TECHNICAL NOTE

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The Effect of Killing and Preservative Solutions on Estimates of Maggot Age in Forensic Cases

REFERENCE: Tantawi, T. I. and Greenberg, B., **"The Effect of Killing and Preservative Solutions on Estimates of Maggot Age in Forensic Cases,"** *Journal of Forensic Sciences*, JFSCA, Vol. 38, No. 3, May 1993, pp. 702–707.

ABSTRACT: Length of the oldest maggots recovered from a body often provide an accurate estimate of the time since death. The length of maggots of *Protophormia terraenovae* (Robineau-Desvoidy) of known age, at peak of feeding, was measured after 5 days immersion in one of 15 killing and preservative solutions, some of which are routinely used at autopsy and in forensic entomology; controls were killed in boiling water. There was shrinkage in all 15 solutions which translated into an underage error of 9.7 h in 70% ethanol, 11 h in San Veino and 16.8 h in formalin. Larvae of *Calliphora vicina* (Robineau-Desvoidy) underwent even greater shrinkage, which resulted in an underage error of 19.2 h in 70% ethanol, 26.4 h in formalin and 28.8 h in San Veino. Young third instar larvae underwent more shrinkage than older ones, with underage errors (in hours) as follows: *P. terraenovae*—70% ethanol, 24 and San Veino, 24; *C. vicina*—70% ethanol, 7.2 and San Veino, 14.4. Maggots killed in boiling water and then placed in preservative solutions did not shrink. Length of the crop, which may be useful in age estimates of postfeeding larvae, was not altered significantly for forensic purposes in these solutions. The highly significant alterations in maggot length underscore a need for standardization in the treatment of maggots collected at the crime scene and at autopsy if their length is to be interpreted in a valid and consistent way. Recommendations are made for treatment of maggots wherever they are collected.

KEYWORDS: forensic science, forensic entomology, blow flies, maggot age, time of death estimation

When blow fly maggots are encountered on a body, they can usually provide the most precise estimate of time of death, especially within the first life cycle [*1*]. The age of actively feeding maggots is determined by measuring their length, after the investigator takes into account their thermal history and species [*1–3*]. Unfortunately, in some homicide investigations, larvae are not collected at all, either at the discovery site or at autopsy. When larvae are collected, they are placed in one of many preservative fluids. Possible shrinkage in some of these solutions and the effect on estimates of larval age and postmortem interval has hitherto been overlooked by forensic entomologists, although some

Received for publication 8 April 1992; revised manuscript received 10 Aug. 1992; accepted for publication 27 Aug. 1992.

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of these formulations have long been used in entomology to kill and preserve immature insects and other arthropods [*4*].

This study compares shrinkage of larvae in various killing and preservative solutions and the potential error in estimates of the postmortem interval. It is evident from our results that there is a need for standardization of treatment and recommendations are suggested.

Materials and Methods

Protophormia terraenovae (Robineau-Desvoidy) and *Calliphora vicina* (Robineau-Desvoidy) were studied from established laboratory colonies. Multiple samples of about 35 third instar larvae each of the former and lesser numbers of the latter were taken from ground beef at peak of feeding at room temperature. The larvae were 100.8 h old, from time of oviposition. Each sample of larvae was immersed in 250 mL of one of a number of killing and preservative solutions for 5 days at room temperature; then the length of each larva was measured. Controls consisted of larvae killed in boiling water and measured immediately. Another group of larvae of *C. vicina* was killed in boiling water, placed into each of the various solutions for 5 days, and then measured. In a separate experiment, larvae of *C. vicina* at peak of feeding, and following 5 days in the above solutions, were dissected and crop length was measured; control crops were from dissections of live specimens.

