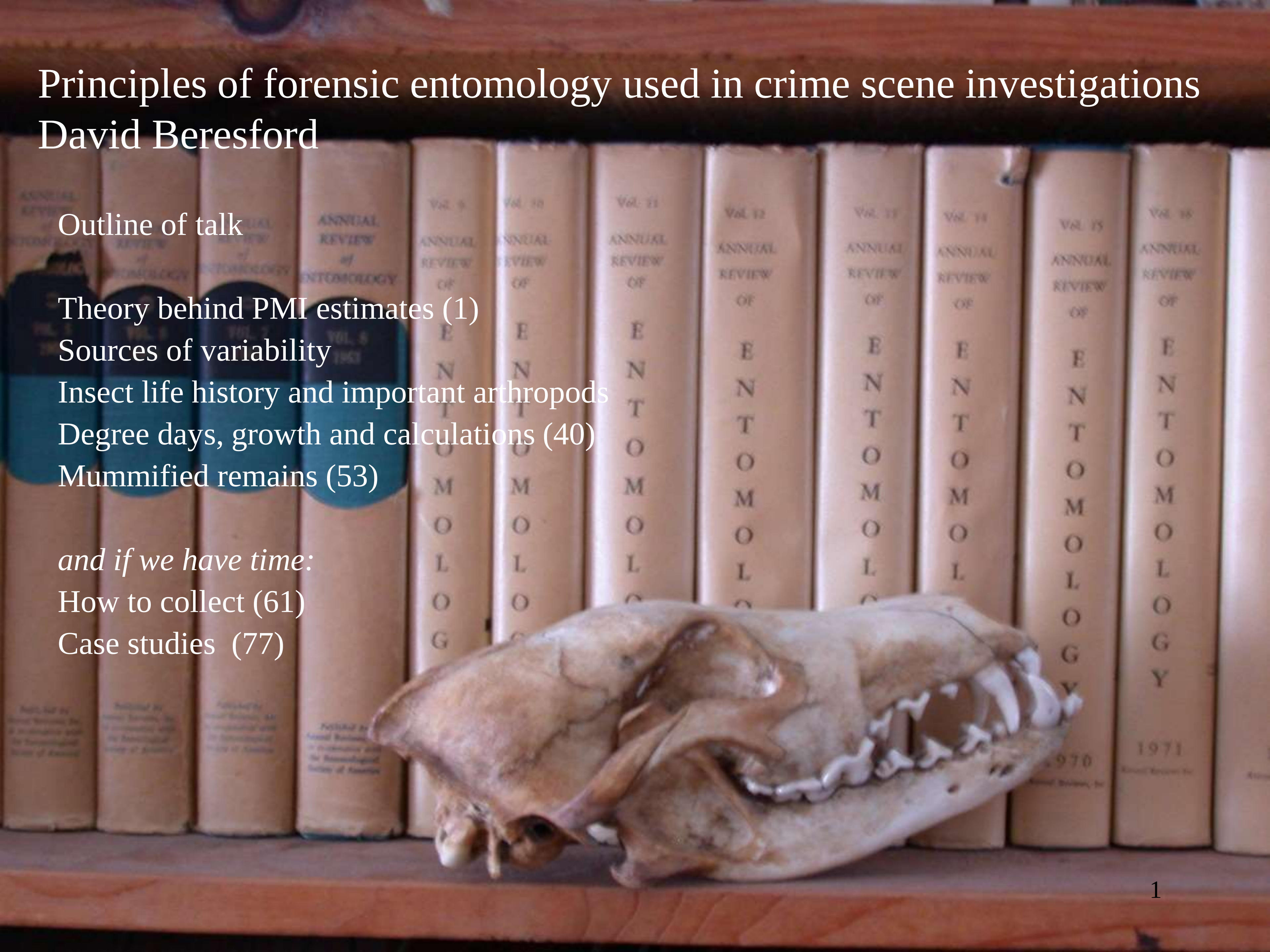
Principles of forensic entomology used in crime scene investigations

David Beresford

Outline of talk

Theory behind PMI estimates (1)
Sources of variability
Insect life history and important arthropods
Degree days, growth and calculations (40)
Mummified remains (53)

and if we have time:
How to collect (61)
Case studies (77)



forensically entomology: identifying time and place of events

commonly for Post Mortem interval of victims of homicides, also used for identification of illicit drugs, other materials

ecology of corpses:

a corpse is a high nutritional package that is suddenly released into the environment, time and placement is random

problem for insects: how to find and colonize these:

need separate life history stages with different requirements:

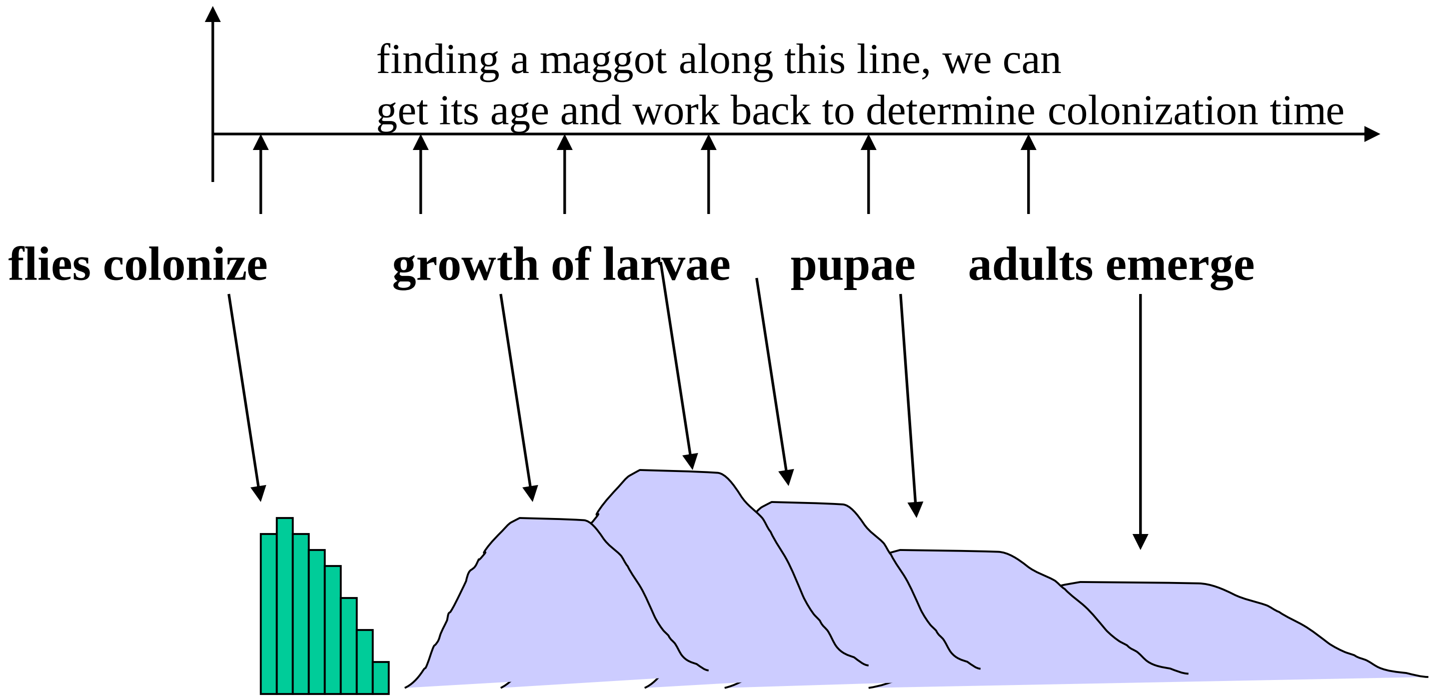
- immatures (called maggots for flies): to obtain lots of food quickly
- adults: to find mates, and disperse and find new carcasses

adaptive peak of each species based on benefits of generalization vs specialization on food source and generalization vs specialization in environment

major problem: availability of food is unpredictable → feast or famine

theoretical outline of the process:

timeline: death → stages of putrefaction → stages of mummification



actual process: not clear demarcations, but distributions

sources of variability:

intrinsic factors:

-arrival times, early, optimal, late colonizers, distance to other nearest corpse, number of flies available, competition from other colonizers

extrinsic factors:

- moisture, sun vs shade, species that arrive, distance to nearest corpse, commonness or rarity of other corpses, predators, parasites, disease

- variability associated with the carcass:

size of corpse, presence of drugs, food, health of individual, amount of fat vs lean meat (and location of that fat), order of organs colonized, order of utilization, exposure of interior organs and body cavity, access to carcass eg partially submerged?

Different insects are in different habitats

WW1: entomologists observed that
sunny portions of the trenches were coated with large bright blue flies, the blue bottles (Order Diptera: Family Calliphoridae, *Calliphora vicina*)

shaded portions of the same trenches coated with green flies, the green bottles (Order Diptera: Family Calliphoridae, *Lucillia* sp)


Research: using animals as surrogates for human corpses, and baited traps for species surveys

Phormia regina

Pollenia sp.

Calliphora vicina

Calliphora vomitoria

Lucilia illustris

Lucilia sericata

Phaenicia sericata (Meigen, 1826)

main early colonizers

life history

holometabolous

eggs and larval stages differ from adult stage

identification based on morphological

features (ie the phenotype)

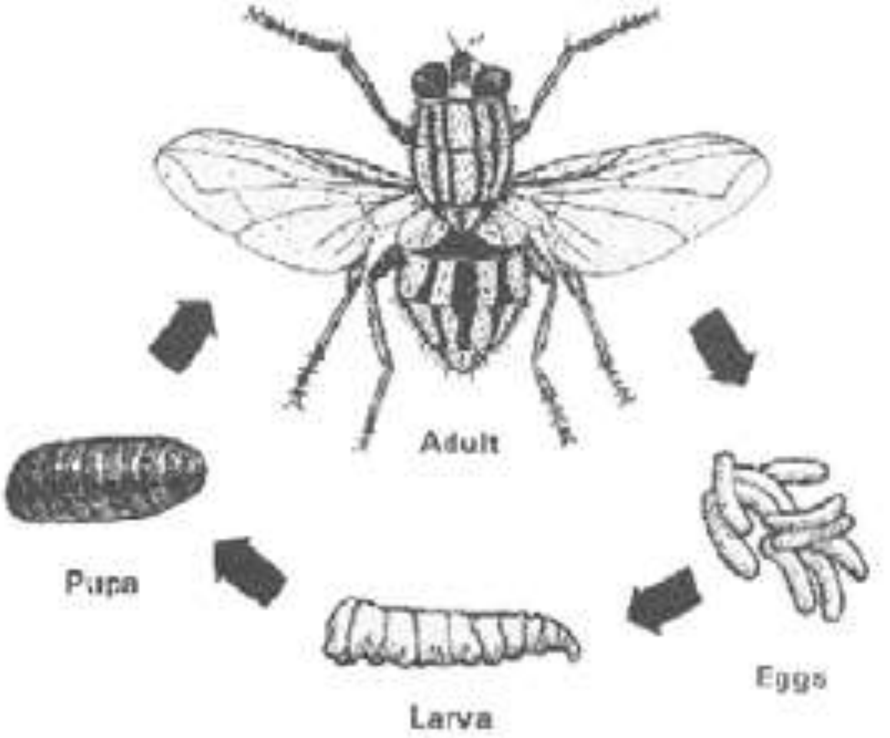
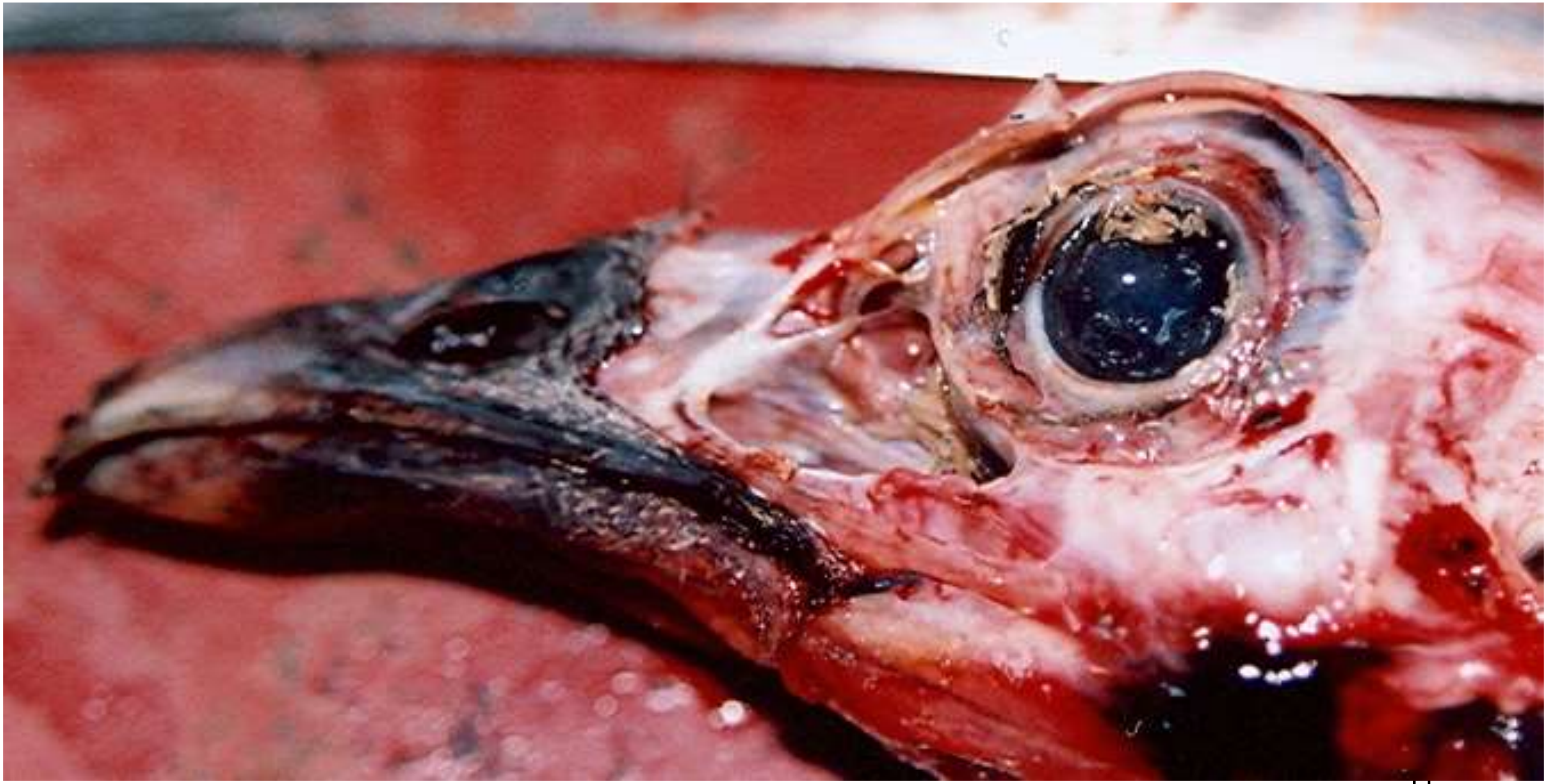


