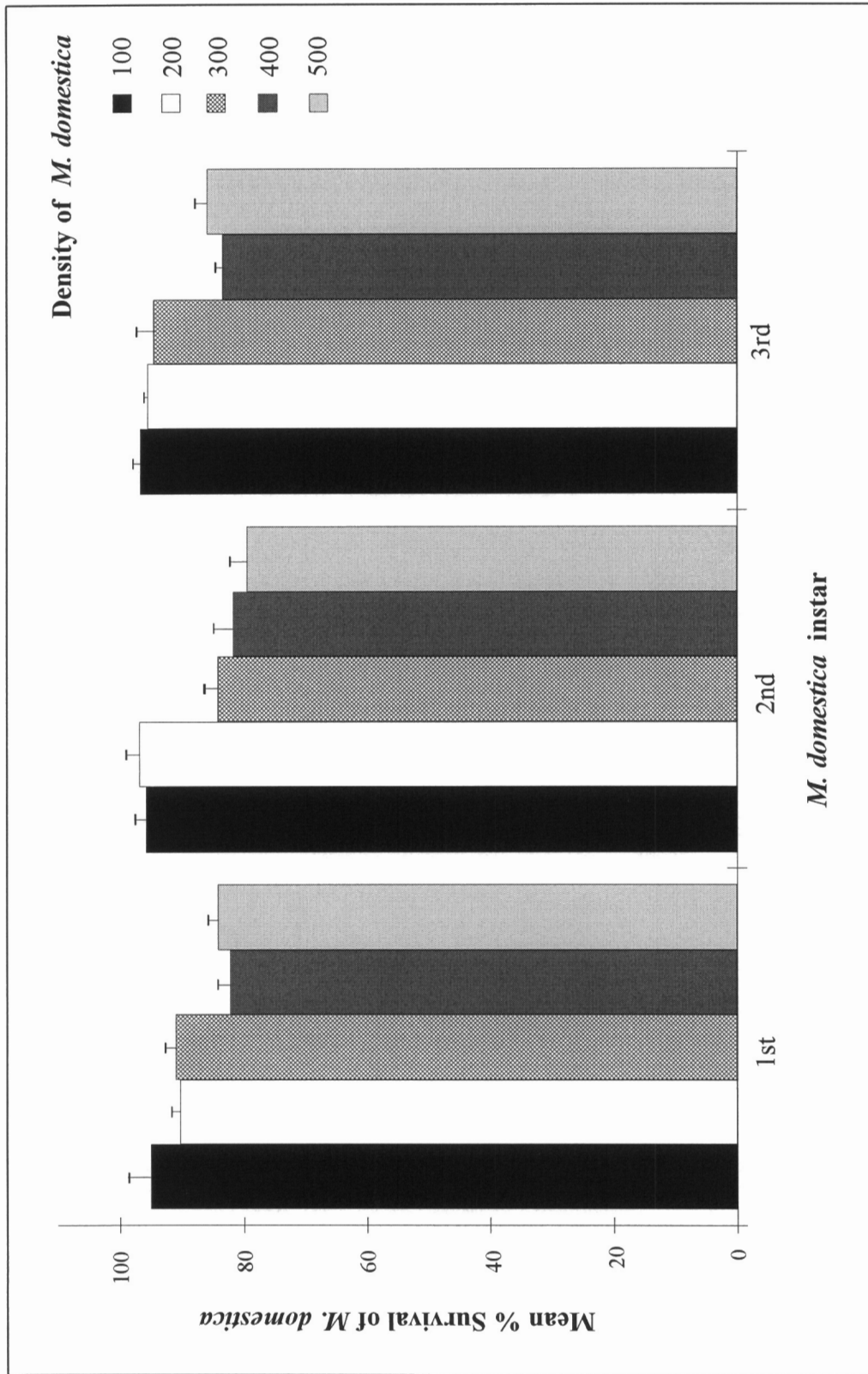


Fig. 5. Mean percent survival (\pm SE) of *Musca domestica* larvae in control treatments at five densities.

Results of ANOVA indicate that density produced several significant effects on survival ($F=18.58$, $df= 4, 30$; $P< 0.001$), while the main effect of the difference in house fly instar was not significant ($F= 1.65$, $df= 2,30$; $P= 0.208$). However, there was a significant interaction between density and house fly instar ($F= 1.88$, $df= 8,30$; $P= 0.1016$), indicating that the effect of density on survival of *M. domestica* larvae was not the same for its three instars. Survival from 90% to 97% was observed for all three instars in the three lower density levels, except for the second instar at the 300 density level.

A significant difference in mean percent survival of first instar house flies occurred between densities 100 and 400 (Table 1). Survival of second instars in the two low densities was significantly higher than survival in the three higher densities. Mean percent survival of second instars in the 100 and 200 density levels was significantly higher than survival in the treatments with 400 and 500 house fly larvae per cup.

Effect of Varying *H. aenescens* Instar and *M. domestica* Density on Survival of First and Second Instar *M. domestica*

Mean percent survival rate of house flies, both uncorrected and corrected for control mortality, at the various densities and *H. aenescens* treatments is shown in Table 1. It is important to emphasize that these values are the mean survival rates of house fly larvae to the pupal stage. In this regard, when a treatment is referred to as house fly first, second or third instar, these refer to the larval instar at which the experiments were initiated, and not the survival of the specific larval stadium.

Figures 6 and 7 show the survival of first and second instar house flies in equal-instar and unequal-instar treatments with *H. aenescens*, and in controls. Results from the ANOVA (Appendix B-1), indicate that the two factors, house fly instar and *H. aenescens*