The killing and preservative solutions were: 70, 80, or 90% ethanol; benzene; kerosene; formalin; 99.7% isopropanol; 70% ETOH/AAG (3 parts 70% ethanol:1 part glacial acetic acid [*5*]); 90% ETOH/AAG (3 parts 90% ethanol:1 part glacial acetic acid [*6*]); KAAD (80 mL 95% ethanol, 20 mL glacial acetic acid, 10 mL kerosene, and 10 mL dioxane [*7*]); KAA (1 part kerosene:1 part glacial acetic acid:30 parts 95% ethanol [*8*]); XAAD (40 mL xylene, 60 mL isopropanol, 50 mL glacial acetic acid, and 40 mL dioxane [*7*]); XA (50 mL xylene and 50 mL 95% ethanol [*7*]); Pampel's fluid (6 parts formalin:15 parts 95% ethanol:2 parts glacial acetic acid:30 parts distilled water [*9*]); and San Veino (Embalmer's Supply Co., Stratford, CT). The latter's constituents are formaldehyde, trichlorethane, phenol, methanol, and synthetic camphor oil; proportions not disclosed.

Age of larvae of *P. terraenovae* and *C. vicina*, based on mean length per sample, was estimated using growth curves (from egg deposition to eclosion) at a laboratory temperature of 23° ± 0.5°C for the former and 22° ± 0.5°C for the latter.

Statistical analysis was performed by the SAS system [*10*]. Larval length of both species and crop size of C. *vicina* in various solutions were compared by a one-way ANOVA.

Results and Discussion

Table 1 compares the relative shrinkage in length of maximum-size larvae of *P. terraenovae* immersed for 5 days in various solutions; boiling water is the control. It will be noted that larvae undergo maximum extension in boiling water, which is comparable to maximum extension of a live larva. Larvae undergo maximum shrinkage in San Veino and formalin. The latter solutions are widely used at autopsy as a surface disinfectant and to kill maggots and other arthropods. This degree of shrinkage makes the maggots appear to be a half-day or more younger than they actually are. Maggots that were killed in 70% ethanol, which is routinely used by entomologists, would appear to be approximately 10 h younger than they actually are. Maggots killed in formalin would appear to be 17 h younger. The ANOVA for body length of *P. terraenovae* larvae in the various solutions was highly significant ($F = 133.5$; $df = 15$; $P = <0.0001$).

TABLE 1—*Effect of killing and preservative solutions on body length of third instar larvae of* P. terraenovae *at peak of feeding.*

Treatment	N[a]	Mean larval length (mm) ± SD	Range (mm)	% shrinkage	Larval age (h)[b]	Deviation from control (−h)
Boiling water	58	15.6 ± 0.6	15.0–17.0	Control	100.8	Control
Kerosene	33	15.1 ± 1.0	13.0–17.0	3.2	98.4	2.4
XA	37	15.0 ± 1.3	12.5–17.0	3.8	98.4	2.4
KAAD	35	14.6 ± 0.5	13.5–15.5	6.4	96.0	4.8
KAA	30	13.6 ± 0.6	13.0–15.0	12.8	93.6	7.2
Benzene	33	13.5 ± 0.7	12.0–15.0	13.5	93.6	7.2
70% ETOH	35	12.9 ± 0.8	11.4–14.0	17.3	91.2	9.7
XAAD	30	12.7 ± 0.9	11.0–14.8	18.6	91.2	9.7
70% ETOH/AAG	35	12.5 ± 0.7	11.0–14.0	19.9	91.2	9.7
80% ETOH	33	12.2 ± 0.5	11.0–13.0	21.8	91.2	9.7
90% ETOH/AAG	32	11.9 ± 0.5	11.0–13.0	23.7	88.8	11.0
Pampel's fluid	30	11.9 ± 0.9	10.5–13.9	23.7	88.8	11.0
90% ETOH	35	11.8 ± 0.7	10.0–13.3	24.4	88.8	11.0
Isopropanol	30	11.5 ± 0.6	10.0–13.0	26.3	88.8	11.0
San Veino	31	11.2 ± 1.2	9.0–14.0	28.2	88.8	11.0
Formalin	33	10.8 ± 0.9	9.0–12.8	30.8	84.0	16.8

[a]Number of larvae examined.
[b]Larval age was determined from a growth curve generated from multiple rearings at 23° ± 0.5°C; data derived from maggots killed in boiling water.