Figure 1. Generalized filth fly life cycle

adult *L illustris* feeding and/or searching for a spot to place eggs


Phormia regina

eggs



eggs

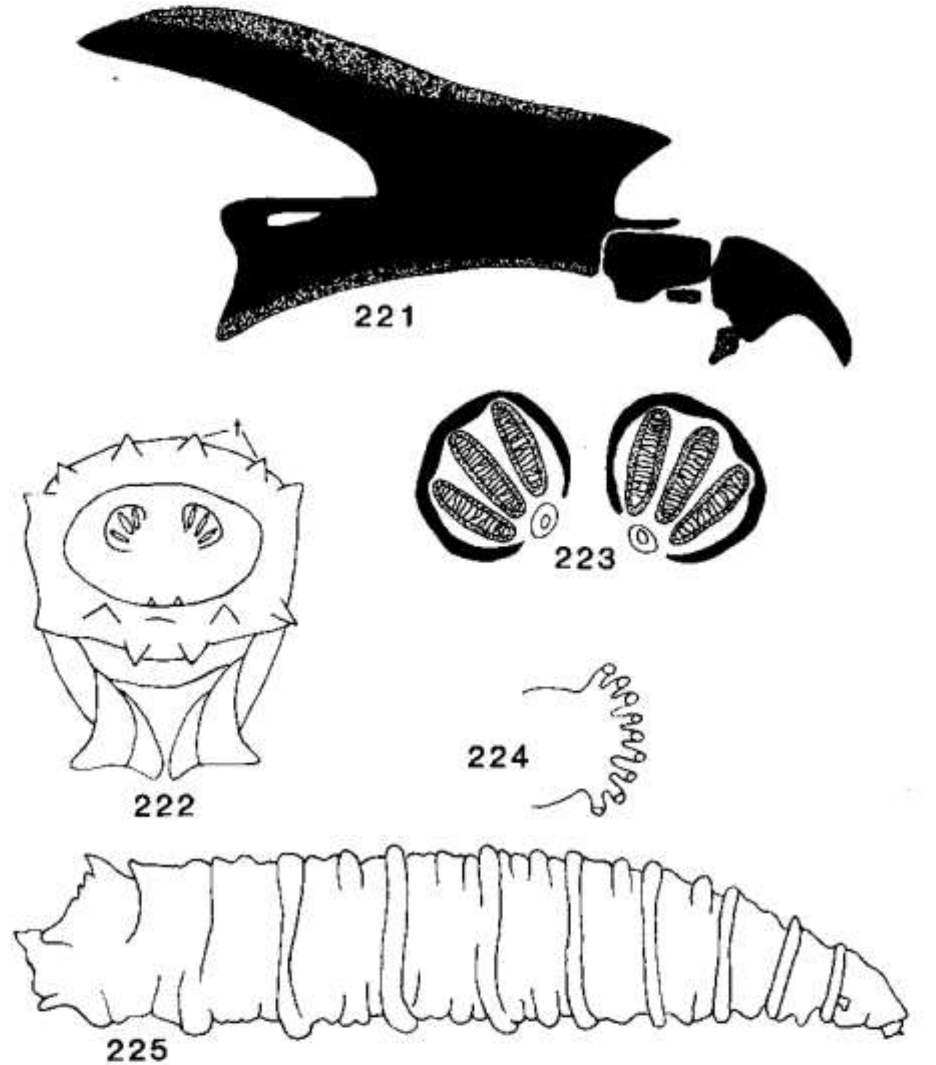
immature stages, the maggots

immature development divided into
- three *instars* or stages
- moult between each stage
- immatures to take advantage of large nutritional packages
- have specialized structures based on habitat

maggots can be hairy, covered in setae (bristles) or smooth

identified by mouthparts and spiracle patterns (breathing tubes)

fannia maggot, latrine fly
www.cmnh.org



Figs 221–225 *Protophormia terraenovae* (Calliphoridae, Diptera), third instar larva. 221, mouthparts, lateral; 222, last segment, end view; 223, posterior spiracle; 224, anterior spiracle; 225, whole larva, lateral.

wandering stage maggots

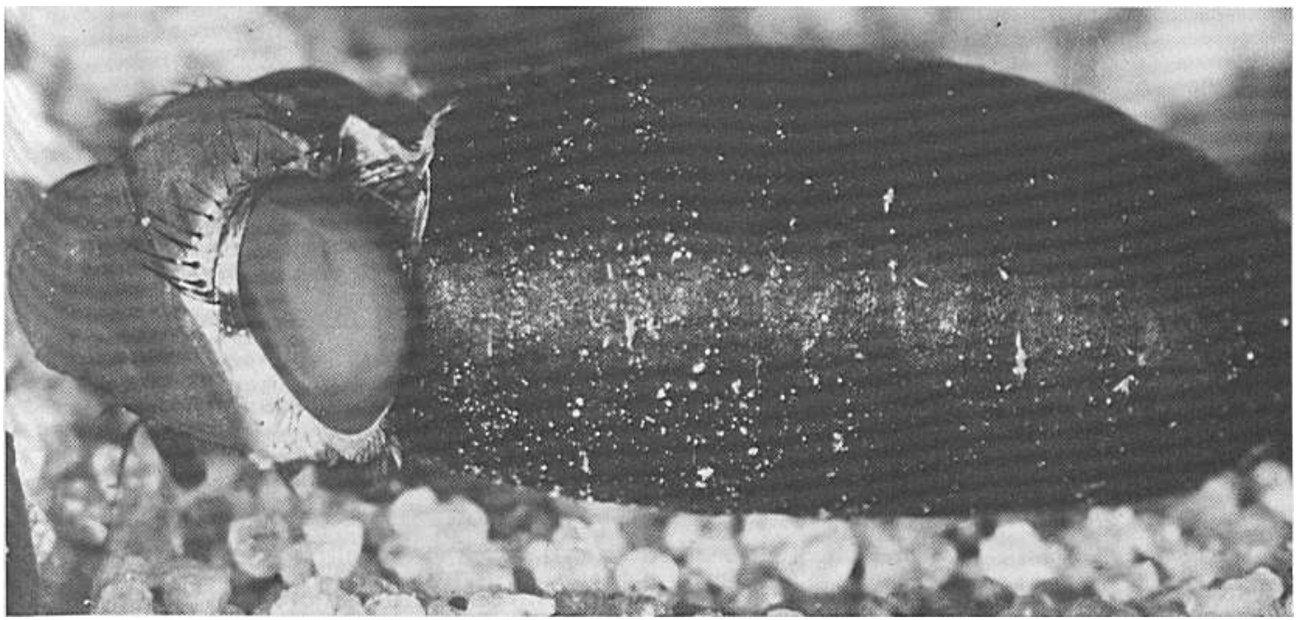
mature maggots crawl away from corpses and bury into the soil

pupae found under corpses, to 3 m away, usually within 1-1.5 m

pupae

adult flies emerge by breaking open pupae with a specialized temporary structure called a ptilinum, swelling on head breaks pupal case

fly emerges, ptilinum shrinks, fly exoskelton harden



flesh fly emerging from pupa

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PAPER

PATHOLOGY/BIOLOGY

Chantal Turpin,[1] B.Sc.; Christopher Kyle,[2] Ph.D.; and David V. Beresford,[3] Ph.D.

Postfeeding Larval Dispersal Behavior of Late Season Blow Flies (Calliphoridae) in Southern Ontario, Canada

place soil in emergence tubs, record hourly temperature

flies (order: Diptera)

most useful order, two wings, hind wings reduced to halteres

primitive flies found on carcasses: crane flies, moth flies, fungus gnats, chironomids (Chironomidae) (moist or aquatic habitats)

advanced flies:

- Calliphoridae (blow flies) first to arrive in cooler climates (here)
- flesh flies (Sarcophagidae, checkered abdomens) first to arrive in warmer regions
- blow flies deposit eggs in folds, eyes, ears etc, flesh flies deposit larvae, same areas
- some blow fly maggots predatory on other maggots
- main role is consuming carrion
- 3 instar stages of maggots, release enzymes to liquify tissue

beetles

beetles (order: Coleoptera):

early stage of decomposition:

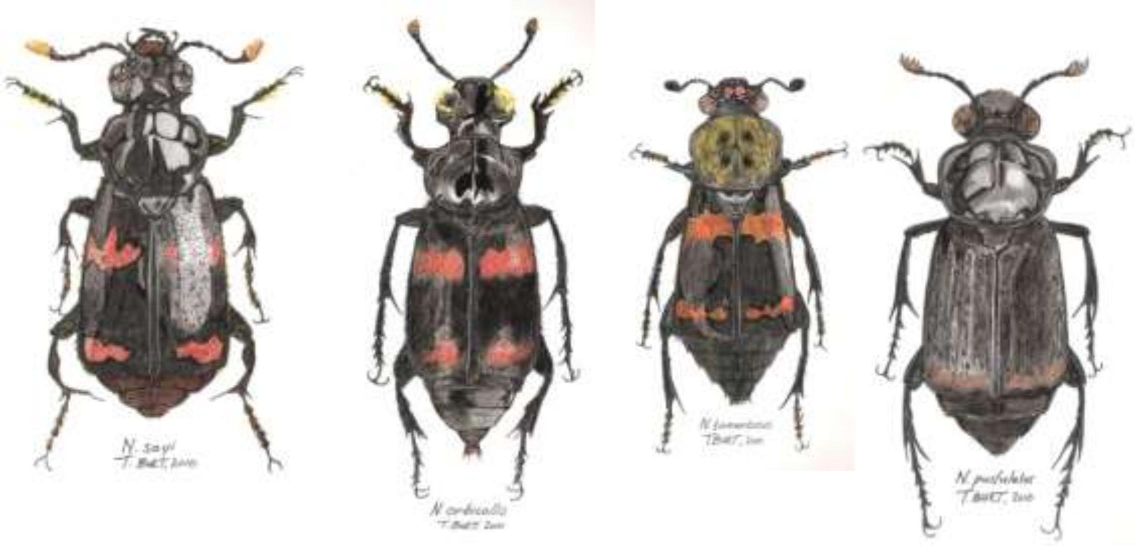
- predatory beetles include Staphilinidae (resemble earwigs), arrive early after death, remain throughout rotting cycle
- scarab beetles often arrive early, commonly after 1-2 days, can be as soon as 15 minutes
- ground beetles (Carabidae) early arrivers, predatory, remain throughout rotting period
- histerid beetles (shiny black robust beetles) arrive in large numbers, feed on maggots, stay throughout decomposition period
- Silphidae (burying beetles)
- arrive early, stay throughout decomposition period, larvae and adults found on corpses
- some authors state that larvae feed on remains and maggots, other authors state that larvae only feed on maggots, adults feed on maggots

mites on hister beetle
Photo Chris Schuster

silphid with mites
http://www.hiltonpond.org/images/BeetleCarrion01.jpg

silphid with grubs
stripe.colorado.edu

The major groups of insects: large carcass beetles, early stages

4 nicrophorines (from left):

Nicrophorus. sayi

Nicrophorus. orbicollis

Nicrophorus tomentosus

Nicrophorus pustulata

four silphines

(bottom row from left):

Necrodes surinamensis

Oxelytrum discicolle

Oxelytrum inaequale

Necrophila americana

paintings by Trevor Burt

beetle development

burying beetle, shows parental care, small carcasses, infants

beetles

middle to late stage of decomposition

- Dermestidae (carpet beetles, museum beetles)
- feed on dried skin, hair, larvae and adults
- common indoor dweller, pest of stored products
- other families also found, but not associated with corpses per se, useful for identifying location
- collection of frass (strung together in links), empty larval casings, etc

dermestid grub
www.entomology.wisc.edu

other insects

www.cals.ncsu.edu

soil insects: the springtails, (order Collembola)

- small <7 mm, jump using a furcula at the end of the abdomen, live in soil all year, (the snow fleas)
- feed on decaying matter (plant and some animal), damp habitats
- damp seepage area underneath decaying corpse is ideal
- species found with corpses associated with damp soil and leaf litter habitats—indicator of top soil, and turf (ie not in dried clay soils or gravel)

silverfish (Thysanura):

- household insect, pest of stored food products
- associated with dried remains, indicative of indoors

entweb.clemson.edu

other insects

cockroaches (order: Blattaria)

- feed on decaying corpses, commonly at later stage remains, not fresh corpses, nocturnal, household pests

earwigs (order: Dermaptera):

- damp habitats, flying insects, predatory
- commonly beneath a corpse as daylight shelter area

