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Oviposition preferences and antennal size in carrion flies

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Abstract

Carrion-feeding flies use odours emanating from the decomposing corpse as cues for oviposition and are described as generalists because the larvae feed on the corpses of diverse species. Whereas several features of the corpse may influence the oviposition choices of these flies, it is not known whether there is a preference for a particular species of corpse. We provided carrion flies with ovipositional (and feeding) choices in a field experiment, in which various odour sources were presented simultaneously. We found novel evidence of broadly consistent choices of carrion by flies from four families. Traps baited with decaying fish flesh captured the greatest number of individuals, whereas traps baited with decaying pig liver typically attracted the least. We also asked whether individuals captured in the various baits vary in antennal size, perhaps reflecting different capacities for odour detection. There was a trend for individuals of *Lucilia sericata* Meigen (Diptera: Calliphoridae) and the platystomatid collected from the traps baited with pig liver to have significantly larger antennae, whereas individuals of *Muscina stabulans* (Fallen) (Diptera: Muscidae) captured in traps baited with marine fish flesh had relatively longer antennae for their body size. Our data reveal a more nuanced pattern of oviposition behaviour in these generalist carrion flies, which may reflect differences in their preference of carrion with different nutrients, and in their capacity to detect particular odours.

Abbreviated abstract (2-3 sentences, max. 80 words)

Carrion-feeding flies use odours emanating from decomposing corpses as oviposition cues, but it is not known whether they prefer a particular species of corpse. Our study presented odour sources in field experiments simultaneously, revealing novel evidence of broadly consistent oviposition choices across carrion flies from four families. We conclude that individual variation in antennal size has an impact on the detection of odours, and that not all species of carrion are the same for carrion flies. [77 words]

Graphic for Table of Contents

figure 2

Introduction

Adult female insects detect appropriate oviposition and feeding sites using chemical cues (e.g., Bernays & Chapman, 1994; Wäschke et al., 2013; Webster & Cardé, 2017) that are typically detected by receptors located on sensilla, which are supported by the antennae (Elgar et al., 2018). The evolutionary significance of the remarkable inter-specific variation in the size, shape, and micro-morphology of insect antennae is surprisingly poorly understood (Schneider, 1964; Chapman, 1982; Bau & Cardé, 2015; Elgar et al., 2018, 2019). Nevertheless, emerging evidence suggests that insect antennal micro-morphology responds to selection exerted by both the nature of the odour, including volatility and complexity, and the context of odour detection, such as foraging, oviposition, mate searching, and social organization (Elgar et al., 2018). The number of sensilla increases with antennal length both within and across some species of insects (Zacharuk, 1985; Elgar et al., 2018), and field experiments reveal that odour detection is correlated with antennal length in bees (Spaethe et al., 2007) and moths (Johnson et al., 2017), and with sensilla density in ants (Gill et al., 2013).

Carrion-feeding calliphorid, sarcophagid, and muscid flies are generalists because the larvae are found on the corpses of different species of vertebrates (Calvignac-Spencer et al., 2013) and invertebrates (Woodcock et al., 2002). Adult carrion flies use the volatile odours produced by the decomposing corpse, endogenous bacteria, or other organisms exploiting the corpse (Tomberlin et al., 2011; George et al., 2012) as cues for oviposition, mate search, and feeding (Amendt et al., 2004). For example, the oviposition behaviour of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) is influenced by two semiochemicals: individuals are initially attracted to sulphur-rich volatiles, originating from bacterial composition products, and then commence ovipositing in response to ammonia-rich compounds and carbon dioxide (Ashworth & Wall, 1994). Trap sampling and behavioural assays reveal that the oviposition choices of carrion flies are influenced by the age and body part of the corpse; presence of larval competitors and predators, and location of the corpse on the substrate (Archer & Elgar, 2003a,b; Gião & Godoy, 2007; Gunn & Bird, 2011; Yang & Shiao, 2012; Charabidze et al., 2015; Gunn, 2016; Martin & Verheggen, 2018).

Remarkably, few studies have investigated whether some species of carrion are preferred over others (but see Bunchu et al., 2008), perhaps because research on carrion flies has a strong focus on forensic entomology (Erzinclioglu, 1983; Amendt et al., 2004). Nevertheless, the macronutrient content of carrion, including protein, carbohydrates, and unsaturated fatty acids is likely to vary between species and thus have an impact on larval survival and growth rates (Simpson & Raubenheimer, 2012). It is widely understood that insects make ovipositional choices that reflect the survival and growth rates of their offspring (Thompson, 1988; Mayhew, 1997; Gripenberg et al., 2010), and this may also be true of carrion flies (see Bunchu et al., 2008). Perhaps the odour emanating from carrion varies with these nutritional profiles, and carrion flies oviposit accordingly.