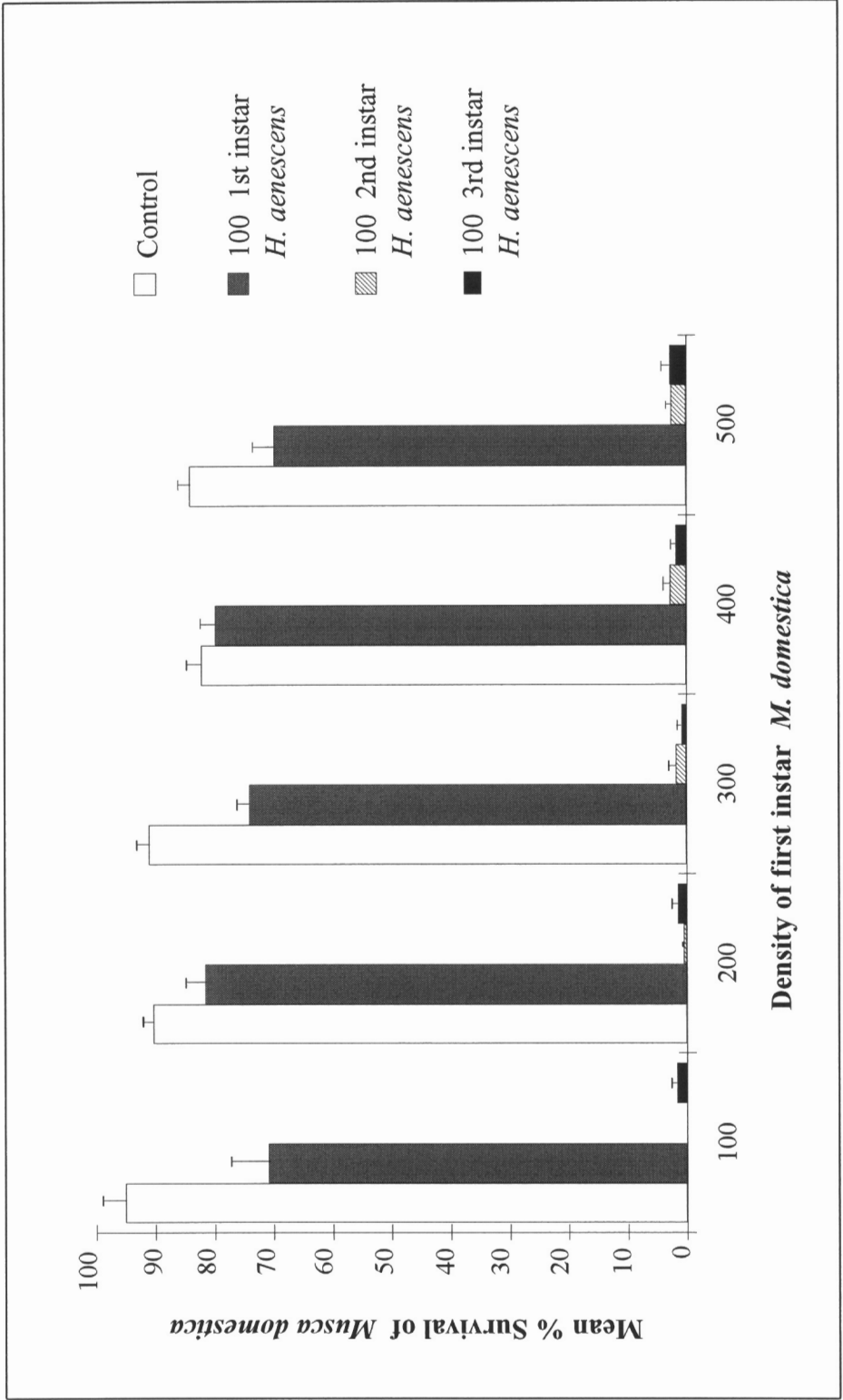


Fig. 6. Mean percent survival (\pm SE) of first instar *Musca domestica* in equal-instar and unequal-instar treatments with *Hydrotaea aenescens*, and in controls.

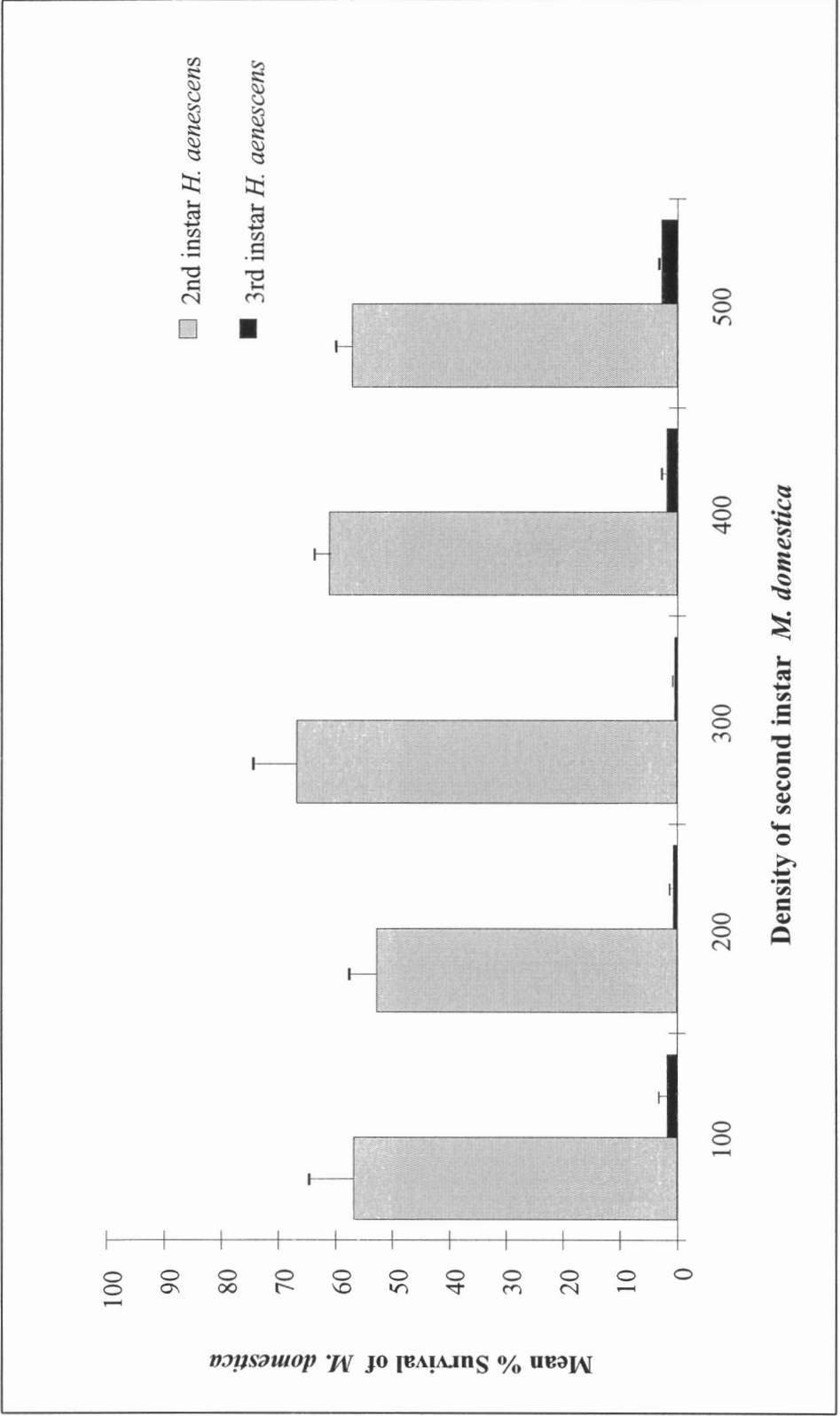


Fig. 7. Mean percent survival (\pm SE) of *Musca domestica* second instars in equal-instar and unequal-instar treatments with *Hydrotaea aeneascens*.