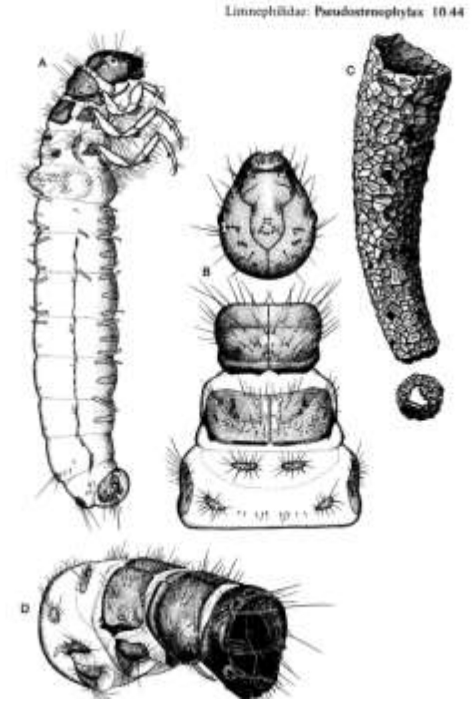
http://news-websites.info/wp-content/uploads/2011/11/Cockroaches1.jpg

agspsrv34.agric.wa.gov.au

other insects

caddisflies (order: Trichoptera):

- immatures live in cases made of sand, twigs, or leaves
- rapid consumer of submerged corpses, omnivorous or carnivorous, consume decaying matter, indicates aquatic habitats, temporary pools, ponds, lakes, streams, or various water quality
- often found in clothing
- aquatic entomologists are required for ID of specimens, specialized aspect of the discipline
- one case, red bloodworms (Chironamidae, midges) from a corpse recovered from a river, first misidentified as carpet fibres
- little work done on this, little baseline data available

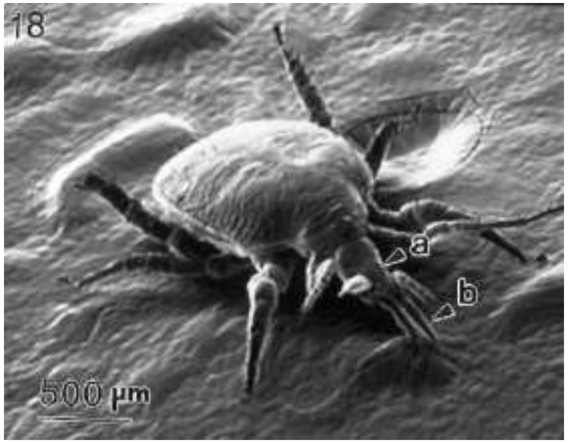


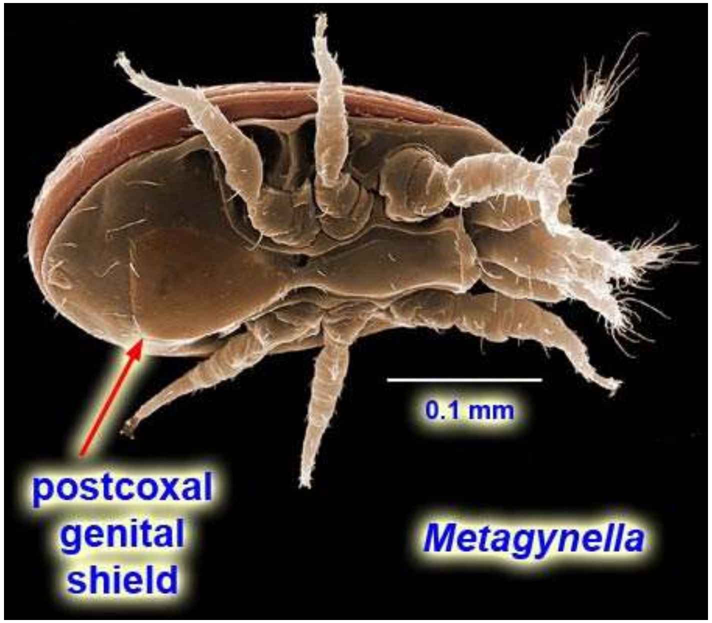
non –insect arthropods:

class Arachnida (the spider like arthropods)

mites (Order Acari):

- small, < 1mm, 1 body part with a small head area attached to body
- adult and nymphal stages different, adults 4 pr legs, nymphs 3 pr legs
- important part of soil community beneath remains, in later stages of decomp.
- 50,000 species described
- estimates of >1,000,000 species in the world





http://keys.lucidcentral.org/keys/v3/mites/Invasive_Mite_Identification/key/Mesostigmata/Media/Html/100Introduction.htm spiders (order: Araneae):

- 8 legs, predatory on insects and other arthropods, corpses are a source of insects prey

pseudoscorpions (order Pseudoscorpionida):

- small, (<5mm) two body parts, abdomen and cephalothorax, pedipalps and mouthparts distinctive, pincher like, called chelate, 8 legs (4 pairs)
- common in late stage decay as predatory on other arthropods
- can disperse by phoresy attached to flies (likely from agricultural areas depending upon species)

Microbisium brunneum, spruce forest

Chelifer cancroides (L.) in house
Peterborough, Ontario (collector: D. Hutchinson
photograph C. Buddle)

sow bugs or pill bugs (order: Isopoda):

- associated with decomposing remains, found at all stages, feed on rotting plant and animal tissue
- possible identifier of location more than time of death

centipedes (class: Chilopoda):

- predatory on other arthropods, some species commonly found indoors, others largely outdoor species

http://www.infestation.ca/insects/centipedes.html

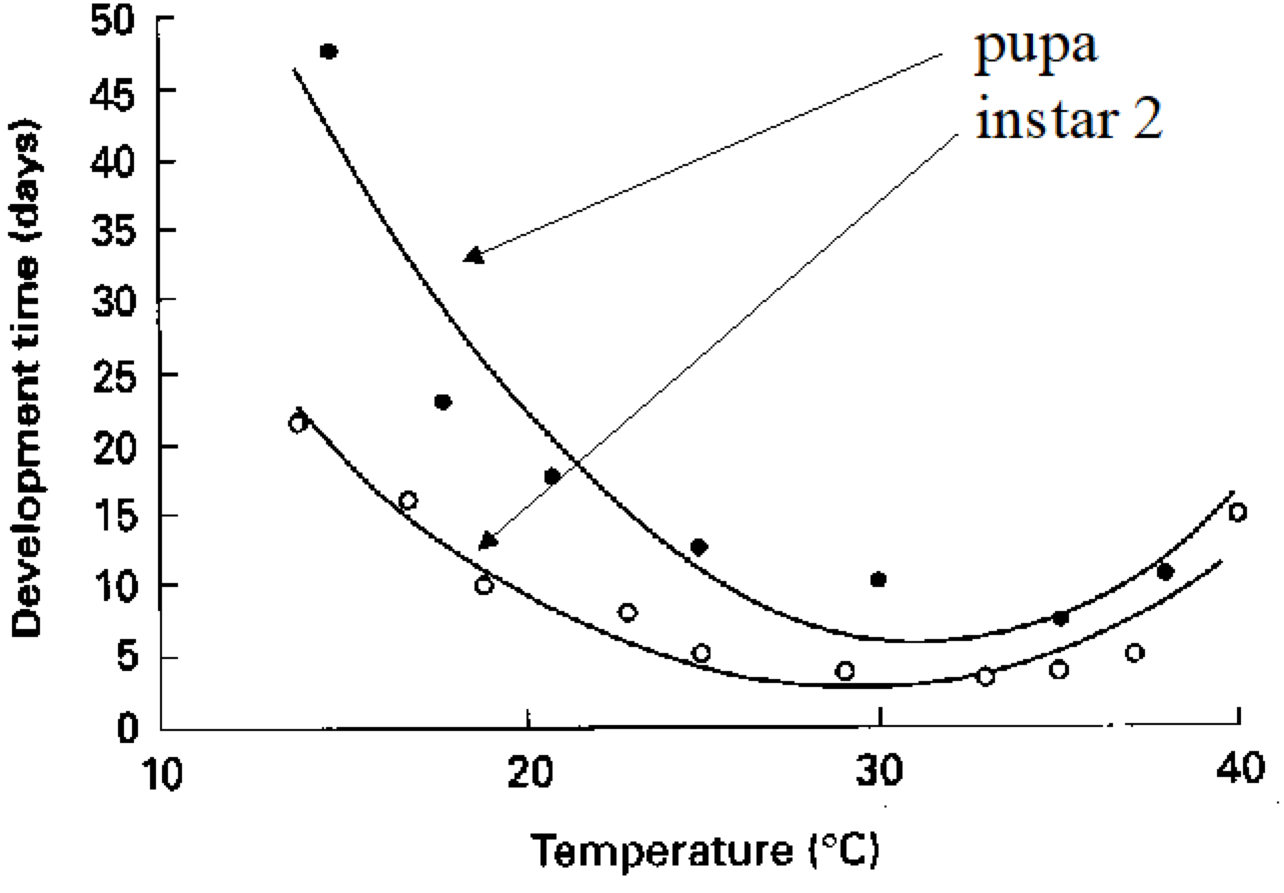
millipedes (class Diplopoda):

- moist habitats, plant feeders but also on decomposing animal tissue, indicator of habitat

http://torontogreen.ca/blog/?p=266

development times

- poikilotherms or ectotherms development linked to temperature
- definition: one degree day = one ^{o}C over 24 hours measures amount of heat over time
- basic idea, think of a car with windows up gathering heat: gets just as hot after 2 hours at 10 ^{o}C as 1 hour at 20 ^{o}C.
- eg typical insect (weevil, Smith and Ward 1995))

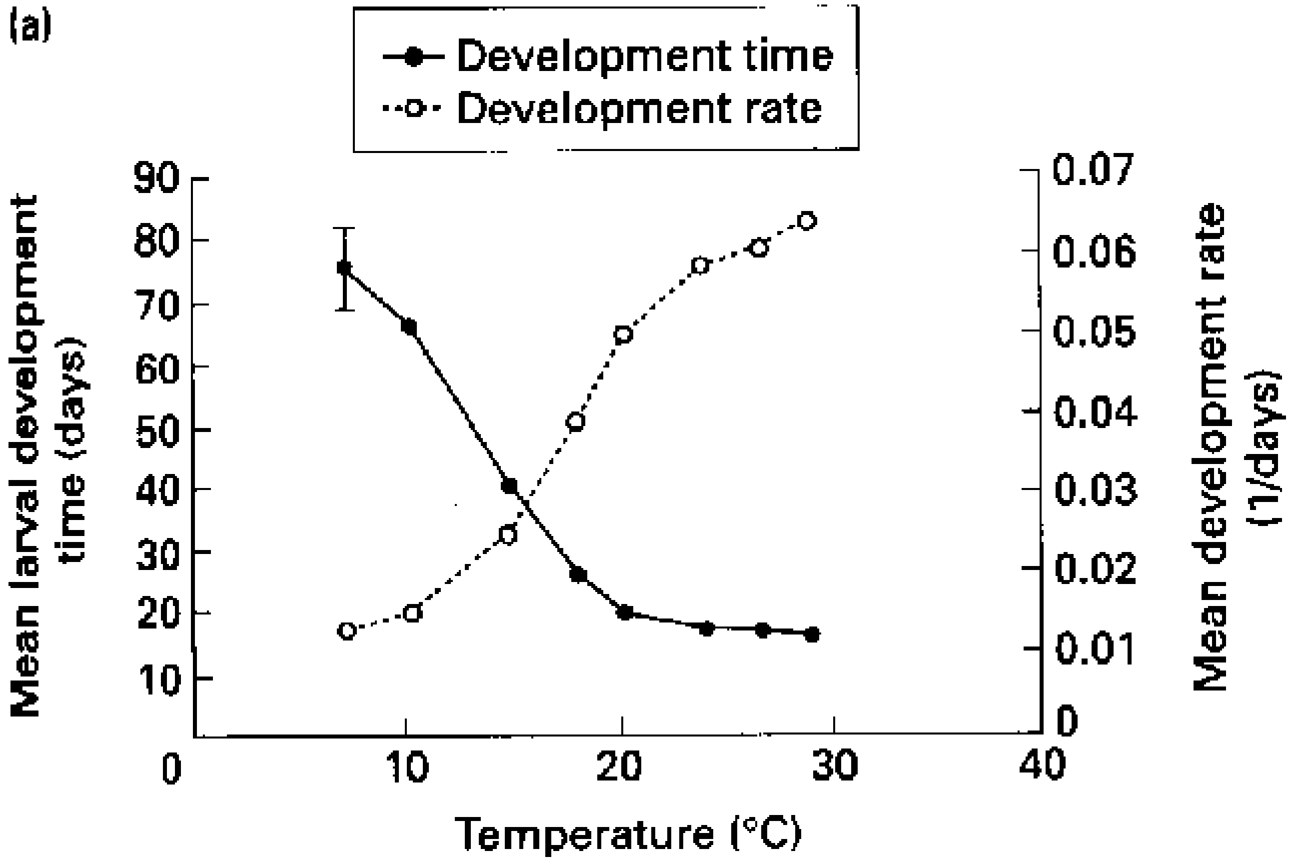


degree days or day degrees (both are used)

(see http://www.ipm.ucdavis.edu/WEATHER/ddds_tbl.html)

symbolized as DD_{temp}, ADH_{temp}, ${}_{temp}ADD$ dd_{temp} , $^{o}D_{temp}$ etc. where *temp* is a threshold below which development does not occur