Table 2 compares the effect of various killing and preserving solutions on the length of maximum-size larvae of *C. vicina*, with boiling water as a control. Actual larval age was 100.8 h, and deviation from the actual age is given in the last column of Table 2. The underage error ranges from 10 h (XA) to 29 h (San Veino). The ANOVA for body length of the larvae in different solutions was highly significant ($F = 38.8$; $df = 7$; $P = <0.0001$). The larvae of *C. vicina* underwent greater shrinkage than those of *P. terraenovae* and this underscores the desirability of testing larvae of other species, as well. Larvae of *C. vicina* did not undergo shrinkage when they were first killed in boiling water and then immersed for 5 days in the solutions listed in Table 2.

Because younger larvae may have thinner, more pliable cuticles, we decided to test young third instars of each species. Samples of 15 larvae, 10 to 12 mm in length, were

TABLE 2—*Effect of killing and preservative solutions on body length of third instar larvae of* C. vicina *at peak of feeding.*

Treatment	N[a]	Mean larval length (mm) ± SD	Range (mm)	% shrinkage	Larval age (h)[b]	Deviation from control (−h)
Boiling water	14	16.8 ± 0.4	16.0–17.2	Control	100.8	Control
XA	7	16.1 ± 0.6	15.2–17.0	4.2	91.2	9.6
70% ETOH	8	15.0 ± 0.5	14.4–16.0	10.7	81.6	19.2
Benzene	10	15.0 ± 0.6	14.0–16.0	10.7	81.6	19.2
Pampel's fluid	6	14.7 ± 0.6	14.0–15.4	12.5	79.2	21.6
Kerosene	10	13.8 ± 0.9	13.0–15.0	17.9	74.4	26.4
Formalin	6	13.6 ± 0.7	13.0–14.7	19.0	74.4	26.4
San Veino	7	13.0 ± 0.8	11.5–14.0	22.6	72.0	28.8

[a]Number of larvae examined.
[b]Larval age was determined from a growth curve generated from multiple rearings at 22° ± 0.5°C; data derived from maggots killed in boiling water.

immersed for 5 days in XA, 70% ethyl alcohol, or San Veino; controls were killed in boiling water as before. Not surprisingly, the percentage of shrinkage was greater for young third instar larvae than for older ones. The underage error (in hours) for *P. terraenovae* was: XA, 7.2; 70% ethanol, 24; and San Veino, 24 (Table 3); for *C. vicina* the underage error in hours was 2.4, 7.2 and 14.4, respectively (Table 4).

There are few, if any, reliable age markers in postfeeding calliphorid larvae. It has been shown, at least in *C. vicina* and *Phaenicia sericata* (Meigen), that the size of the crop can serve as a good age indicator [*1*]. When the maggot stops feeding the crop downsizes at a predictable rate and therefore, any artifactual shrinkage due to preservation becomes a relevant concern. Table 5 summarizes data on crop length in live larvae of *C. vicina* at peak of feeding and after preservation. Exposure of larvae to boiling water or 70% ethanol actually increased the length of the crop, whereas the other solutions caused shrinkage. The ANOVA for larval crop size in different fluids was highly significant ($F = 12.9$; $df = 8$; $P = <0.0001$). The amount of shrinkage, however, does not exceed what normally occurs in the first 3 h after the larva stops feeding [*1*], so the effect of the different solutions on crop length is not useful for forensic purposes.

It is obvious from our results that the kind of solution in which maggots are killed or preserved has a significant effect on their length and therefore their estimated age, and hence can lead to a miscalculation of the postmortem interval. Therefore, we suggest that boiling water should be used by criminal investigators, medical examiners, forensic pathologists, coroners, and forensic entomologists as a standard killing solution for maggots of forensic importance. The larvae can then be preserved in 70% ethanol. Boiling appears to greatly lessen autolysis by destroying digestive enzymes and the gut flora.