instar, were significant ($F= 13.61$, $df= 1, 44$; $P<0.05$, and $F= 1025$, $df= 1, 44$; $P<0.01$, respectively), and that density was not significant ($F= 0.0122$, $df= 4, 44$; $P= 0.1077$). However, there was a significant interaction between house fly instar and *H. aenescens* instar ($F= 21.17$, $df= 1, 44$; $P<0.01$). When interactions occur between main effects, it is not possible to determine the influence of each effect separately on the dependent variable (% survival of house flies). The significant interaction above indicates that the effect of *H. aenescens* instar on the survival of house flies is different between house fly first and second instars. Therefore, *t*-tests ($\alpha= 0.05$) were used to examine survival of *M. domestica* for both instars over the two levels of *H. aenescens* instar (equal-instar and unequal-instar) as well as survival of house flies from each level of *H. aenescens* instar over both levels of house fly instar. Table 2 shows the treatment means and Table 3 shows *t*-test results. Mean percent survival of first instar house flies exposed to equal-instar *H. aenescens* was significantly higher than that of second instars in same treatments. Survival was significantly reduced when both first and second instar house flies were exposed to *H. aenescens* instars which were one stadium higher. Regardless of house fly instar levels, there was no significant difference in mean percent survival between the unequal-instar treatments. It is interesting to note that there was no survival of house fly larvae when the first instars were exposed to second instar *H. aenescens*.

Effect of Density of *M. domestica*, *H. aenescens* Instar on First instar *M. domestica* Larvae.

The results of the two-way ANOVA which tested the effect of density and *H. aenescens* instar on only first instar house flies are presented in Appendix B-1. Fig. 6 shows the treatment means from this analysis. Here again, the effect of *H. aenescens* instar was significant ($F=493.03$, $df=2, 30$; $P<0.01$) and the effect of density was not ($F=2.50$, $df=4,30$; $P=0.0638$). Results from Tukey's HSD tests ($\alpha=0.05$) on the mean

Table 2. Mean % survival of first and second instar *Musca domestica* exposed to *Hydrotaea aenescens* larvae of equal and advanced larval stadia.

Treatment number	Treatments		n=15	
	<i>M. domestica</i> Instar	<i>H. aenescens</i> Instar	\bar{X} %	SE (\bar{X})
1	1st	1st	75.25	1.97
2	1st	2nd	1.56	0.41
3	2nd	2nd	58.84	2.44
4	2nd	3rd	1.51	0.34

Table 3. Results of pair-wise *t*-tests on mean % survival of *Musca domestica* from the treatments in Table 2.

Comparison	df	<i>t</i>	<i>P</i>
1 vs 2	28	21.221	<0.01
1 vs 3	28	5.155	<0.01
2 vs 3	28	25.260	<0.01
2 vs 4	28	0.244	0.81

survival of the first instars exposed to *H. aenescens* instars of equal stadium, one stadium higher, and two stadia higher (Table 4) show that survival (all densities included) was highest (ca. 75%) for the first instars in the equal-instar treatments and decreased to ca. 2% in treatments with unequal-instars. There was no significant difference in survival between the latter treatments.

Effect of Density and Instar on Survival of Each Instar of *M. domestica* Exposed to Equal-instar *H. aenescens* larvae.

Survival of *M. domestica* first, second, and third instars which were exposed to equal-instar *H. aenescens* is shown in Fig.8. The results of the two-way ANOVA (Appendix B-3), indicate that the effects of both house fly instar and density were significant ($F=12.95$, $df=2, 30$; $P=0.0031$, and $F=3.03$, $df=4, 30$; $P=0.0326$, respectively). Mean percent survival of first instar house flies in these equal-instar treatments with *H. aenescens* was significantly higher than survival of second and third instar house flies in these same treatments (Table 5). There was no significant difference in mean percent survival between the latter two treatment groups.

Although an ANOVA detected a significant effect of density on survival of house fly larvae in the equal-instar treatments, Tukey's HSD tests failed to detect differences between these treatment means at 0.1 level of significance (Table 6). Survival increases up to density 400 and then decreases. However, Fisher's Least Significant Difference (LSD) test, a more liberal multiple comparison procedure, did detect a significant difference in survival between the 100 and 400 density treatments at the 5% significance level.

Table 4. Results of Tukey's tests on mean percent survival of first instar *Musca domestica* exposed to equal-instar, and unequal-instar treatments with *Hydrotaea aenescens* larvae.

Treatments		n=15	
<i>M. domestica</i> Instar	<i>H. aenescens</i> Instar	\bar{X} % survival	SE (\bar{X})
1st	1st	75.25 a *	1.97
1st	2nd	1.56 b	0.41
1st	3rd	1.75 b	0.34

* Means followed by the same letter within a column are not significantly different ($P > 0.05$).