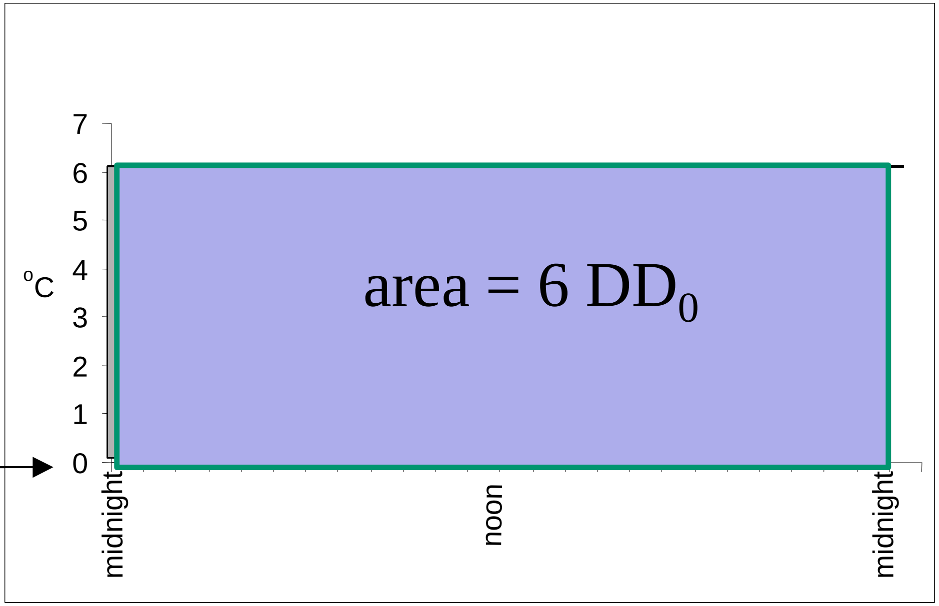
usually called accumulated degree hours (ADH) or accumulated degree days (ADD) by forensic entomologists, (heat units in agriculture eg corn growing days)



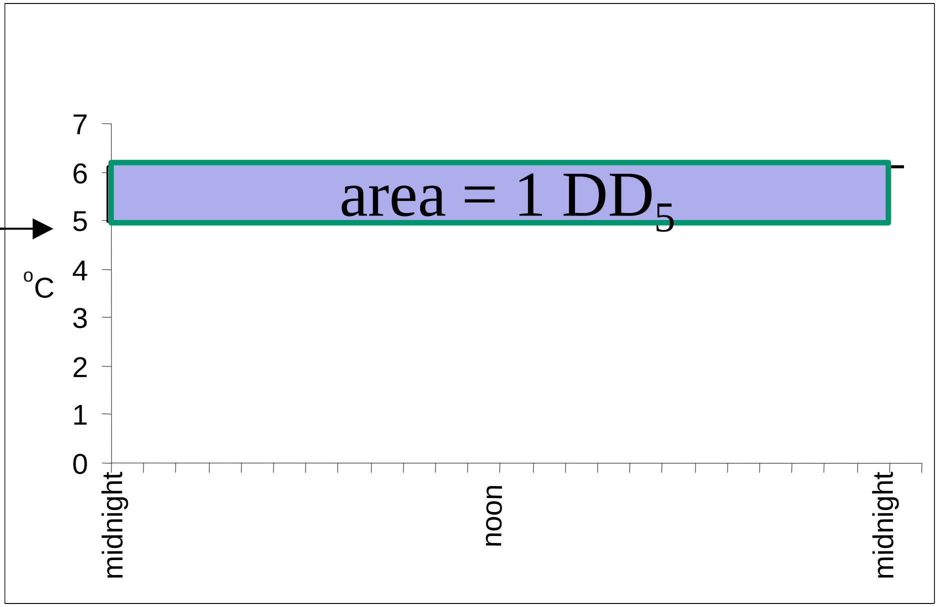
examples

- if an insect with a developmental threshold of 6 C is held for 48 hours at 8 C, it has been held for 4 degree days (written as $4DD_6$)
- if an egg requires 18 degree days > 10 C before hatching this will take:
  - 9 days at 12 C
  - 6 days at 13 C
  - 2 days at 19 C
  - or 1 day at 28 C

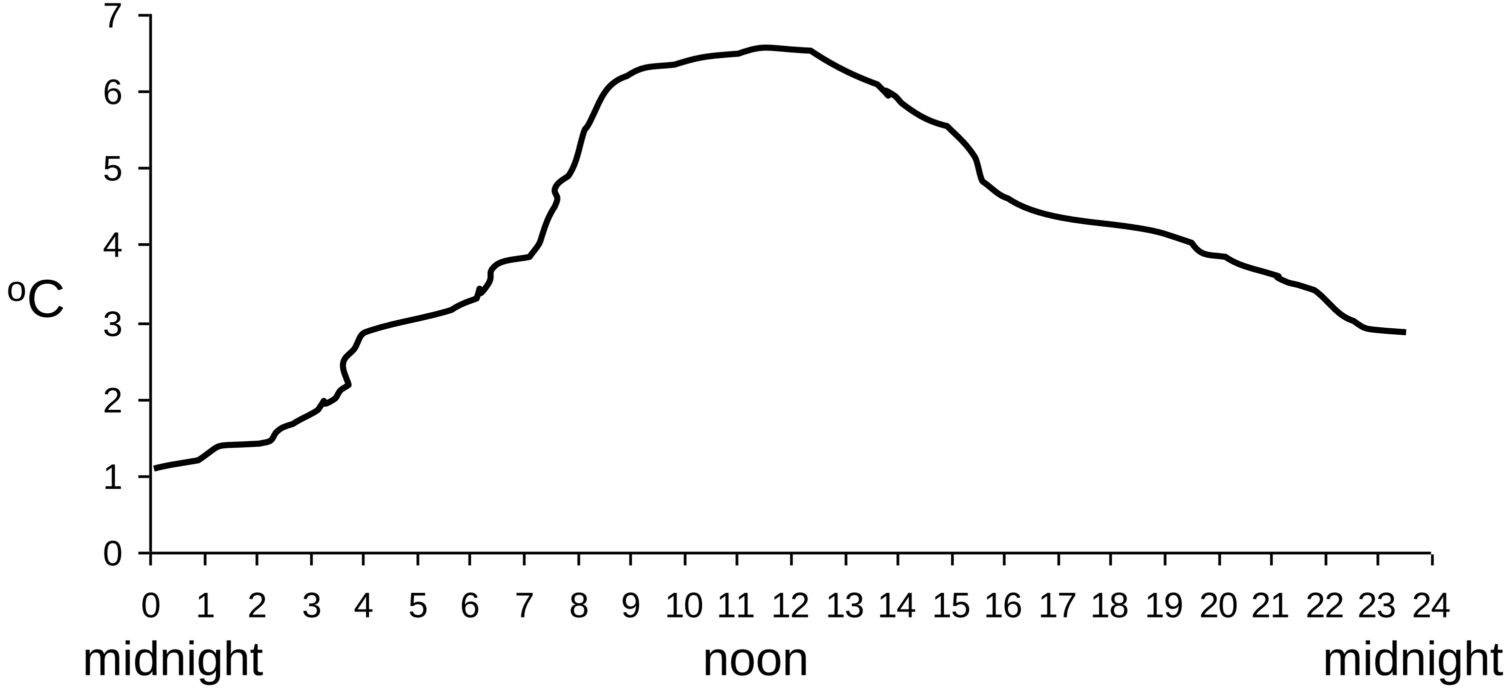
developmental
threshold



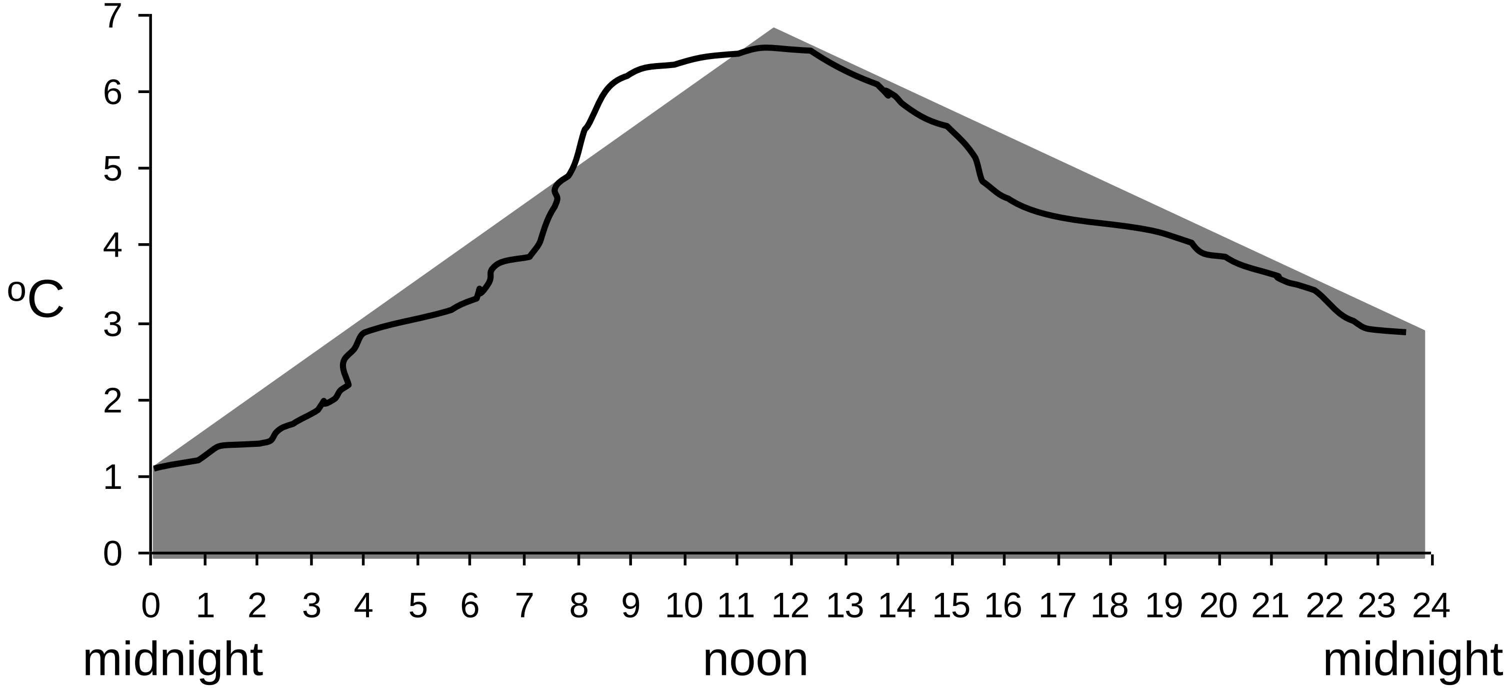
developmental
threshold

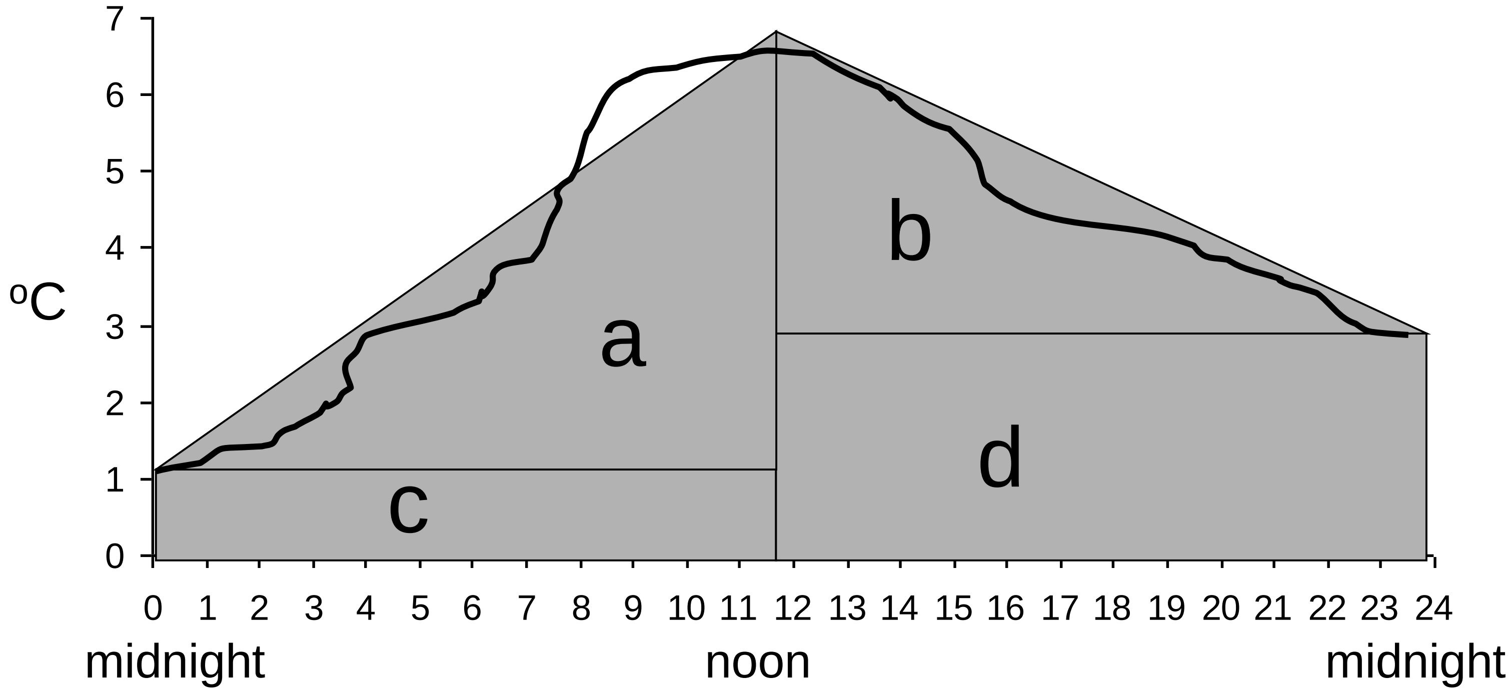


actual daily temperatures:
DD still based on area under the curve, but the curve is more difficult to manage



method 1: simplify curve as a triangle (this is fine, works well)





triangle area = ½ (base x height)