Here, we use field choice experiments to explore the variation in response of several taxa of carrion flies to traps baited with carrion of species that vary in both nutritional value and local abundance. We assume that the relative number of flies in each trap reflects a combination of detection of and preference for particular carrion, primarily as oviposition sites but we also recognize that some flies may anticipate feeding. Accordingly, we expect to capture on average more flies in traps baited with carrion of greater nutritional value, as the available nutrients are likely to influence larval survival and growth rates (Gripenberg et al., 2010). We ask whether the variation in capture numbers reflects differences in odour detection by measuring the size (body length) of flies collected from different traps. Following Johnson et al. (2017), we assume that traps containing carrion that emits less easily detected odours (e.g., they occur at a lower concentration) will capture flies with larger antennae, which support more receptors (e.g., Smallegange et al., 2008). Our field experiment, in which free-living carrion flies is offered a simultaneous choice of traps baited with carrion from different species, allows us to ask (1) whether different taxa of carrion flies are similarly attracted to different species of carrion, and (2) whether there are intra-specific differences in the length of the antennae of individuals that are attracted to these different baits.

Materials and methods

Trapping

The carrion flies were lured to fly traps, set over 10 days between July and August 2017, on the campus of Beijing Forestry University (Haidian District, Beijing, China), using three baits: marine ribbon fish (*Trichiurus lepturus* L.) flesh, frog leg, and pig liver, each obtained from commercial outlets. These species of carrion were selected as baits as they differed in nutritional content (Figure 1), and their likely frequency of natural exposure to the flies. The nutritional content of frog leg is much lower than that of marine fish flesh, but the flies are more likely to encounter the carrion of frog leg than marine fish because the ocean is about 160 km from the traps. Freshwater fish may be more abundant, but we do not know whether marine and freshwater fish carrion produce the same odour. The baits may differ in other ways, including the concentration of particular volatiles released during the decay process, although the chemical composition of the odour released from these three types of carrion has not been documented.

The fly trap (Shanghai Lv Wei Daily Chemical Co., Shanghai, China) consists of a cylindrical ‘flight cage’ (25 cm diameter, 32 cm high) that is suspended above the bait pot with the appropriate bait placed inside (Figure 2). The tapered, conical opening at the base of the fly cage directs the upward movement of the flies into the small opening into the cage, where they remain captive.

The bait comprised 2 cm^3 cubes of carrion that had been left at room temperature for 24 h before use and were used for 1 day only. The three fly traps were placed at least 50 m from each other and suspended 0.3 m from the ground. The traps were simultaneously set between 09:00 and 17:00 hours each day. The captured flies were collected at the end of each day and stored in ethanol for identification and analysis.

Measurements

The flies collected from the traps were sorted and identified using local taxonomic keys. We excluded male flies from our sample as our focus was on female oviposition behaviour. The body length (from the tip of the head to the tip of the abdomen) of each fly was measured using a micrometer under microscopic magnification. The left antenna (pedicle and funiculus) of each specimen was excised, assigned with an individual number, and mounted on a slide with glycerol. Digital images of the slides were obtained using an Olympus SZX16 stereoscopic microscope (Olympus, Tokyo, Japan), using the same resolution, and the antennal length of each specimen was measured using Nano Measurer 1.2 (Fudan University, Shanghai, China). We defined the length of the antenna as the distance between the proximal tip of the pedicle and the distal tip of the funiculus.

Statistical analysis

The statistical analyses were conducted using JMP v.13 (SAS Institute, Cary, NC, USA). We used ANOVA to investigate between-taxa variation in both antennal and body length, and separate bivariate regression models to investigate relationships between body and antennal length for each taxon. We used a mixed effects model to investigate the variation in the number of individuals caught in each trap (log transformed), with fly taxon and bait as fixed effects, and collection day as a random effect with the variance partitioned using restricted maximum likelihood (REML). We used mixed effects models to investigate, for each fly taxon, the variation in absolute antennal length and body length, with bait as a fixed effect, and collection day as a random effect with the variance partitioned using REML. Finally, we used mixed effects models to investigate, individually for each fly taxon, the variation in antennal length corrected for body size (henceforth, relative antennal length), with bait (fish flesh, frog leg, or pig liver) and body size as fixed effects, and collection day as a random effect with the variance partitioned using REML.