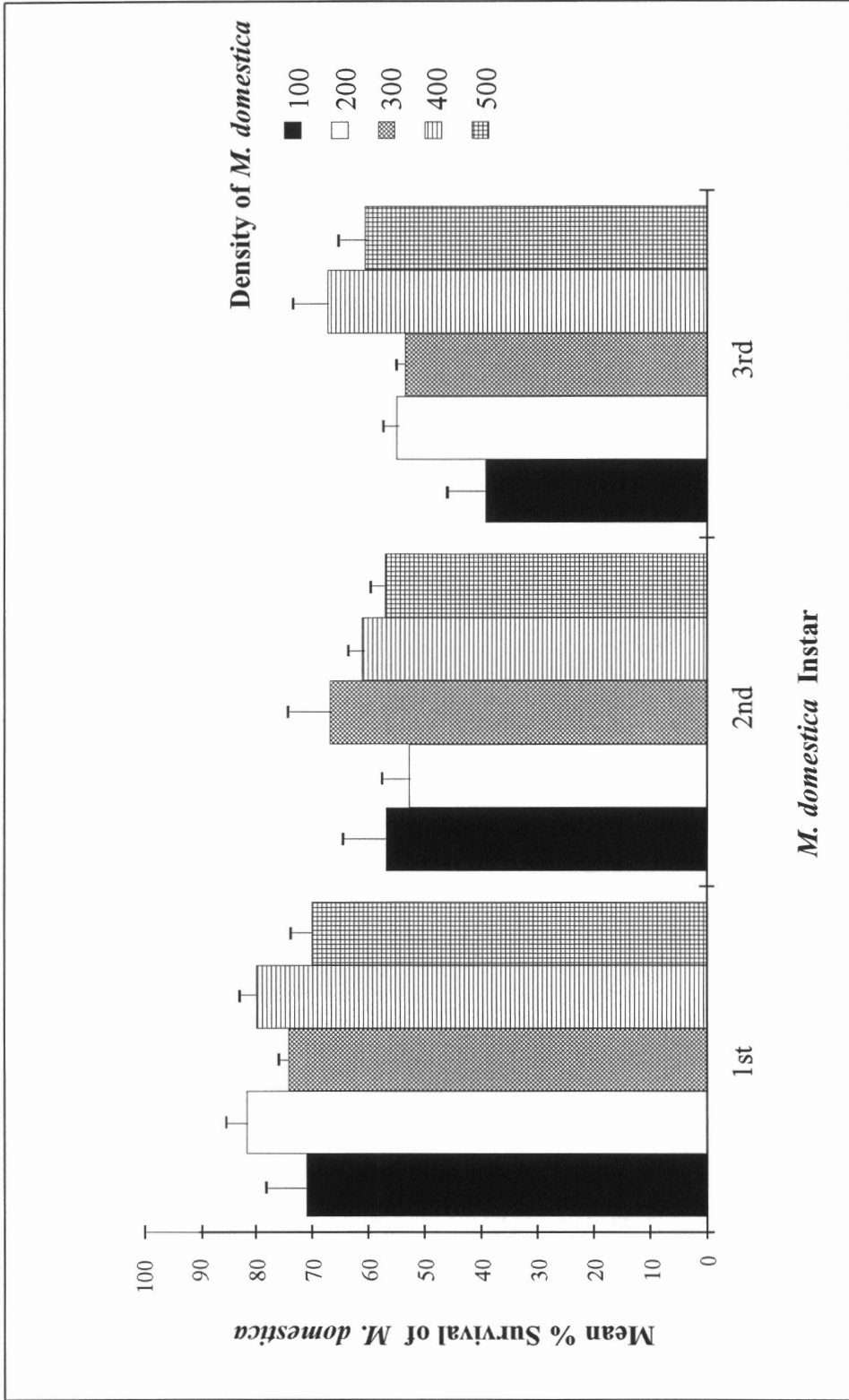


Fig. 8. Mean percent survival (\pm SE) of *Musca domestica* first, second, and third instars at five densities.

Table 5. Results of Tukey's tests on mean percent survival of *Musca domestica* larvae exposed to *Hydrotaea aenescens* of equal larval stadium.

Treatments		n=15		
<i>M. domestica</i> Instar	<i>H. aenescens</i> Instar	\bar{X} %		SE (\bar{X})
1st	1st	75.25	a	1.97
2nd	2nd	58.84	b	2.44
3rd	3rd	55.06	b	3.03

* Means followed by the same letter within a column are not significantly different ($P>0.05$).

Table 6. Results of Tukey's test on mean percent survival of *Musca domestica* first, second, and third instar larvae exposed to *Hydrotaea aenescens* larvae of equal stadium

Density	\bar{X} (n=10)	SE (\bar{X})	
100	55.62	5.76	a *
200	63.06	4.98	a
300	64.75	3.78	a
400	69.34	3.41	a
500	62.48	2.72	a

* Means followed by the same letter within a column are not significantly different ($P>0.05$).

Discussion

The high overall survival of all three instars of house flies in the controls along with the decrease in survival at the higher densities was expected. Decreasing survival was most likely due to larval crowding and competition for the limited resources in the containers. Other studies report varying survival of house flies in control treatments when similar moisture and temperature levels were used. However, they differ from the present study in terms of larval density, type and volume of media used, in addition to the fly stage counted. In one test, during which adult emergence was recorded, Koller (1989) used 100 g poultry manure as the medium, and initial first instar densities of 25, 50, and 75 per cup of 474 ml capacity. Survival was reported to be 73%, 74.6%, and 73% for the three densities which were held without predators at 70% moisture, 50% relative humidity, and 27°C. In a second test using similar conditions, Koller reported control survival for the same densities of first instars, as 85%, 88%, and 88%. Hogsette (1979) conducted competition studies between *H. aenescens* and house flies using 15, 30, and 45 first instar larvae per 90 ml of larval medium. Survival to the adult stage was reported to be 98% in the treatment containing 15 and 30 larvae per container, and 97% for 45 larvae per container. Olkers & Hulley (1984) studied predation by third instar *H. capensis* on second instar *M. domestica* in 200 ml of larval medium. They varied the density of house fly larvae from 5-80/cup. Control survival of the house flies was a uniformly high 97% for each of the treatments in the density range. Muller (1982) conducted similar predation studies with *H. aenescens* and house flies in 200 g of medium, using densities of 100, 200, 300, 400, and 600 first instars per container. Survival to the adult stage was reported to be 77%, 82%, 89%, and 72% for the densities respectively. Although these survival estimates are lower overall than those observed during the present study, a decrease in survival with increased density is apparent.

Density also produced a significant effect on survival of the three house fly instars when they were exposed to *H. aenescens* which were of equal stadium to the house flies. The reason this occurred in these equal- instar treatments is not known. If the means from each density of the equal-instar" treatments are isolated and subject to ANOVA, the results show that density is not significant ($F=1.26$, $df=4$, 44 ; $P=0.30$).