add areas of a, b, c, d to obtain total

a   ½ (0.5 x 6) = 1.5

b   ½ (0.5 x 4) = 1

c   1 x 0.5 = 0.5

d   3 x 0.5 = 1.5

total DD_0 = 4.5

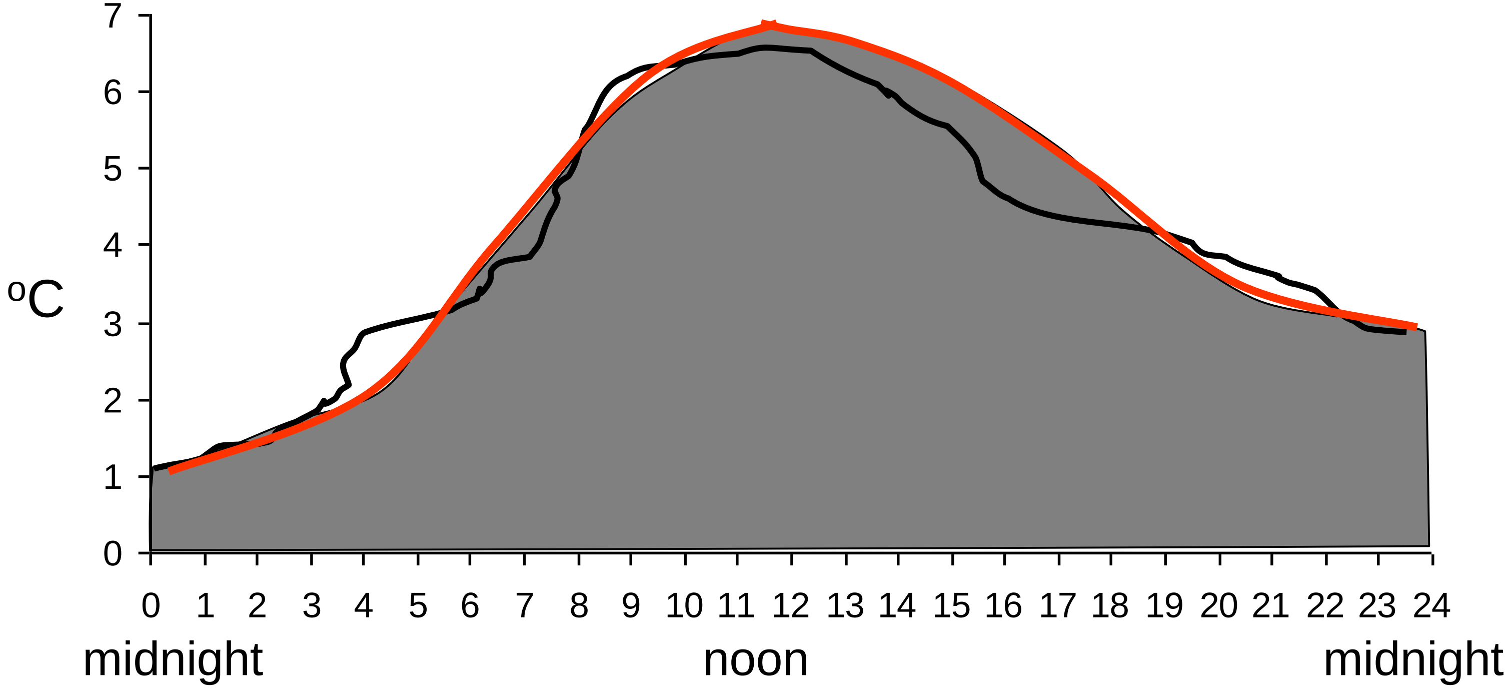
method 2: sine wave method

integrate are under a sine curve with amplitude defined by max and min, and wave length defined as 12 hours or 0.5 days

classic references:

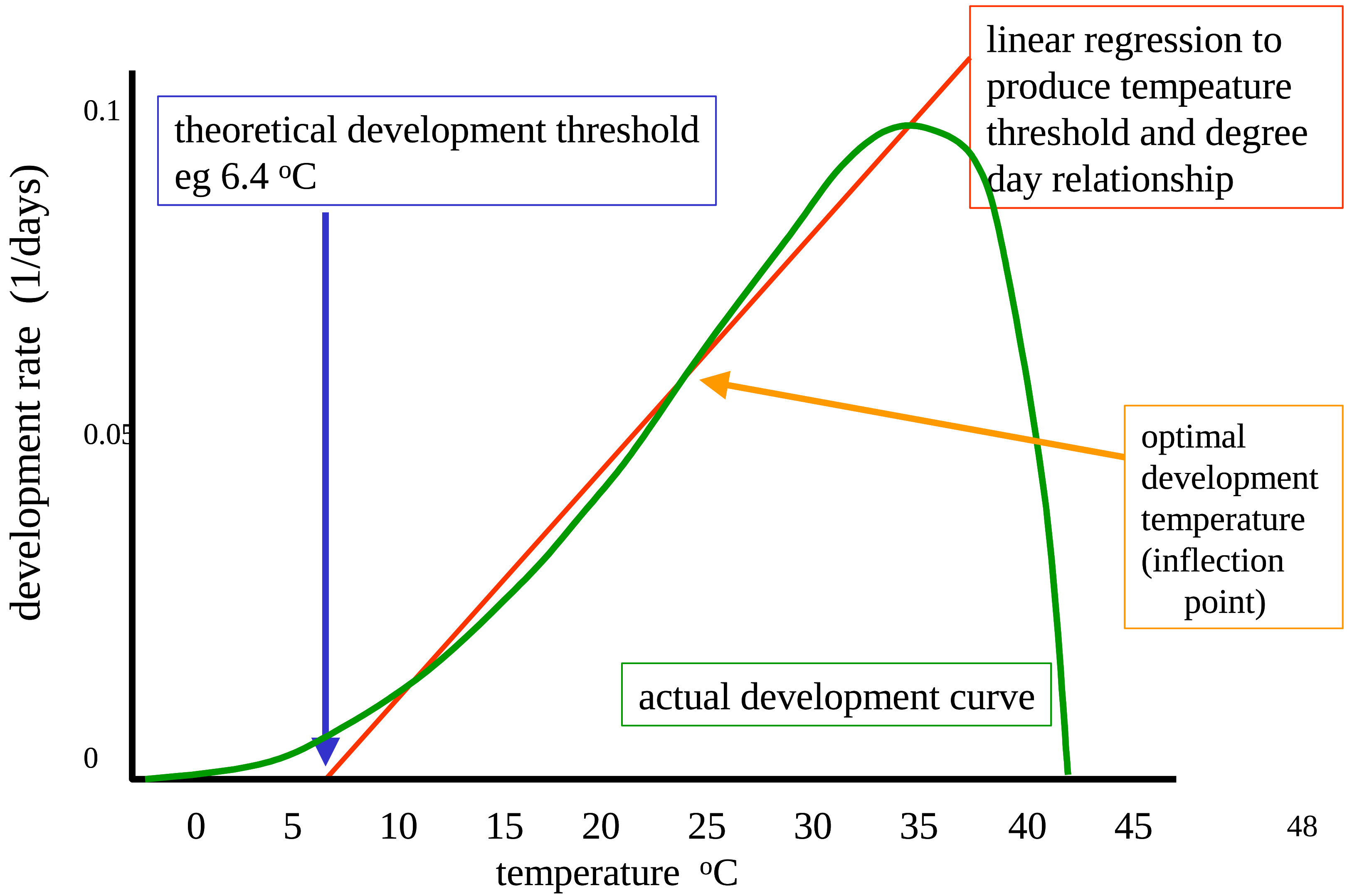
Allen, J. C. 1976. A modified sine wave method for calculating degree days. Environmental Entomology 5:388-396.

Baskerville, G. L., P. Emin. 1969. Rapid estimation of heat accumulation from maximum and minimum temperatures. Ecology 50:514-17.



development assumed to be linear with respect to temperature across a range of temperatures typical of the habitat

this assumption is embodied in the degree-day approach to measuring developmental time

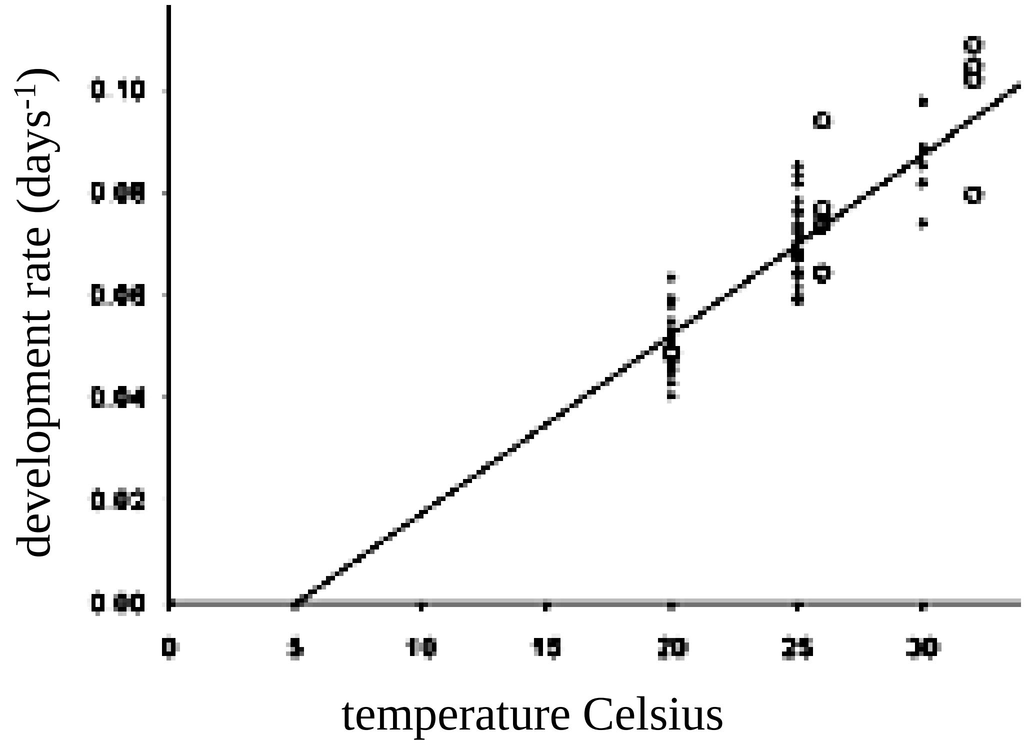


example

Phormia regina

grown at different temperatures, and then plotted against developmental rate to get threshold temp of 5.4 °C

from NABITY ET AL.: *P. regina* DEVELOPMENT USED IN FORENSIC ENTOMOLOGY



developmental rates can be modelled:

note error bars, and decrease in maggot length

- an 11 mm maggot could be 9 days (216 hours) to 15 days (360 hours) old.

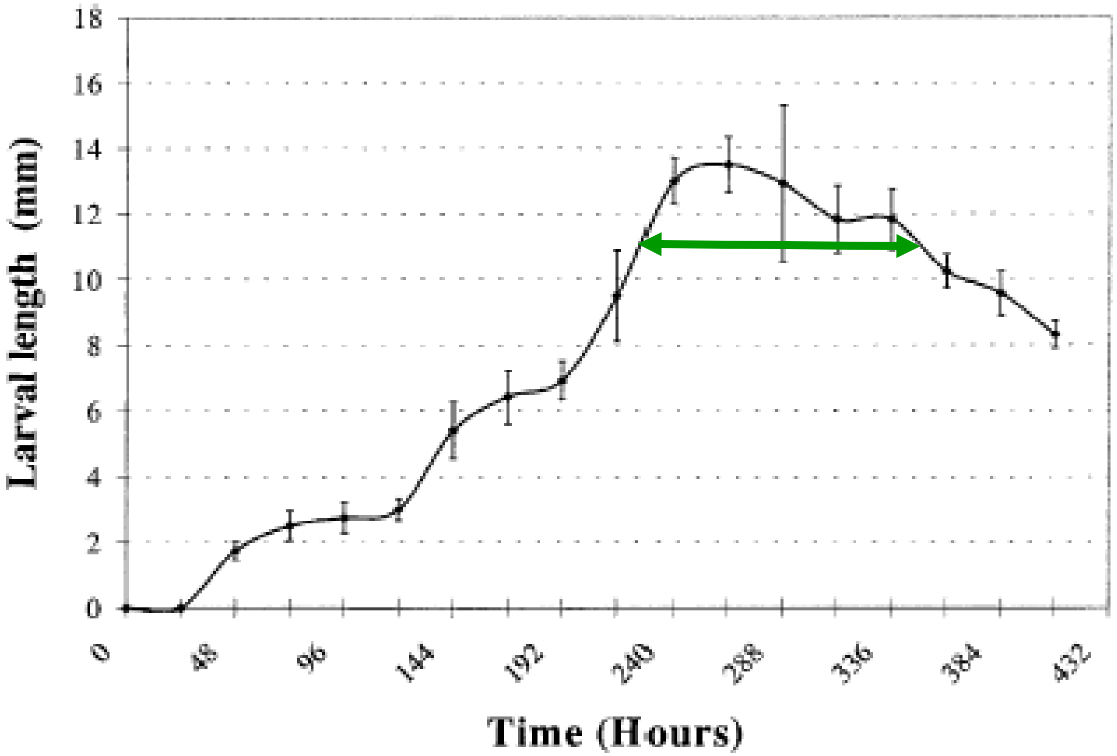
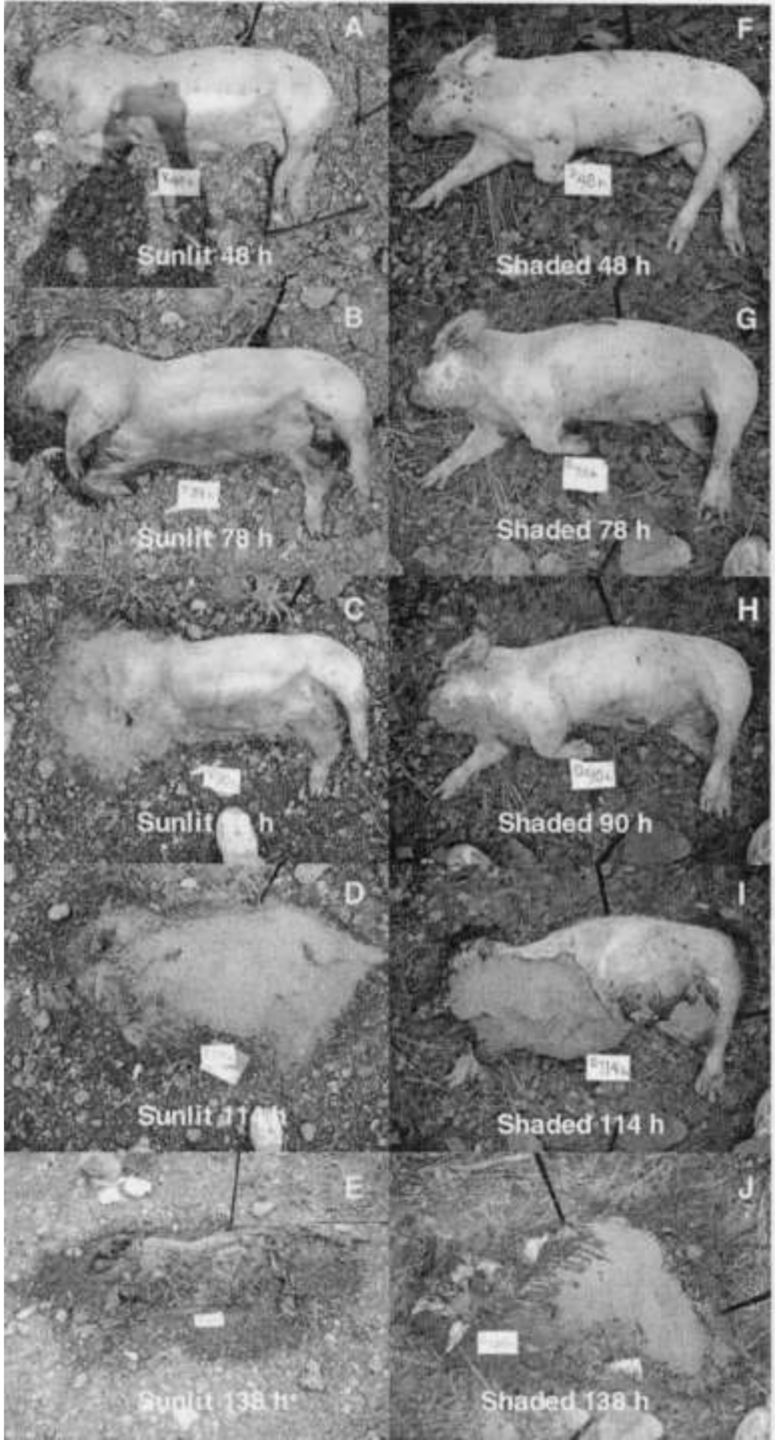