Results

Taxonomic differences in attraction to baits

Our analysis is confined to four taxa (Diptera) that were consistently collected from all three baits: the green bottle fly, *L. sericata* , the false stable fly, *Muscina stabulans* (Fallen) (Muscidae), flesh flies *Sarcophaga* spp. (Sarcophagidae), and an unidentified, single species of signal fly (Platystomatidae). The sample included several species of *Sarcophaga* as the females of this genus cannot be easily distinguished by morphological characters. There was inter-specific variation among these taxa in both body length ($F_{3,626} = 401.35$) and antennal length ($F_{3,626} = 1\,870.15$, both $P<0.0001$; Table 1).

The variation in the number of flies collected from the traps was explained by the type of bait ($F_{2,150.8} = 12.03$, $P<0.0001$), but not by fly taxa ($F_{3,150.8} = 2.23$, $P = 0.09$). In general, larger numbers of flies were caught in the traps baited with fish flesh than in traps baited with either frog leg or pig liver (Figure 3), a pattern that was broadly similar across the fly taxa and collection dates (fly taxa*bait interaction: $F_{3,150.9} = 1.30$, $P = 0.27$; 4.7% of the variance explained by the date of capture, Wald $P = 0.35$).

Body size, antennae size, and type of bait

In all fly taxa, the length of the antennae was significantly correlated with the size of the female (Figure 4), although there were substantial inter-specific differences in explained variance – platystomatid: $r^2 = 0.18$, $\beta = 0.027 \pm 0.004$, $F_{1,192} = 43.06$; *M. stabulans*: $r^2 = 0.24$, $\beta = 0.026 \pm 0.005$, $F_{1,70} = 23.11$; *Sarcophaga* spp.: $r^2 = 0.41$, $\beta = 0.041 \pm 0.003$, $F_{1,224} = 155.98$; and *L. sericata*: $r^2 = 0.60$, $\beta = 0.077 \pm 0.005$, $F_{1,136} = 207.72$, all $P<0.0001$. The body length of *M. stabulans* varied across the traps ($F_{2,69} = 7.728$, $P<0.001$; Table S1, Figure S1), with individuals caught in the trap baited with pig liver being longer than those captured in the traps baited with fish flesh. However, the body length of *L. sericata*, *Sarcophaga* spp., and the platystomatid was not explained by the type of bait (Table S1, Figure S1).

The type of bait did not consistently predict the variation in either antennal length (Table S2) or relative antennal length (Table S3). The variation in antennal length was marginally explained by the type of bait in *L. sericata* ($F_{2,135} = 2.91$, $P = 0.058$) and the platystomatid ($F_{2,191} = 2.66$, $P = 0.073$), with individuals of both taxa collected from the traps baited with pig liver having larger antennae (Figure 5). The variation in relative antennal length was explained by the type of bait in *M. stabulans* only ($F_{2,69} = 5.35$, $P = 0.007$), with individuals collected from traps baited with fish flesh having the longest relative antennal length (Table S3).

Discussion

Our field experiments revealed novel patterns of attraction to particular odour cues by carrion flies, which were broadly consistent across four families. Traps baited with decaying marine fish flesh captured the most individuals, whereas traps baited with decaying pig liver typically attracted the least. This largely consistent pattern of relative preferences for different sources of odour has not been widely reported for carrion flies in natural populations, perhaps reflecting the more typical forensic entomological research focus on how oviposition behaviour is influenced by variation in the characteristics of the same carcass species (Archer & Elgar, 2003a,b; Gião & Godoy, 2007; Gunn & Bird, 2011; Yang & Shiao, 2012; Charabidze et al., 2015; Gunn, 2016; Martin & Verheggen, 2018; but see Bunchu et al., 2008). We also found that individuals of *L. sericata* and an unidentified platystomatid collected from the traps baited with pig liver tended to have longer antennae than those collected from the other traps. Individuals of *M. stabulans* collected from traps baited with pig liver were larger than those collected from the other traps, and individuals captured in traps baited with marine fish flesh had relatively longer antennae (for their body size) than those captured in the other traps.

The differences in the capture rates of fly taxa across the traps baited with carrion is broadly consistent with the widely held view that female insects make ovipositional choices that optimize offspring growth and survival (e.g., Gripenberg et al., 2010). Flesh of the marine fish, which has a relatively high protein, unsaturated fatty acid, and lipid content attracted the highest number of flies from any taxon. However, although these macronutrients are more abundant in pig liver than in frog leg, traps with the former consistently attracted the fewest flies. The relative proportion of macronutrients that optimize larval growth is likely to vary between species (Simpson & Raubenheimer, 2012), and clearly this requires verification for the fly and carrion taxa included in our study. Nevertheless, the number of flies trapped by baits will also depend upon the capacity of fly taxa to detect the odours released by the decaying material, irrespective of their nutritional content.