When first and second instar house fly larvae are exposed to equal instars of *H. aenescens*, survival between the two house fly instars was not the same. However it was similar for first and second instar house flies exposed to unequal-instar *H. aenescens* that were one stadium higher. Survival was higher for first instar house flies exposed to *H. aenescens* of equal instar than for second instar house flies exposed to equal-instars of *H. aenescens*. Because house flies have a faster development rate, initiating experiments with first instar house flies and first instar *H. aenescens* would probably allow many house flies to evade predation. Survival of house flies from containers initiated second instar *H. aenescens* would be lower than those initiated with first instars, as it has been reported that second larval stadium of *H. aenescens* lasts ca. 20 hours under optimum conditions (Schumann 1982). Second instar house flies require from 24 hours to several days to develop (West 1951). If the second instars of both species are comparable in development rate, then it is possible that *H. aenescens* would molt to the third instar fairly quickly and predation would commence.

After control mortality is removed, survival of house fly larvae decreases when they are exposed to *H. aenescens* larvae of advanced larval stadium. This effect was demonstrated for both first and second instar house flies exposed to *H. aenescens* one instar advanced, and for first instar house flies exposed to *H. aenescens*, two instars advanced. These results agree with results from similar studies conducted by Mueller (1982) and by Farkas & Papp (1990). Olkers & Hulley (1984) reported similar results in

laboratory studies of predation by *H. capensis* on house flies. The information obtained from the present study is important when comparisons of predator effectiveness based on laboratory studies are made between *H. aenescens* and other predators of house flies. When identical instars of *H. aenescens* and house flies are combined, as opposed to the combination of different larval stadia, predator effectiveness of *H. aenescens* will likely be underestimated because in the field (manure pits of poultry houses) there are overlapping generations of *H. aenescens* and house flies.

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Summary and Conclusions

Existing literature on control of pest Diptera in high-rise cage layer poultry houses provide highly variable data with respect to the performance of the various predatory arthropods. Much of this variability was obviously due to the different experimental conditions used by different investigators. Previous work by Muller (1982) Nolan & Kissam (1985), and Rueda et al. (1990) had indicated that *H. aenescens* was a better predator than was often supposed in much of the literature. Conversely, this study was based on the belief that *C. pumilio*, which many described as the "ideal" predator, did not live up to its reputation. This information suggested the possibility that laboratory test conditions were masking the true picture of what happens in the field.

The studies reported here tested the effects of various biological and ecological phenomena on the predator *H. aenescens* (Weidemann) (Diptera: Muscidae) and other selected arthropods (including *C. pumilio*) that typically inhabit manure of high-rise cage layer poultry houses. The conclusions therefore address the main concerns, which are; 1) simulating field conditions more exactly to investigate predator biology and performance, 2) assessing the performance of *H. aenescens* as a predator, and 3) providing a basis of information to support IPM programs for poultry farms.

Experimental Conditions

Several aspects of the studies conducted illustrate the importance of achieving experimental conditions that closely replicate field conditions. In the field, both manure moisture and prey density vary, and the predators and prey are able to migrate to the conditions they prefer. It became apparent when comparing the effectiveness of *H. aenescens* and *C. pumilio* in different conditions of manure moisture and prey density, that these factors interact to produce more than simple effects. For example, although *H. aenescens* preferred very wet conditions, this insect performed better as a predator at

50% moisture, but has a higher survival rate at 70% moisture in the absence of prey. Mature house fly larvae move to drier conditions to pupate and *H. aenescens* can follow. This indicates a greater adaptability of *H. aenescens* larvae under field conditions, than has been previously assumed.

The larval stadia of both *H. aenescens* and house flies used in lab studies was shown to be another important factor. Studies which use larvae of equal age would underestimate the predator's effectiveness. Because overlapping generations always occur in the field, initiating treatment with *H. aenescens* that are at a more advanced larval stadium than the house flies offered as prey produces optimum levels of predation for *H. aenescens*. This arrangement more likely represents field conditions than use of the two fly species at the same stadium. These data support the recommendation for mass releases of *H. aenescens* into cage layer houses as second instars, especially in wetter areas.

The medium used in laboratory studies is also an important factor to consider when regarding survival rates. Survival of both house flies and *H. aenescens* was higher in the study in which an artificial substrate was used than when unadulterated manure was used. If this factor is ignored, then any experimental results might be compromised.

Performance of *H. aenescens*

H. aenescens is able to exert a dramatic predatory pressure on house flies. This was shown in the study in which instar synchrony was investigated, where house fly mortality was as high as 100%, depending on the larval stadium at which the tests were initiated.

Of the predators examined, *H. aenescens* was the only one positively correlated with house fly larvae. This correlation may be partially an artifact of the mass releases that were conducted throughout the study, but the time from release to next sample

collection provided the opportunity for dispersal. *H. aenescens* larvae appear to remain in areas with high house fly populations and thus can provide effective control.

Another positive aspect of *H. aenescens*' performance is its relationship with *C. pumilio*. Although it was found in one of the studies that *C. pumilio* predation on house flies was comparable to levels reported in the literature, another study made it apparent that *C. pumilio* abundance was not correlated with manure moisture, and was negatively correlated with both house fly and *H. aenescens* larvae. This means that, in the field, *C. pumilio* might not be found in high numbers where house fly and *H. aenescens* eggs and larvae are found. It is felt that these data would negate any earlier concern that *C. pumilio* might reduce *H. aenescens* numbers. This also applies to the other arthropod predators that were studied and which also showed negative correlation with house flies and *H. aenescens*.

Information to Support IPM Programs

The manure sampling scheme that was devised for these studies is one that is simple and can be used by extension agents, researchers, and poultry producers themselves for assessing and monitoring the status of the arthropod populations in the manure. As the desired situation is to have high arthropod heterogeneity in the manure pits, sampling from discrete manure moisture areas will allow for comparison between houses or between farms with respect to specific arthropod fauna such as predators. Many of the predators of flies are very easy to distinguish, and a poultry operator could determine the status of the manure arthropod community both in between and after manure cleanouts.

The temperature-dependent development study also provided important baseline information which can be developed further and incorporated into computer-based expert systems. Such information is essential for computer-aided decision making which

promises to become an important IPM tool. By incorporating information on *H. aenescens* into computer models, more accurate answers can be provided to questions commonly asked by poultry operators initiating *H. aenescens* releases. Such questions include: 1) How long will it take for *H. aenescens* to become dominant over the house fly population that is already established in the houses? 2) How many *H. aenescens* should be released on each release date? and 3) When can *H. aenescens* releases be discontinued?