Fig. 8. Larval growth of *Phormia regina* under cyclic temperature regime 15–25°C (±1°C).

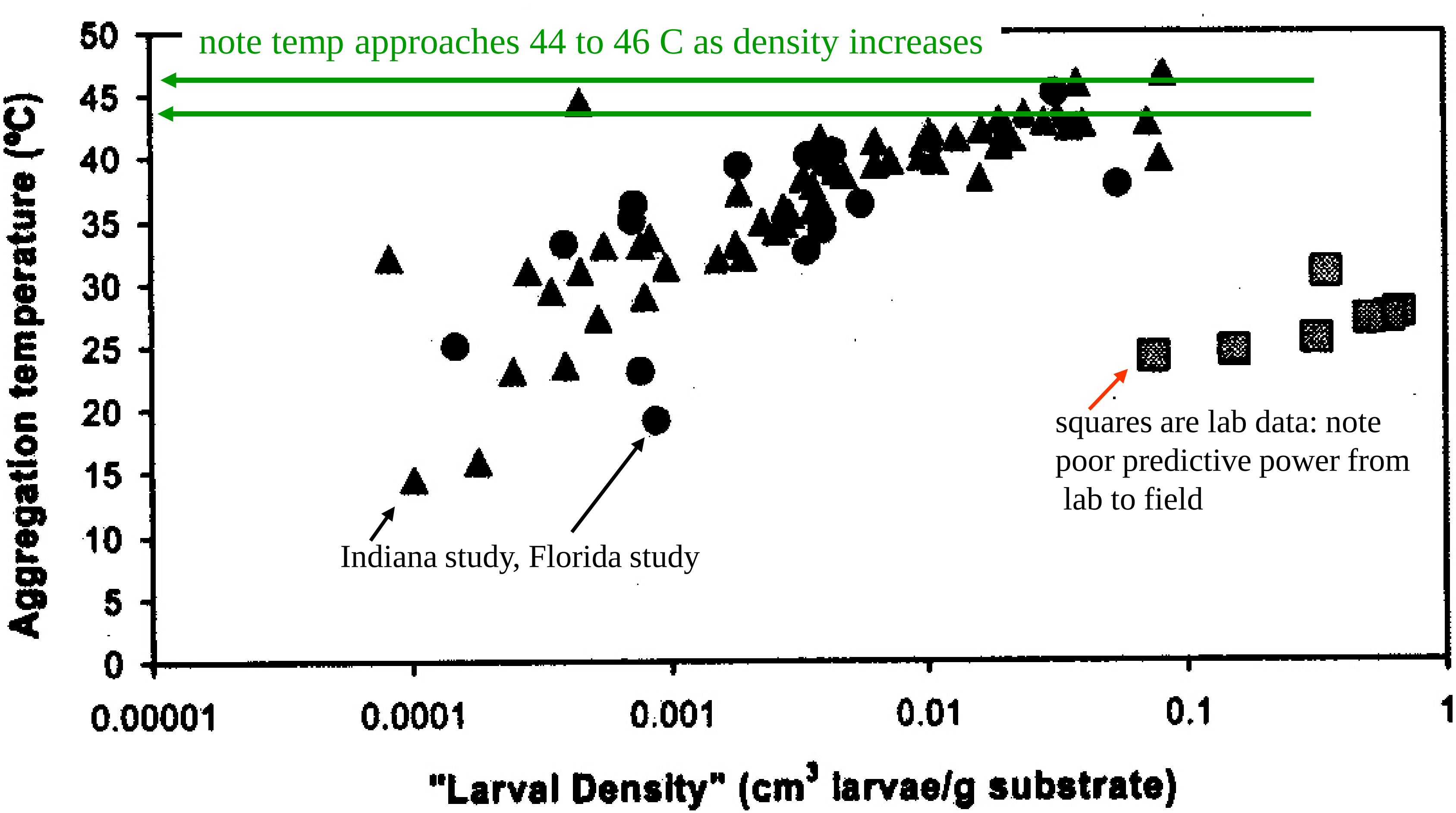
total AHD during 10 day experimental period:

- shaded = 4891 or 489.1/day or 20.4 DD0
- sunlit = 5155 or 515.5/day or 21.5 DD0
- no difference in maggot mass temps between sites!
- yet more rapid decay in sunlit than shaded site, almost twice as fast
- *P regina* arrived after 84 hours at both sites
- 3rd instars at 48 hrs[sun], 60 hrs [shade]
- *Lucilia coelruleiviridis* arrived 84 hours both sites, 3rd instars 48 hrs[sun], 60 hrs [shade]
- sun: *L. c.* 3rd instars most common up to 132 hrs, *P. r.* after 132 hours
- shaded, similar pattern but less obvious

- *P. regina* is considered a cool weather and cool climate species, but is prevalent in mean daily temps of 24 C



temperature of maggot mass is related to maggot density, high density produces constant temperatures higher than air temperature




pig after 3 weeks summer

mummified beaver after 5 weeks summer

coyote, 6 weeks, summer

coyote, 10 weeks, summer, bones scattered

clothes moth typical of mummified remains (stored product pests)

basics of insect searching behaviour

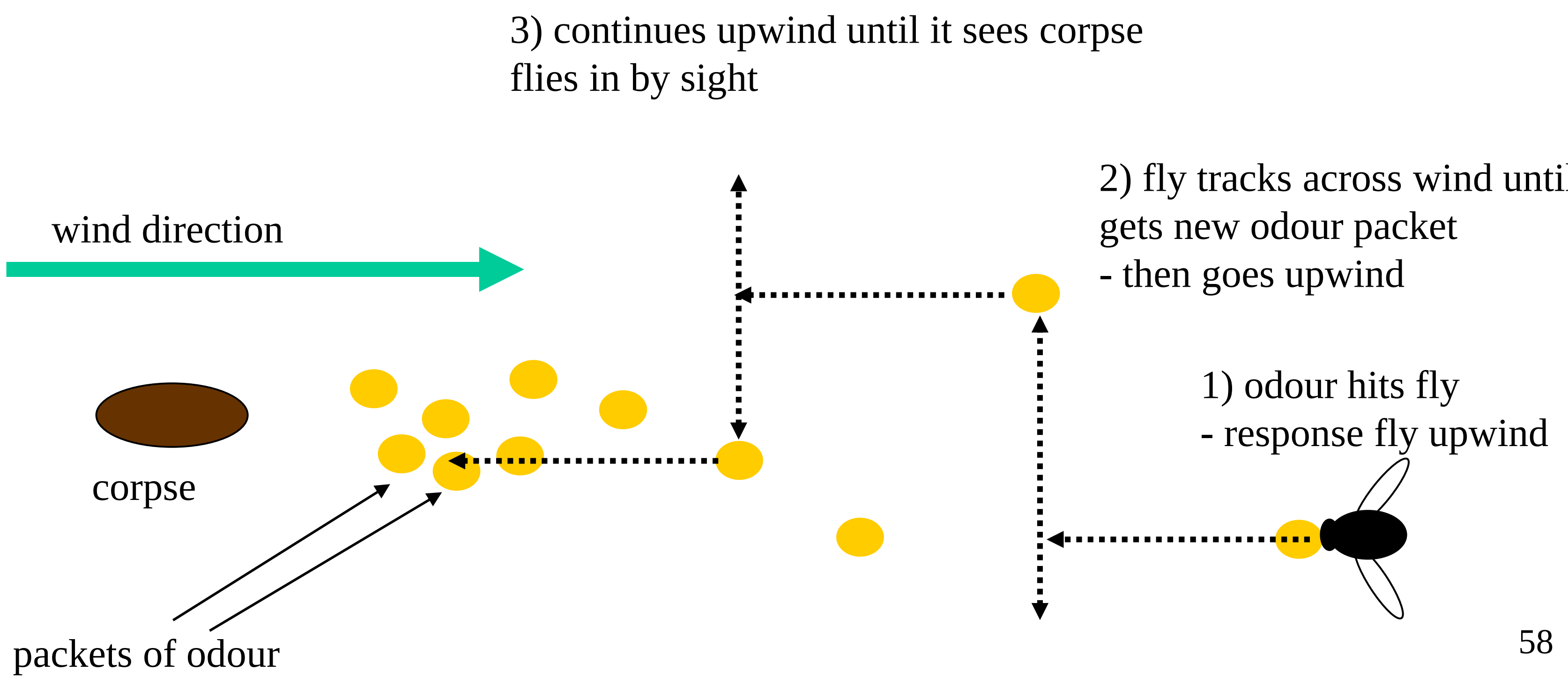
each fly species is distinct, has distinct behaviour:

Calliphora vicina and *Phormia regina* as different as a cougar from a bobcat, or a blue jay and a crow – cannot generalize from one species of fly to another

need for surveys of extant species:

1) geographic variation of species composition
2) seasonal difference in species composition

surveys are often based on trapping flies in a region—need to understand trap response of flies



Flight in the dark (Wooldridge et al. For. Sci. Int. 2007)

generally: calliphorids do not fly in the dark, or oviposit

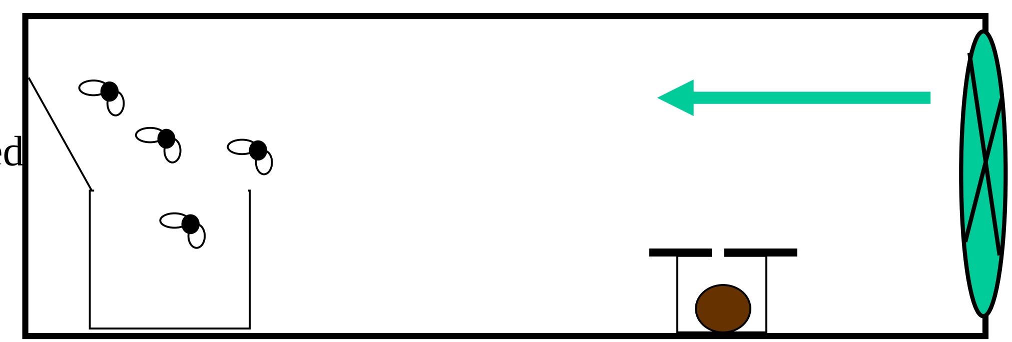
recall study, some oviposition at night, whether crawling flying

caged *Calliphora vomitoria* (rural blue bottle fly) and *Lucilia sericata* (green bottle) held in large cages

wind tunnel experiment, caught on sticky panels, number based on light intensity, 30 flies per experiment, 3 hour sessions

Results:

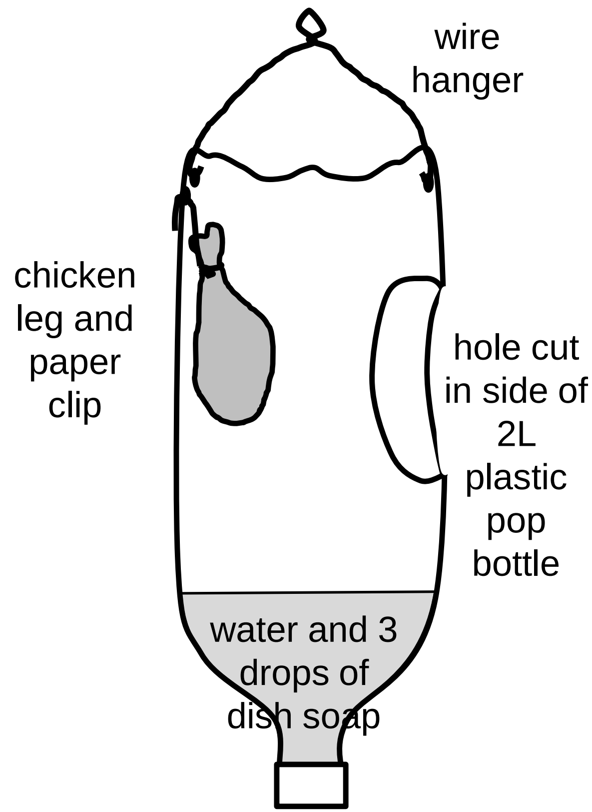
	number of flies (and %) (range is baited and unbaited samples)	
	L. sericata	*C. vomitoria*
full illumination (345 $W.m^2$)	10-15 [33%-50%]	7–14 [23%-47%]
32% illumination (110 $W.m^2$)	12-20 [40%-67%]	7-10 [23%-33%]
12% illumination (40 $W.m^2$)	6 [20%]	3-8 [10%-27%]
0 illumination (0 $W.m^2$)	4-7 [13%-23%]	2-5 [7%-17%]



NB

- total dark, still some flight, even with no bait
- night time is not total darkness, street lights, moon provide light: therefore oviposition and flight occur at night, with increased likelihood if some light present→ adds to variability of PMI estimates
- observations in wind tunnel→ not less flies *but* less able to find baited station in dark

sampling blow flies



hang up away from cats or dogs, to get the catch, open the pop bottle lid and drain into a bowl or pail

Studies on the Effectiveness of Coroplast Sticky Traps for Sampling Stable Flies (Diptera: Muscidae), Including a Comparison to Alsynite

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Department of Biology, Trent University, 1600 West Bank Drive, Peterborough Ontario, Canada K9J 7B8

J. Econ. Entomol. 99(3): 1025–1035 (2006)

More biases: removing flies caught on sticky traps
Alsynite traps, flies counted each hour
Coroplast traps, counted each hour, and counted each hour and then removed

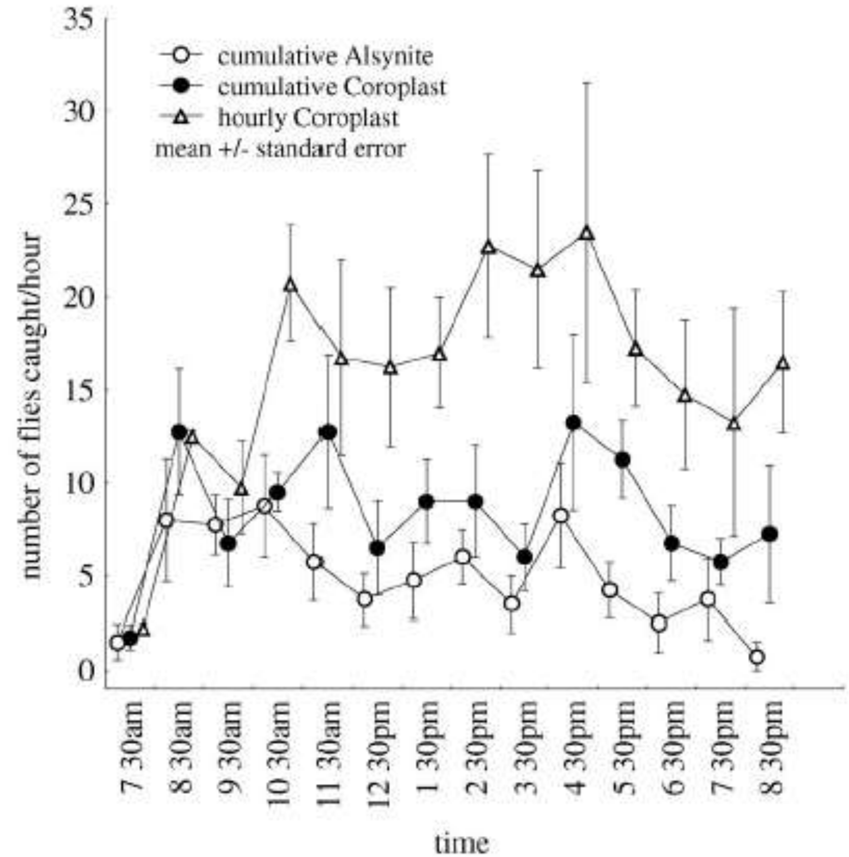


Fig. 3. Means and standard errors of the number of stable flies caught each hour on Coroplast and Alsynite traps. On the hourly Coroplast trap, all stable flies caught were removed after counting every hour; on the cumulative traps (open and closed circles), the stable flies caught during previous hours were allowed to remain on the traps.

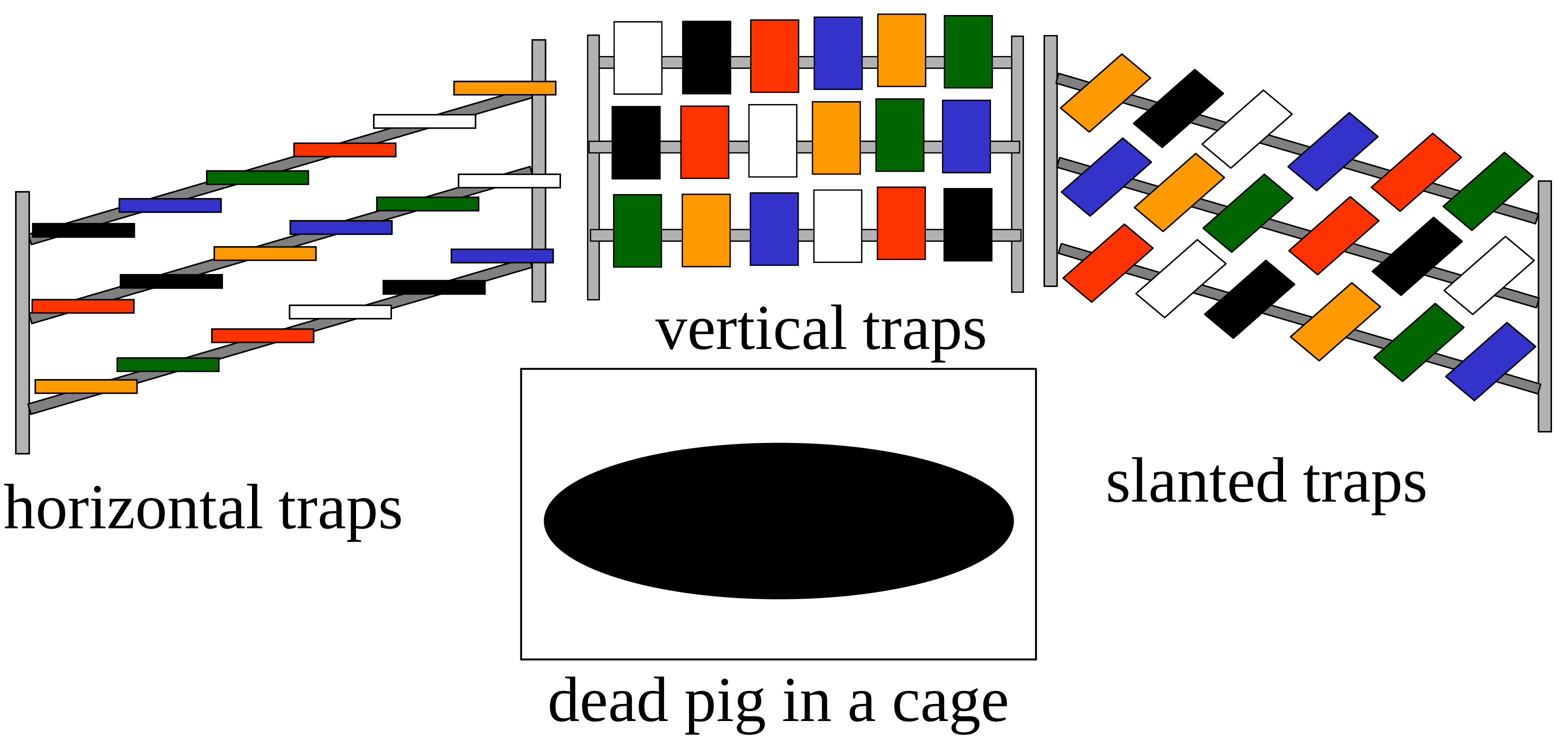
Can. Soc. Forensic Sci. J. Vol. 43. No 4 (2010) pp. 181–190

response to trap orientation, colour, and height

ARTICLES

SAMPLING ADULT BLOW FLIES (*Diptera: Calliphoridae*) AT PIG CARCASSES WITH STICKY TRAPS: EFFECTS OF TRAP COLOUR, HEIGHT, AND INCLINATION

VICKY BILANIUK[1] AND DAVID V. BERESFORD[1,2]



trap heights
30 cm apart, and 30 cm apart on each cross beam
Latin squares design, 6 trials, one day each, 6 different configuration of traps and orientations

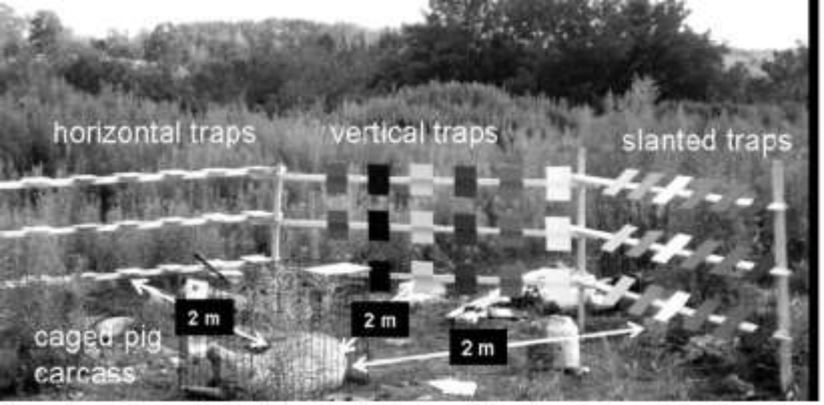


Figure 1. Photograph taken July 22, 2008, showing the location of the wooden hurdles and trap placement around the study carcass, Trent University campus.

results:

similarity based on colour height, orientation

expect different types of traps needed for these three groups

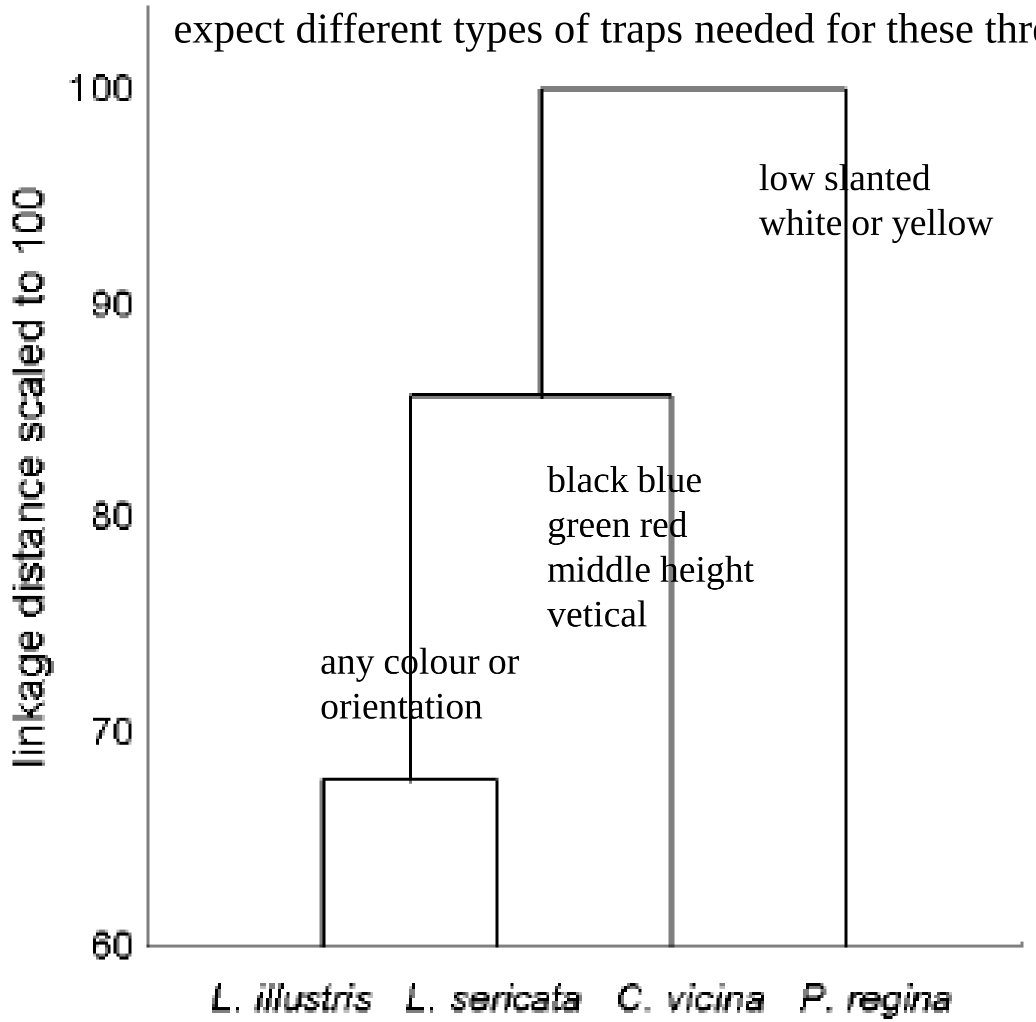


Figure 2. Cluster analysis showing the similarity of trap response between the four relevant Calliphoridae species caught. Linkage distances (scaled to 100) were obtained from unweighted pair-group averages of Euclidean distances, based on the number of flies caught by each sampling configuration in all experiments (n = 54, 3 angles × 3 heights × 6 colours).