Odour detection under natural conditions depends, in part, on antennal morphology

(Spaethe et al., 2007; Gill et al., 2013; Johnson et al., 2017), and this may additionally explain the variation in the numbers of flies captured in the different traps. Individuals with larger antennae typically have more sensilla (Chapman, 1982; Smallegange et al., 2008; Elgar et al., 2018), which suggests at least two, non-mutually exclusive, explanations. First, larger antennae may simply allow individuals to detect smaller quantities of odour (Johnson et al., 2017). The pig liver bait, despite having a high nutritional content, may have attracted the fewest flies because it emitted very little odour. Intriguingly, individuals of *L. serricata* and the unidentified platystomatid that arrived at these traps had the longest antennae. The absence of an effect in *Sarcophaga* spp. may reflect the inclusion of several species in the same taxa. Second, larger antennae may support larger numbers of receptors that detect 'rare odours'. Females of *M. stabulans* caught in the fish-baited traps had relatively longer antennae for their body size, which may allow those individuals to detect odours that are unlikely to be frequently encountered. Perhaps the diversity of sensory receptors on individual antennae is locally adapted (sensu Kawecki & Ebert, 2004), with the number of receptors capable of detecting a particular odour reflecting its relative, local abundance. For example, the weaker olfactory response to moistened sheep fleece by Australian *L. sericata* compared with those found in Britain (Cragg & Cole, 1956) may reflect the relative scarcity of such a cue in a more arid environment.

It is not clear why the pattern of variation in relative antennal length with type of bait is confined to *M. stabulans*, but the life-history patterns of these flies, which arrive at the carrion at different stages of decomposition, may be significant. Blow flies, such as *L. sericata*, and flesh flies *Sarcophaga* spp. are typically the first to arrive at a carcass (Joseph et al., 2011), and selection may favour a capacity for rapid detection of carrion rather than distinguishing between carrion. These flies likely respond to odours generated in the autolysis stage of decomposition, derived from protein or amino acids, which are simple, small molecules (i.e., propionic acid, lactic acid, methane, hydrogen sulfide, and ammonia) that are less species-specific. Interestingly, *L. sericata* and *Sarcophaga* spp. are large flies with long antennae, perhaps reflecting strong selection on rapid detection. In contrast, muscid flies, including *M. stabulans*, typically delay oviposition until the carcass reaches the 'bloat' stage of decomposition (Joseph et al., 2011). The chemical odours emitted at this stage of decomposition derive from the actions of microorganisms, and therefore differ according to the composition of tissues, thereby allowing these flies to select suitable oviposition sites across the decomposing corpse. Little is known about the biology of platystomatid flies, but their striking preference for marine fish flesh over the other baits warrants investigation.

In summary, our field experiment reveals that different species of carrion consistently attracted different numbers of diverse carrion flies. Interestingly, marine fish flesh attracted the most flies, even though this carrion is locally rare. Our data suggest a more nuanced pattern of oviposition behaviour in carrion flies than their generalist moniker suggests, which may reflect differences in their preference for carrion with different nutrients, and their capacity to detect particular odours.

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Figure captions

Figure 1 Macronutrient content in 100 g of raw fish flesh, pig liver, and frog leg, using data provided by the United States Department of Agriculture (https://fdc.nal.usda.gov/).

Figure 2 Illustration of the fly trap.

Figure 3 The number of individuals of (A) *Lucilia sericata*, (B) *Muscina stabulans*, (C) an unidentified platystomatid, and (D) *Sarcophaga* spp. captured per day from traps baited with the remains of fish flesh, frog leg, or pig liver. Note the y-axis scale is not the same in each panel. The first and fourth quartiles are indicated by the whiskers, and the second and third quartiles are enclosed within the box, the median is indicated by the line within the box, and '×' indicates the mean.

Figure 4 Relation between antennal length and body length of individuals of (A) *Lucilia sericata*, (B) *Muscina stabulans*, (C) an unidentified platystomatid, and (D) *Sarcophaga* spp. captured from traps baited with the remains of fish flesh, frog leg, or pig liver. Note the y-axis scales differ between panels.