The study of diversity illustrated the importance of manure management as an essential practice in house fly IPM. Sampling from a brand-new house at a new farm showed that it takes many months for a poultry house to acquire a stable diverse arthropod population with a healthy balance of predators and prey. If the house is started in the winter, the time taken to acquire this balance is even longer, although this disadvantage must be contrasted with the high number of house flies that typically infest a house started in the summer. Based on these results, I would recommend that a poultry house started in the winter should be augmented with natural enemies of Diptera. Many of these are readily available commercially.

Recommendations for Conducting *H. aenescens* Releases

Releasing second instar *H. aenescens* is a good general rule for conducting mass releases into manure pits, when the releases are being used for controlling existing house fly populations. House fly breeding areas, ("hot-spots") can readily be located and *H. aenescens* larvae applied directly to the spot. In this way the second instars will exhibit maximum predation. *H. aenescens* can also be used prophylactically, however, populations should be monitored regularly using the moving-tape method of Turner & Ruzler (1989) and if needed *H. aenescens* should be augmented regularly.

When conducting prophylactic releases the prevailing manure conditions should be known in order to determine the most appropriate fly stage to release. After a manure cleanout (ideally a partial or staggered one), releases of any larval stage can be made onto the fresh accumulating manure, or pupae can be released onto the drier manure. If the desire is to maintain an existing *H. aenescens* population in a poultry house with good manure conditions (i.e. where the manure is piled-up and fairly dry), then again pupae should be released. This will allow the adult females the opportunity to locate ideal oviposition sites. In very wet conditions, releasing early instars is advisable since it is under these conditions which house flies prefer to oviposit. In this way *H. aenescens* will be advanced in age over newly hatching house fly larvae.

Further investigations should center on exploring the interactions between the other arthropod predators highlighted in the moisture correlation study, and other life stages of the Diptera. This should reveal other predators that could be used in IPM programs. Two of the predators, pseudoscorpions and earwigs, which were found at the longer-established farm and not at the newer one, are known as scavengers but also exhibit predatory behavior which may be significant. Additionally their absence from the newer facility after 1 1/2 years may indicate that they become abundant when the manure reaches a certain age and the arthropod community reaches a certain stability. It follows that these two arthropods, and potentially others, would be able to be used as ecological indicators of a "healthy" arthropod community. Furthermore pseudoscorpions and earwigs may be able to be mass reared and released and, since they prefer drier habitats, they would not affect *H. aenescens* predation on house flies.

Valuable information could also be obtained from studying the diversity of arthropods in houses that have not had *H. aenescens* released into them. This information could then be contrasted with that obtained from the diversity study

presented here, to give a fuller picture of the changes that occur in the naturally acquired arthropod populations under a setup scheme that involves mass releases of *H. aenescens*.

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Appendix A. Ordination table resulting from Canonical Correlation Analysis of data collected from Houses #3, and #4 at Glenwood Farms, Jetersville, Va.