collecting data, immature flies

Place maggots in hot water, (just under boil, we are not trying to poach them) until dead, (10-20 sec)

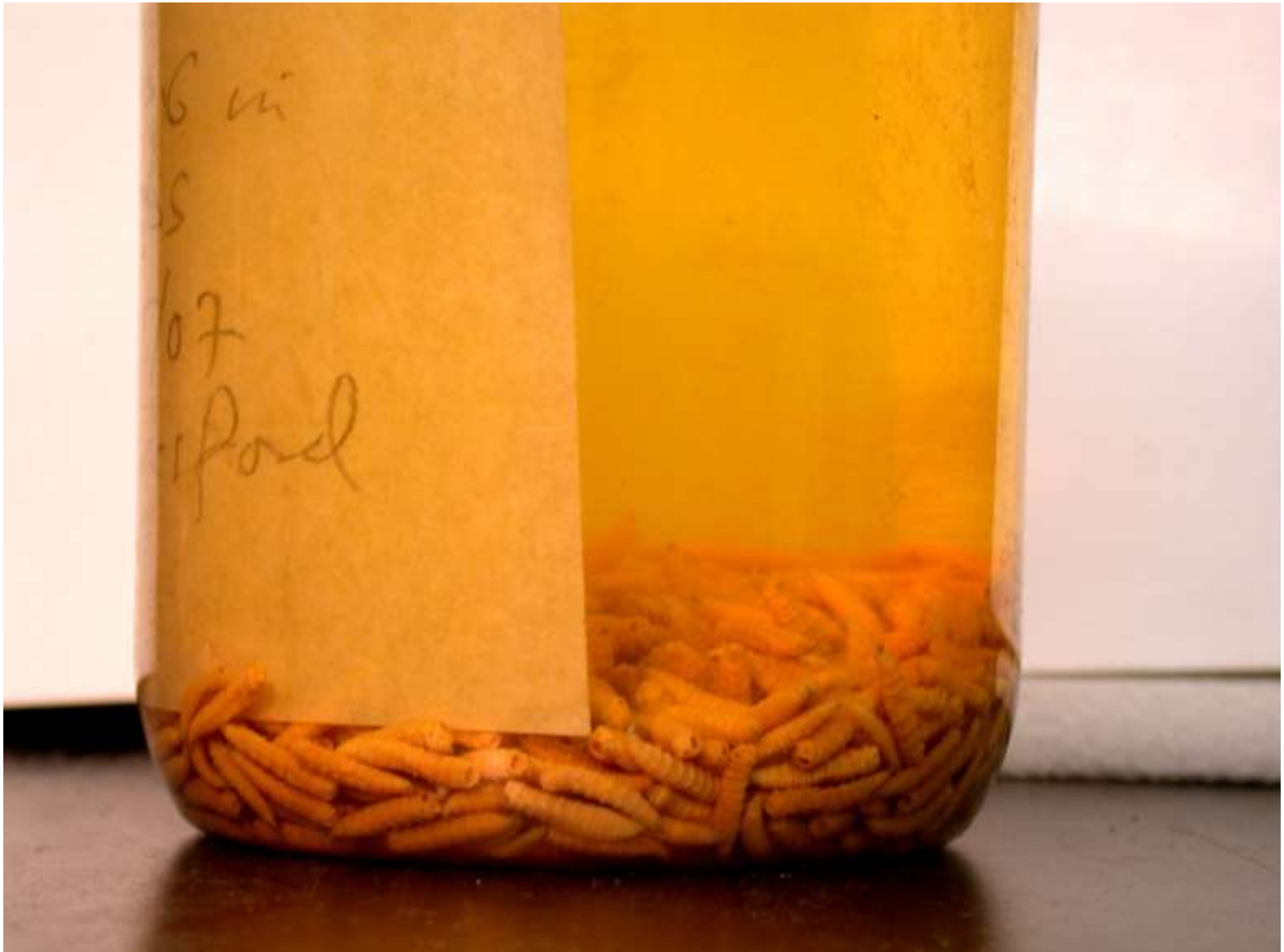
Hot water can be brought into the field in a thermos, or produced with a Kelly kettle or camp stove.

A tin of water and some canned heat (Sterno) will work well.

Collect maggots, and place in a strainer, dip into how water for a few seconds until they are quiescent (and likely dead). Place maggots into 70 to 80% ethanol. Record the length and width of the longest maggots by photographing a group with a scale, and the date, time, and location recorded.

Transferring live maggots will not allow fluid to reach interior of maggots, their gut contents will rot and they will turn brown

transfer maggots to 70% or 80% ethanol (160 proof rum),



collecting pupae

Shortly after the maggots reach third instar stage, just after wondering stage has been reached

dig daily soil samples (3 – 5) from near the carcass

Samples should be a core 2 inches in diameter and 5 inches deep.

collect any pupae you find

rear pupae in sand in a rearing container (a Mason's canning jar with screen in the lid, or a plastic jar with cloth mesh held on with an elastic)

record how long they take to emerge as adults

preserve other pupae in 70% to 80% ethanol

maggot wandering

collection of soil around site

sampling insects at the scene of the crime

materials: rubber gloves and protective clothing

forceps, potting trowel, artist's paint brush, for collecting maggots

aspirator or pooter, bulb type or blowing types are best, not mouth-sucking type, and not to be used on corpse itself

nets, aerial or water, depending upon location

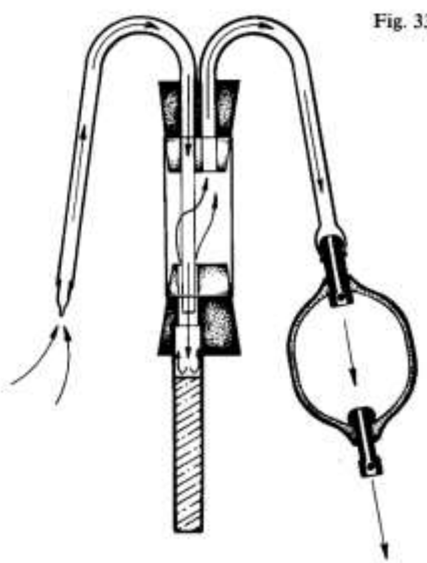
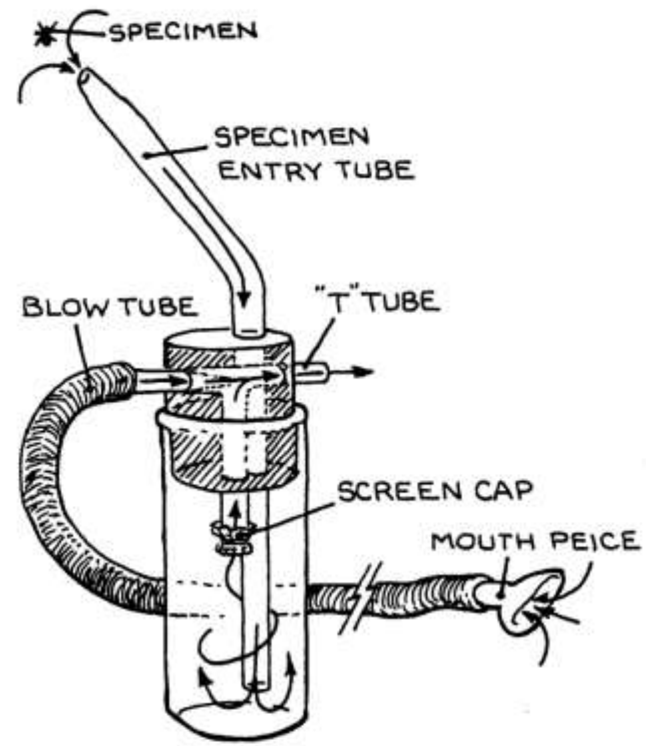


Fig. 33. An aspirator.

Fig. 31.



7.5
BLOW-TYPE ASPIRATOR
(CUTAWAY DIAGRAM)

killing adults is done with ethyl acetate in a plastic container, ideally a tube with a cork for one-handed use, or plastic bottle (be careful, ethyl acetate can dissolve some plastics).

Soak cotton or cloth in ethyl acetate and put in with insect for an hour.

Ethyl acetate is just cheap nail-polish remover

other killing agents are dangerous (ie old books recommend cyanide, bad idea) store adults in 79-80% ethanol or ethyl alcohol, denatured (ie with some trace methanol added) is ok, with labels in pencil in the bottle—alcohol causes ink to run.

pinning is only used for reference collections, not necessarily needed for identification of collections from corpses

it is labour intensive

Protophormia terranovae (James Bay)	*Phormia regina* (Trent Univ)
Calliphora vomitoria, (Warsaw Ont)	*Sarcophagidae* (Warsaw Ont)

at the site, [from E. Paul Catts and Neal H. Haskell. 1990. Entomology and death: a procedural guide. Joyce's Print Shop, Inc. Clemson, South Carolina.]

- do not touch the corpse, entomological evidence is only one aspect of the investigation, do not ruin other evidence at the site
- once permission is granted to collect from official in charge proceed with

1. visual data collection, notations of the site
2. begin collecting climate data at site
3. collect specimens from body
4. collect specimens from area beneath and surrounding body

Disappearing evidence: note that winged insects will leave in the presence of the researcher, you wills scare the flies away if you approach the corpse

Note

- the number and kind of flying or crawling insects
- location of large infestations
- insect predators, ants, beetles, wasps, bees
- exact position of body, compass directions of main axis etc, sunlight and shaded portions, which orifices are exposed or covered
- insect activity in 20 foot radius of body, ie maggot migration, pupae, adults, larvae, adult blow flies will be attracted to areas where there is blood on the ground
- take lots of photographs

Climate data collection

Note temperature, sunlight, aspect, geographic description of site

Need to collect temperature and relative humidity at site

1) Ambient air temp at 1 and 4 foot heights near body (30 and 130 cm)
2) Ground surface temperature required slightly above surface ground cover
3) Body surface temp on skin surface
4) Maggot mass temp, insert end of thermometer into middle of maggot mass
5) Soil temp under body as soon as body is removed, and 3 to 6 feet from body, and at 10 and 20 cm depths

Always shade thermometer from sunlight when taking temperatures

Note when and how much sun, scattered sun, and shade during the day.

RH obtained using we and dry bulb hydrometer or psychometer, these allow estimate of dew wetting: even with dry conditions, a body may be wet from dew each day

Take temperature readings every 3 hours for 3 – 4 days to enable temperature corrections for use with weather station data: or use a data logger at site

Hobbo data logger (good, expensive)

or I-buttons (good cheap, not always water proof)

Removal of specimens from body

- Only take away insects: do not produce artifacts in tissue of clothing by damaging or moving these
- After body is removed from bag, insects should be collected from this, but
- examine bag for insects before use, some residual insects may be there which will contaminate data
- Collect eggs, 20 to 100 of the largest maggots and preserved, and a second collections of maggots for rearing
- Use maggot rearing cups: make out of aluminum foil and add liver: place this in a 1 pint tub (500 ml ice cream tub) with a screened lid and 1 inch (2-3- cm) vermiculite or sterile sand for pupae to migrate into
- Keep at constant temperature for maturing, and ID timing of events

some case studies: place of death

Calliphora vicina abundant in cool seasons, *Phaenicia sericata* (or *Lucilia sericata*) prevalent in warm months, *Lucilia illustris* prefers sunlight, *Phormia regina* prefers shade

[Note the niche partitioning!, only by timing, not spatial]

- murder victim thrown into disused well and covered with tires and debris
- search begun for victim in general region, site of corpse unknown to investigators
- during search, thousands of flies observed congregating over a pile of discarded tires and refuse
- digging through junk revealed corpse at bottom of old well
- advanced decomposition, but no insects due to material obstructing fly access, in spite of strong attractive odours

some case studies: place of infestation

- young woman attacked in Chicago by man in ski mask
- suspect caught, police found ski mask in home of suspect
- suspect said mask was for ski-ing the previous winter and not used since then
- police found cocklebursburs on mask and other pant material
- given to entomologist for examination who found small caterpillars in the burs, identified these
- 1 year life cycle, moth lays eggs in spring, caterpillars develop in summer, pupate in fall for overwintering to emerge in spring/early summer
- proved mask had been outside that same summer around time that rape had occurred
- suspect confessed, convicted

a nice example of entomological evidence linked to vegetation

case study: myiasis

- several young children taken to hospital, had diaper rash, malnutrition, and appeared neglected
- anal and genital areas infested with maggots
- flies collected and preserved
- larvae indicate that they were on children at least 4-5 days, indicated amount of time since diapers were changed, or properly cared for or cleaned
- entomological evidence was the only evidence that was able to indicate mistreatment of the children
- used to place children into foster care

case study: drugs and development

- female, 20 stabbed, body found Oct 12 in pine woods Washington State
- early bloat stage of corpse, maggots on face and torso, blackening face and upper body
- maggots refrigerated for 5 days, then given beef kidney
- adults began to emerge on Nov 10, two species, *Phaenicia (Lucilia) sericata* and *Cynomya cadaverina*
- first species oviposits 1 to 2 days after death
- second species oviposits within 24 hours, in sunlit conditions
- mean maggots size used to identify PMI, but, one very large maggot was found, 17.7 mm long
- this size indicated it was 3 weeks old, *which was impossible with this corpse*
- maggot was found in a nasal pocket, victim was identified as a cocaine user and had snorted cocaine immediately prior to her heath
- evidence that cocaine accelerates maggot development

corpse in a well experiment (dead raccoon)


questions