TABLE 3—*Effect of killing and preservative solutions on body length of young third instar larvae of* P. terraenovae.

Treatment	N[a]	Mean larval length (mm) ± SD	Range (mm)	% shrinkage	Larval age (h)[b]	Deviation from control (−h)
Boiling water	15	10.4 ± 0.5	10.0–11.5	Control	81.6	Control
XA	15	8.5 ± 0.5	8.0–9.5	18.3	74.4	7.2
70% ETOH	15	5.6 ± 0.6	5.0–7.0	46.2	57.6	24.0
San Veino	15	5.5 ± 0.5	5.0–6.5	47.1	57.6	24.0

[a]Number of larvae examined.
[b]Larval age was determined from a growth curve generated from multiple rearings at 23° ± 0.5°C; data derived from maggots killed in boiling water.

TABLE 4—*Effect of killing and preservative solutions on body length of young third instar larvae of* C. vicina.

Treatment	N[a]	Mean larval length (mm) ± SD	Range (mm)	% shrinkage	Larval age (h)[b]	Deviation from control (−h)
Boiling water	15	10.8 ± 0.5	10.0–12.0	Control	64.8	Control
XA	15	10.2 ± 0.7	9.0–11.0	5.6	62.4	2.4
70% ETOH	15	9.1 ± 0.8	8.0–10.0	15.7	57.6	7.2
San Veino	15	7.0 ± 0.4	6.0–8.0	35.2	50.4	14.4

[a]Number of larvae examined.
[b]Larval age was determined from a growth curve generated from multiple rearings at 22° ± 0.5°C; data derived from maggots killed in boiling water.

TABLE 5—*Effect of killing and preservative solutions on crop length in third instar larvae of* C. vicina *at peak of feeding.*

Treatment	N[a]	Mean crop length (mm) ± SD	Range (mm)	% extension or shrinkage
Live	12	7.7 ± 0.3	7.5–8.2	Control
Boiling water	14	8.7 ± 0.5	7.9–9.8	13.0[b]
70% ETOH	8	8.0 ± 0.6	6.8–9.0	3.9[b]
Kerosene	10	7.5 ± 0.9	6.7–9.0	2.6[c]
Pampel's fluid	6	7.4 ± 0.5	7.0–8.0	3.9[c]
Formalin	6	7.3 ± 0.5	7.0–8.0	5.2[c]
San Veino	7	7.3 ± 0.5	6.7–8.0	5.2[c]
Benzene	10	7.2 ± 0.4	7.0–8.0	6.5[c]
XA	7	6.3 ± 0.5	6.0–7.0	18.2[c]

[a]Number of larvae examined.
[b]Extension.
[c]Shrinkage.

This procedure improves precision in still another way. Maggots that are first killed in solutions such as 70% ethanol retain some elasticity and extensability of the cuticle. This can lead to further error in measuring body length, whereas maggots killed in boiling water are fully extended and rigid. Hot water can be brought to the crime scene in a thermos and can be boiled on site by means of a sterno can or an electrical heating device plugged into a car's ignition or some other source of electricity. If dry ice is available, larvae should be cooled (not frozen) until they are returned to the laboratory and then killed in boiling water. If none of these alternatives are available, XA can be substituted for boiling water, taking into account later, about 4% shrinkage for mature third instars, and more for younger larvae, in this fluid. The use of San Veino and other solutions containing formalin for killing larvae should be avoided. It is noteworthy that preservatives including formalin and alcohol have no effect on opiates, cocaine or barbiturates which may be sequestered by larvae. Marijuana is degraded only at pHs below 4 and above 9[3]. In all the stated procedures it is understood that, in addition to preservation, a sample of larvae will be kept alive and reared to the adult stage for species confirmation.

Acknowledgment

The authors would like to thank Alan Parman for his statistical assistance and advice.

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