Figure 5 Absolute antennal length (mm) of individuals of (A) *Lucilia sericata*, (B) *Muscina stabulans*, (C) an unidentified platystomatid, and (D) unidentified *Sarcophaga* spp. captured from traps baited with the remains of fish flesh, frog leg, or pig liver. Note the y-axis scales are not the same in each panel. The first and fourth quartiles are indicated by the whiskers, and the second and third quartiles are enclosed within the box, the median is indicated by the line within the box, and '×' indicates the mean.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1 Mixed models explaining variation in the body length of four dipterans (*Muscina stabulans*, *Lucilia sericata*, *Sarcophaga* spp., and an unidentified platystomatid), arriving at traps with different baits (fish flesh, frog leg, or pig liver)

Table S2 Mixed models explaining variation in the absolute antennal length of four dipterans (*Muscina stabulans*, *Lucilia sericata*, *Sarcophaga* spp., and an unidentified platystomatid), arriving at traps with different baits (fish flesh, frog leg, or pig liver)

Table S3 Mixed models explaining variation in the antennal length, corrected for body size, of four dipterans (*Muscina stabulans*, *Lucilia sericata*, *Sarcophaga* spp., and an unidentified platystomatid), arriving at traps with different baits (fish flesh, frog leg, or pig liver)

Figure S1 Body length (mm) of individuals of (A) *Lucilia sericata*, (B) *Muscina stabulans*, (C) an unidentified platystomatid, and (D) *Sarcophaga* spp. collected from traps baited with the remains of marine fish flesh, frog leg, or pig liver. Note the y-axis scales are not the same in each panel. The first and fourth quartiles are indicated by the whiskers, and the second and third quartiles are enclosed within the box, the median is indicated by the line within the box, and '×' indicates the mean.

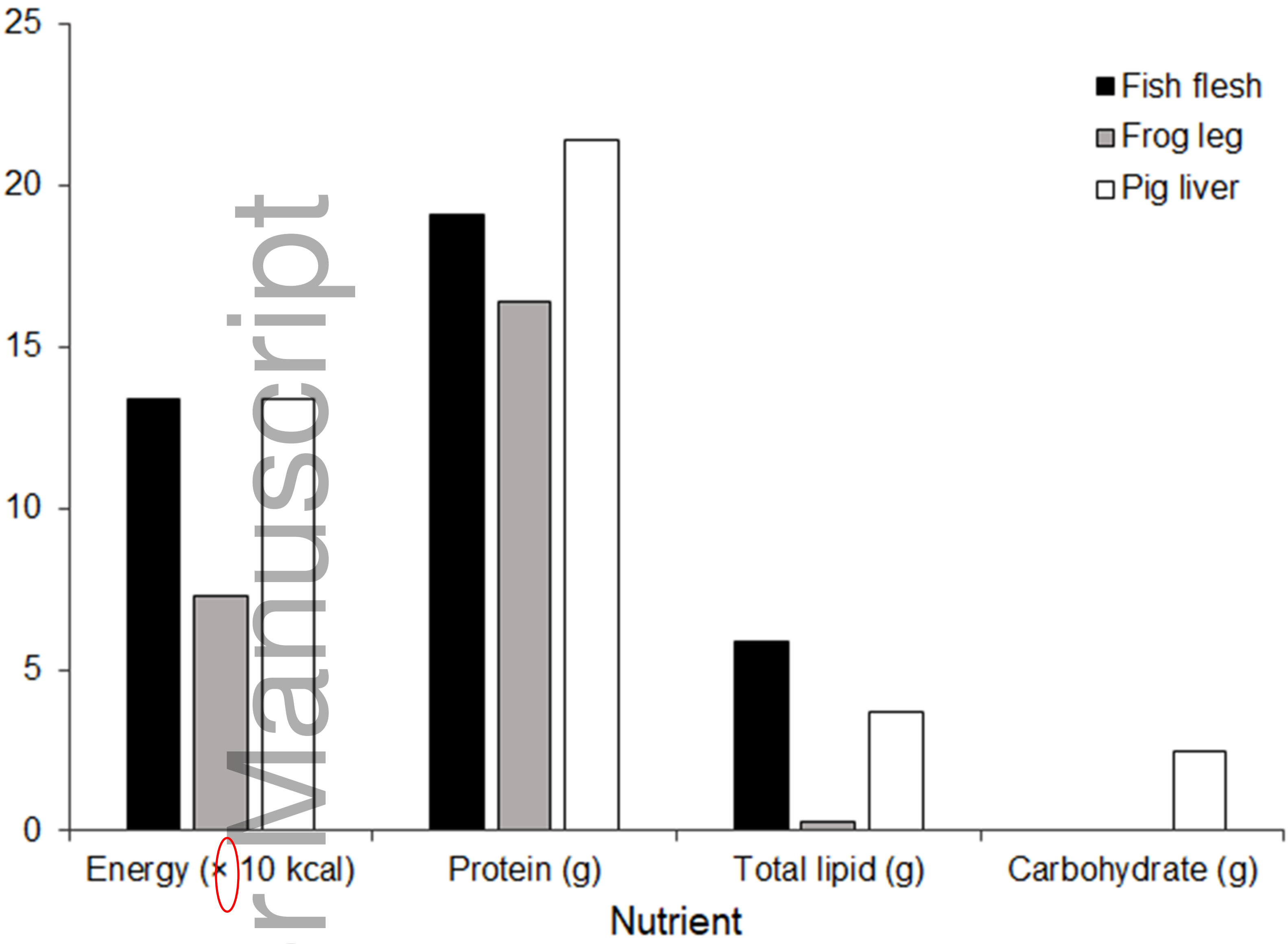
Table 1 Overall mean (± SD) body and antennal lengths (mm) of the four fly taxa collected from traps with different baits