Obs	Sphaeroceridae	<i>M. domestica</i>	<i>H. aeneus</i>	<i>C. pumilio</i> immatures	<i>C. pumilio</i> adults	Pseudoscorpionida	Dermoptera	Anthodoridae	<i>A. diaperinus</i> immatures	<i>A. diaperinus</i> adults	Density1*	% Moisture
1	0.00	0.00	0.81	0.00	0.00	0.00	1.04	1.90	1.77	1.28	-2.40	16.55
2	0.00	0.25	0.00	0.75	0.25	0.00	1.51	0.69	1.86	1.86	-1.87	20.00
3	0.00	0.00	0.00	1.49	0.70	0.30	0.48	1.46	2.25	1.97	-1.86	27.79
4	0.27	0.00	0.27	0.55	0.55	0.64	1.49	1.11	1.50	1.27	-1.84	20.07
5	0.25	0.25	0.00	0.25	0.99	0.41	0.41	1.58	2.00	0.99	-1.83	14.48
6	0.00	0.00	0.32	0.93	1.35	0.87	1.81	2.08	1.93	2.04	-1.83	26.95
7	1.32	0.31	0.00	0.72	1.10	0.50	1.35	2.03	2.22	2.04	-1.78	20.34
8	0.24	0.00	0.00	0.51	0.00	0.00	1.36	0.00	2.37	1.41	-1.73	15.82
9	0.53	0.00	0.00	1.68	0.34	0.34	1.33	1.28	2.33	2.08	-1.70	25.89
10	0.43	0.00	1.08	0.27	0.43	0.27	1.52	0.43	2.26	1.51	-1.64	19.18
11	0.78	0.00	0.00	1.69	0.48	0.85	1.12	1.74	2.09	2.07	-1.60	39.98
12	0.64	0.00	0.00	2.01	0.00	0.82	1.41	1.17	1.89	1.74	-1.54	31.52
13	0.79	0.00	0.00	1.39	1.35	2.03	0.96	1.27	2.34	1.50	-1.54	23.83
14	0.86	0.00	0.00	1.16	1.02	0.00	0.00	1.82	2.13	0.86	-1.52	20.50
15	0.96	0.00	2.72	1.51	1.00	0.00	1.67	2.05	2.39	2.07	-1.48	27.10
16	0.52	0.00	0.00	1.86	1.14	0.00	1.36	0.60	2.54	2.43	-1.45	46.21
17	0.67	0.00	0.00	1.57	0.00	0.00	1.40	1.16	1.53	1.60	-1.44	23.08
18	0.00	0.26	0.00	1.37	0.82	0.00	0.26	0.00	1.92	0.00	-1.38	1.22
19	0.31	0.00	0.00	0.53	0.00	0.00	0.00	0.58	1.29	0.56	-1.36	15.53
20	0.36	0.55	1.10	1.43	0.55	0.36	1.47	0.87	1.91	1.52	-1.33	34.62
21	0.70	0.00	0.00	1.04	1.23	0.00	1.35	0.60	2.14	2.14	-1.31	32.97
22	0.44	0.00	0.00	0.66	0.00	0.00	1.58	0.44	0.91	1.35	-1.29	20.00
23	0.63	0.00	0.00	1.63	0.26	0.26	1.83	0.42	1.77	1.98	-1.23	25.17
24	1.22	0.00	0.00	1.50	1.56	0.00	1.40	1.40	1.65	0.71	-1.20	36.71
25	1.31	0.00	0.33	1.84	1.26	0.00	0.75	1.90	2.69	2.41	-1.15	25.13
26	1.27	0.00	0.00	1.64	0.83	1.46	0.89	0.99	2.59	1.78	-1.14	36.42
27	0.93	0.00	0.72	0.98	0.80	0.72	0.00	1.14	2.01	1.41	-1.05	25.30
28	1.77	0.00	0.00	1.56	0.84	1.24	1.72	1.35	2.19	2.05	-0.88	37.41
29	0.58	0.00	0.00	0.75	1.40	0.28	1.88	0.97	1.88	1.88	-0.88	36.35
30	1.87	0.00	0.00	1.83	1.50	1.24	1.97	0.68	2.62	2.12	-0.70	35.39
31	1.48	0.37	0.37	1.62	0.00	0.70	2.01	0.89	1.23	1.97	-0.63	34.82
32	1.57	0.00	0.72	1.54	0.72	1.14	1.46	0.49	1.88	1.54	-0.61	35.68
33	1.48	0.00	1.85	1.32	0.53	0.00	0.66	0.53	2.15	1.32	-0.51	53.12
34	1.76	0.00	0.00	1.86	1.72	1.43	1.25	0.36	2.32	1.85	-0.41	35.8
35	0.32	0.00	1.66	1.07	1.53	0.00	0.00	0.00	0.80	0.80	-0.38	57.27
36	1.35	0.35	1.62	1.47	1.24	0.00	1.60	0.00	1.51	1.32	-0.14	54.94
37	1.63	0.00	2.24	1.92	1.46	2.17	0.76	0.34	2.21	2.13	-0.04	51.57
38	1.99	0.00	1.77	0.00	0.00	0.31	0.78	0.49	0.00	0.00	0.00	49.38
39	1.52	0.00	0.00	1.87	1.08	1.95	0.62	0.00	1.55	2.31	0.06	48.53
40	1.78	0.00	1.96	1.96	1.02	1.69	0.72	0.62	1.83	2.34	0.07	54.95
41	2.04	0.00	0.95	1.45	1.08	2.04	0.00	0.95	1.08	1.11	0.09	37.27
42	1.12	0.34	1.84	1.58	1.22	0.00	0.00	0.00	0.85	0.00	0.11	60.10
43	2.01	0.00	1.09	1.46	0.29	0.88	0.58	0.00	1.27	0.88	0.16	57.36
44	2.05	0.59	0.00	1.87	0.59	1.49	0.46	0.82	2.55	2.19	0.21	62.68
45	1.72	0.00	2.68	1.16	0.00	0.00	0.00	0.82	0.82	1.61	0.21	42.58
46	1.96	0.00	1.73	0.28	0.56	0.00	0.00	0.00	0.74	0.00	0.26	61.50
47	2.06	0.00	2.15	1.79	1.45	1.59	0.91	0.00	2.04	0.00	0.27	56.92
48	1.78	0.00	0.00	2.00	0.81	1.56	0.00	0.00	2.01	2.55	0.27	57.02
49	1.19	0.00	1.26	1.34	1.44	0.00	0.00	0.00	0.00	0.28	0.35	62.20
50	1.47	0.00	1.51	1.60	0.45	0.81	0.58	0.00	0.00	0.81	0.36	67.04
51	1.75	0.00	3.59	1.26	1.23	1.46	0.37	0.00	1.15	0.56	0.37	57.93
52	2.17	0.00	1.17	1.88	0.69	1.53	0.30	0.00	1.57	1.31	0.42	65.09
53	1.74	0.00	0.87	1.92	1.10	0.00	0.00	0.00	1.77	2.04	0.43	57.95
54	2.16	0.00	1.81	0.00	0.00	0.00	0.00	0.00	0.29	0.00	0.43	60.97