Species	Body length (mm)		Antennal length (mm)	
	Mean ± SD	CV	Mean ± SD	CV
Lucilia sericata (n = 138)	13.59 ± 1.73	12.7	1.34 ± 0.17	12.7
Muscina stabulans (n = 72)	8.39 ± 1.05	12.5	0.70 ± 0.06	8.6
Platystomatid (n = 194)	8.20 ± 0.86	10.5	0.42 ± 0.05	11.9
Sarcophaga spp. (n = 226)	11.32 ± 1.19	10.5	0.91 ± 0.12	13.2

n, number of individuals per taxon; CV, coefficient of variation

Supporting Information

Tables S1-3 provide full details of the mixed models used to explain the variation in body length (Table S1), antennal length (Table S2), and antennal length corrected for body size (Table S3) of flies captured at traps baited with different kinds of decaying flesh, and plots describing the variation in the body length of flies collected from the different traps (Figure S1).



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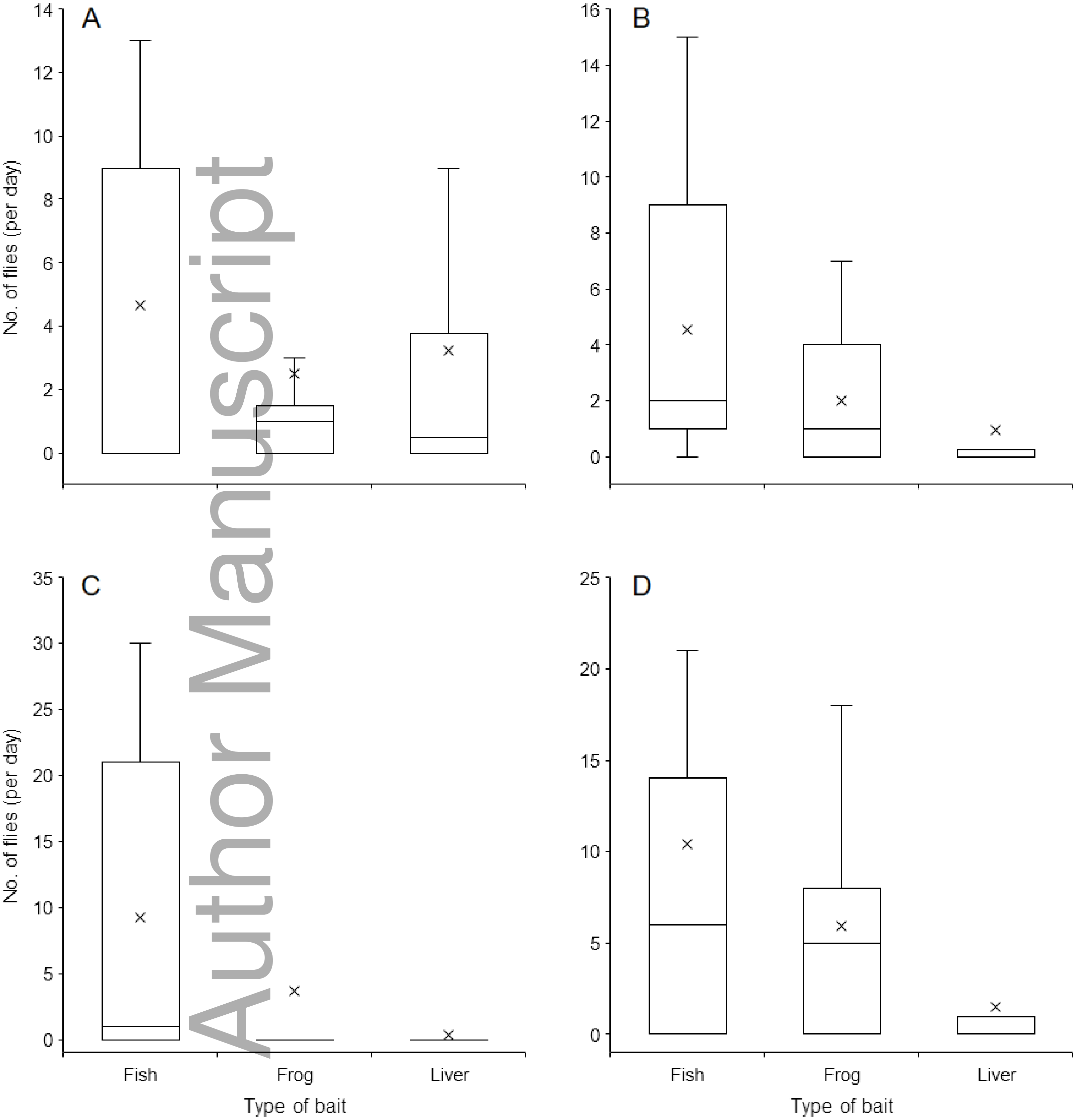

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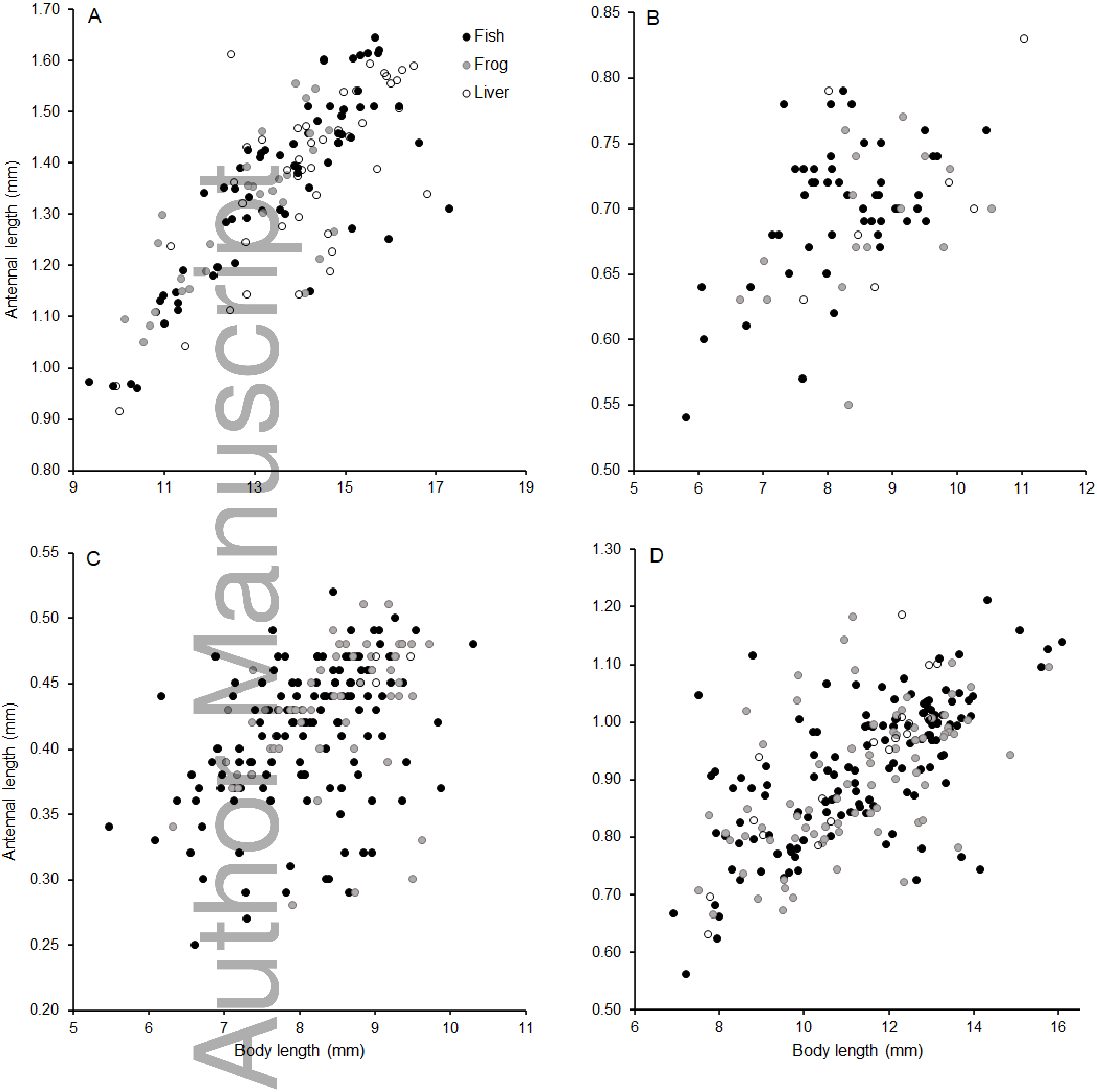