55	1.81	0.00	1.76	0.86	0.77	2.18	0.00	0.00	0.77	1.29	0.44	61.56
56	1.85	0.00	1.96	0.85	0.27	0.85	0.00	0.00	1.02	1.54	0.45	63.98
57	2.10	0.00	2.39	1.02	0.43	1.37	0.00	0.00	1.19	0.89	0.47	70.00
58	2.28	0.00	1.81	0.80	0.31	0.31	0.00	0.31	0.00	0.31	0.47	49.87
59	1.63	0.00	2.56	1.06	0.00	0.00	0.00	0.00	0.73	1.50	0.50	61.50
60	2.08	0.00	1.87	0.00	0.31	0.00	0.00	0.00	2.00	0.00	0.54	62.60
61	2.03	0.00	1.79	2.23	1.20	1.56	0.82	0.00	2.00	2.79	0.56	55.12
62	2.27	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.57	66.20
63	1.92	0.00	1.76	0.61	0.80	0.00	0.00	0.00	0.00	0.00	0.60	70.63
64	2.05	0.75	2.20	1.26	0.00	1.24	0.52	0.00	1.28	1.41	0.61	62.99
65	1.96	0.00	2.19	1.92	1.27	1.84	0.28	0.00	1.84	2.37	0.61	59.28
66	2.18	0.00	2.62	1.03	1.03	0.73	0.00	0.00	1.24	0.73	0.62	60.57
67	1.93	0.00	1.70	1.51	1.36	0.00	0.00	0.00	0.92	0.71	0.64	60.17
68	2.03	0.93	0.00	1.67	0.26	1.37	0.00	0.00	1.52	1.76	0.65	63.77
69	2.30	0.00	1.05	1.21	1.00	1.31	0.00	0.00	1.35	0.52	0.66	65.86
70	1.88	0.00	2.81	1.47	0.32	0.00	0.32	0.64	0.31	1.40	0.66	46.19
71	1.95	0.00	1.10	1.62	0.80	1.42	0.00	0.00	0.31	0.72	0.67	64.93
72	1.69	0.00	1.65	1.53	1.42	0.00	0.00	0.00	0.00	0.00	0.67	64.03
73	2.42	0.00	2.20	0.87	0.00	0.00	0.32	0.00	0.32	0.00	0.68	73.76
74	2.04	0.00	2.07	1.11	1.14	0.84	0.00	0.00	0.97	1.14	0.68	59.04
75	2.08	0.00	2.54	0.81	1.15	1.08	0.00	0.00	0.63	0.50	0.69	60.09
76	1.51	0.72	2.74	1.41	0.93	1.33	0.00	0.00	0.98	1.28	0.69	59.99
77	2.47	0.00	1.65	0.99	0.29	0.00	0.00	0.00	0.59	0.00	0.70	55.67
78	2.39	0.00	1.62	1.06	0.00	0.00	0.00	0.00	0.46	0.00	0.71	60.75
79	2.31	0.00	1.54	0.00	0.81	0.00	0.00	0.00	0.59	0.46	0.73	60.00
80	2.09	0.00	2.30	0.87	0.98	0.00	0.62	0.00	0.00	0.32	0.74	50.53
81	2.52	0.00	2.62	1.03	1.03	0.73	0.32	0.00	1.24	0.73	0.75	60.53
82	2.39	0.00	1.15	1.64	0.60	1.64	0.30	0.00	0.60	1.04	0.80	64.03
83	2.60	0.00	1.33	0.34	0.00	0.34	0.00	0.00	0.00	0.00	0.81	62.10
84	2.21	0.33	2.11	1.42	0.85	1.17	0.33	0.00	0.75	0.90	0.81	70.90
85	2.11	0.00	1.53	1.26	0.83	0.00	0.29	0.00	0.00	0.47	0.83	67.70
86	2.38	0.47	1.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.85	74.09
87	2.14	0.00	1.84	1.52	0.82	0.00	0.00	0.00	0.00	0.00	0.86	69.49
88	1.76	0.61	1.72	1.47	1.41	0.00	0.00	0.00	0.48	0.48	0.86	68.18
89	1.85	0.44	1.77	1.13	1.31	0.56	0.00	0.00	0.44	0.86	0.87	61.21
90	2.16	0.00	2.46	1.45	0.74	1.84	0.33	0.00	0.00	0.51	0.87	64.48
91	2.36	0.47	1.31	0.29	0.29	0.00	0.00	0.00	0.00	0.00	0.88	73.45
92	2.29	0.00	0.77	1.79	1.68	0.00	0.00	0.00	0.77	0.59	0.89	65.40
93	2.07	0.00	1.81	1.20	1.50	0.00	0.00	0.00	0.00	0.00	0.90	67.41
94	2.36	0.31	1.33	1.41	1.01	1.27	0.00	0.00	1.33	1.63	0.90	71.39
95	2.23	0.00	1.75	1.29	0.94	0.00	0.00	0.00	0.00	0.00	0.92	70.14
96	2.32	0.34	1.85	1.11	0.91	1.15	0.34	0.00	0.00	0.00	0.93	65.92
97	2.08	1.00	1.00	0.00	1.17	0.00	0.00	0.00	0.00	0.00	0.95	71.81
98	2.41	0.00	1.11	1.51	1.69	0.00	0.00	0.00	0.73	0.50	0.96	66.76
99	2.39	0.00	1.12	1.64	0.82	1.44	0.00	0.00	0.32	0.74	0.96	64.42
100	2.28	0.95	0.00	1.36	0.27	0.73	0.44	0.00	0.56	1.38	0.96	64.39
101	2.20	1.19	1.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	73.50
102	2.23	0.81	1.24	1.44	1.22	0.57	0.28	0.00	1.01	1.17	1.01	65.40
103	2.51	0.38	2.44	0.97	0.97	0.97	0.00	0.00	0.82	0.72	1.07	61.25
104	2.47	0.00	1.72	1.19	0.82	0.00	0.00	0.00	0.32	0.32	1.11	70.32
105	2.10	0.00	1.59	1.20	1.59	0.00	0.00	0.00	1.20	1.20	1.17	62.50
106	2.38	1.47	2.13	1.43	0.00	0.00	0.55	0.00	1.11	1.36	1.21	70.40
107	2.25	1.23	3.17	1.02	0.80	0.31	0.00	0.00	1.35	1.26	1.23	67.83
108	2.24	1.49	2.05	1.00	0.00	0.00	0.00	0.00	0.91	1.59	1.32	66.16

* First Canonical Variable

Appendix B1. Results of analysis of variance on mean percent survival of five densities of *Musca domestica* first and second instars exposed to *Hydrotaea aenescens* equal-instar, and unequal-instar treatments.

Source	df	SS	F	P
A (<i>H. aenescens</i>) instar	1	0.11	13.61	0.02
B (<i>M. domestica</i>) instar	1	11.12	1025.09	<0.01
AB	1	0.13	21.17	<0.01
C	4	0.05	2.02	0.11
AC	4	0.03	1.35	0.27
BC	4	0.04	1.80	0.15
ERROR	44	0.01		

Appendix B2. Results of analysis of variance on mean percent survival of five densities of *Musca domestica* first instars exposed to *Hydrotaea aenescens* equal-instar, and unequal-instar treatments.

Source	df	SS	F	P
A (<i>H. aenescens</i>) instar	2	8.93	493.03	<0.01
B (Density)	4	0.04	2.50	0.06
AB	8	0.07	2.02	0.08
ERROR	30	0.13		

Appendix B3. Results of analysis of variance on mean percent survival of five densities of *Musca domestica* first, second, and third instars exposed to *Hydrotaea aenescens* equal-instar, and unequal-instar treatments.

Source	df	SS	F	P
A (<i>M. domestica</i>) instar	2	0.20	12.95	0.003
B (Density)	4	0.02	3.03	0.03
AB	8	0.01	41.94	0.09
ERROR	30	0.01		

VITA

Perian Lenore Dillon was born on March 16, 1960, in Sacramento, California. She moved to Philadelphia, Pennsylvania, at the age of 5 where she attended Charles W. Henry Elementary School, and subsequently the Philadelphia High School for Girls from which she graduated in 1978. She received a B.S. in Marine Science from Hampton University in 1983. During her senior year she took her first entomology course under Dr. Barbara J. Abraham and although she was fascinated by all living thing since childhood, it was this course that sparked her interest in this field as a career. She received M.S. in Environmental Science from Hampton University in 1987. Throughout her Bachelors and Masters degrees she worked her way through with jobs such as waitress, dormitory resident assistant, and shop worker, yet she had the opportunity to complete several exciting internships such as with the National Oceanic and Atmospheric Administration in Woods Hole, Massachusetts, the North Carolina Marine Resources Center in Manteo, North Carolina, and the United States Fish and Wildlife Service in New Orleans, Louisiana. In August 1987 she began the pursuit of a Ph.D. degree in entomology at Virginia Tech, with specific interest in medical and veterinary entomology. She completed her degree requirements in March 1994. After receipt of her Ph. D. degree, Ms. Dillon will be seeking a research or teaching position in entomology.