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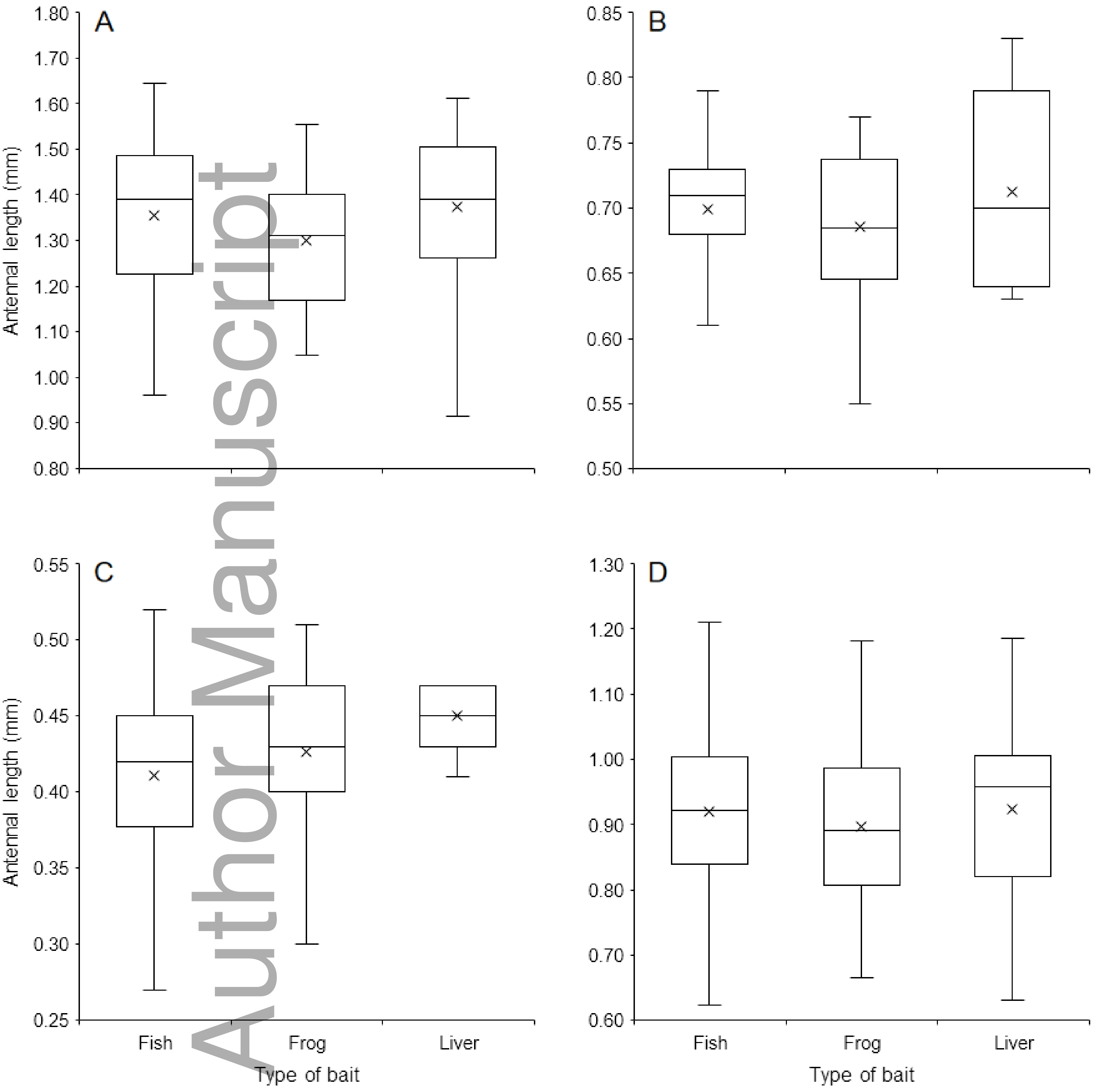

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