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The effects of feeding regime on the growth and reproduction of the medicinal leech *Hirudo medicinalis*

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SUMMARY

1. The feeding frequency, the size of meals, the number of meals required to attain reproductive maturity and the number of meals taken between iteroparous reproductive bouts were determined in the laboratory under optimal conditions for the medicinal leech *Hirudo medicinalis* fed exclusively on mammalian (bovine) blood. In addition the number of bouts of reproduction and the numbers of cocoons and hatchlings per cocoon produced were determined.
2. The average time for *H. medicinalis* to reach reproductive maturity at 20°C was 289 days, at an average wet biomass of 8143 mg with two–nine separate bouts of cocoon production. The number of meals to first reproduction was 8.9 (mean meal size of 3066.7 mg), with a significant correlation between total mass of blood ingested and the numbers of reproductive bouts and number of cocoons produced. Mean lifetime cocoon production per individual was 12.43, with 3.9 hatchlings per cocoon.
3. The significant positive relationships between ingestion, fecundity and developmental rate observed support the hypothesis that declining abundances of field populations of *H. medicinalis* are the result of lower available energy for growth, reflecting leeches now feeding predominantly on amphibian blood of lower energetic value than mammalian blood.

Introduction

Many sanguivorous leeches feed extensively on vertebrates, with a few species, including the European medicinal leech *Hirudo medicinalis* Linn., adapted to feeding on mammals (Herter, 1936; Kaiser, 1954). Despite their long, historic use for blood letting (phlebotomy; Young, 1984, 1986), their more recent use in microsurgery (West, Nichter & Halpen, 1991) and as a source for physiological active substances (Markwardt, 1985; Sawyer, 1986; Rigbi *et al.*, 1987; Adams, 1988), relatively little is known about the ecology of field populations of *H. medicinalis* and how the number, size and quantity of blood meals affects growth and reproduction.

In Europe, wet grazing pastures were probably the most common habitat for *H. medicinalis*, and significant

reductions in this habitat due to changes in agricultural practice and cattle management have had an effect on encounter rate with potential mammalian hosts (Mann, 1955; Wilkin & Scofield, 1990). In addition, the commercial market for medicinal leeches is still primarily supplied by field collections rather than culturing, although not at historic levels when, for example, the annual import of medicinal leeches between 1817 and 1843 to France primarily from eastern Europe ranged from nineteen to fifty-seven million (Sawyer, 1981). Gathering leeches for scientific use, in combination with the reduction in wetland habitats, has resulted in *H. medicinalis* being placed on the endangered species list, although it is still recorded from twenty-four European countries (Wells, Pyle & Collins, 1983).

Even with conservation and maintenance of wetlands, the potential exists for a continuing decline in populations of *H. medicinalis* (Elliott & Tullett, 1984).

Hirudo medicinalis can ingest eight to nine times its own body biomass of blood, and can survive for up to 1 year between meals (Lent & Dickinson, 1984). During and immediately following a blood meal *H. medicinalis* shows a significant reduction in responsiveness to external stimuli (Dickinson & Lent, 1984). Although specialized for detecting and feeding upon mammals, *H. medicinalis* has a broad range of alternative hosts, the most important being frogs, toads, fish and waterfowl (Blair, 1927; Bennike, 1943; Hoffman, 1960; Sawyer, 1986). Previous laboratory studies suggest that changes in availability of hosts can affect the population structure and dynamics of *H. medicinalis* (Sineva, 1944; Zapkuvne, 1972a,b; Sawyer, 1986; Wilkin & Scofield, 1990, 1991a). Fed only on frog blood, *H. medicinalis* reaches maturity after seven–nine feedings in 17–20 months at 17–19°C, but adding mammalian blood to the diet reduces the time and increases the size at maturity (Sineva, 1944). With the first two meals on amphibians and all subsequent meals on mammals, *H. medicinalis* reaches sexual maturity after six meals in 12–18 months (Sawyer, 1986). Based upon observations of developmental rates and fecundity of *H. medicinalis* fed blood from different natural hosts (Sineva, 1944; Zapkuvne, 1972a,b; Sawyer, 1986), and evidence that the diet of *H. medicinalis* in the field shows an increasing reliance on amphibian hosts (Wilkin & Scofield, 1990, 1991a), it was hypothesized that the decline in abundance of *H. medicinalis* in the field is the result of increased developmental times and reduced fecundity of leeches feeding predominantly on amphibians.

In this study, the feeding frequency of *H. medicinalis*, the size of meals, the number of meals required to attain reproductive maturity and the number of meals taken between iteroparous reproductive bouts were determined in the laboratory with *H. medicinalis* fed exclusively on mammalian (bovine) blood. In addition, the number of bouts of reproduction and the numbers of cocoons and hatchlings per cocoon produced were determined, and the demographic implications of changes in availability of natural hosts are discussed.

Materials and methods

Commercially obtained *Hirudo medicinalis* ($n = 30$) were maintained in 5000-cm³ aquaria containing

1000 cm³ dechlorinated City of Calgary tap water at 20°C with a 12 : 12 h light : dark regime and a flow-through filtration system to remove solid waste and nitrogenous compounds. The leeches were fed every 30 days with fresh cattle blood (37°C) in a pig gut casing. Commercial pig gut casings (sausage skins) were soaked for 48 h in distilled water to remove excess salts. A 20-cm section knotted at one end was filled with fresh bovine blood and closed by knotting. Each 'blood sausage' was attached vertically to the inner wall of an aquarium containing a single *H. medicinalis* and the leech was allowed to feed. If the leech attached and fed through the upper half of the 'blood sausage' it was inverted to ensure an *ad libitum* blood supply. Bovine blood was offered every 30 days to each leech. If an individual did not feed, blood was reoffered on days 31 and 32. All leeches fed with a frequency no greater than every 30 days and no lower than every 32 days.

Once every week containers were checked for cocoon production and hatchlings. Cocoons and hatchlings were removed and maintained in conditions identical to the adults. All life history characteristics are based on individuals hatched in the laboratory. Of the second-generation leeches, only leeches that hatched on the same day and took their first meal on the same day were subsequently monitored ($n = 7$). Consumption per feeding was obtained by subtracting the wet biomass (± 0.1 mg) of an individual leech prior to feeding from the wet biomass immediately having fed to satiation. Growth rates were based on the incremental wet biomass of leeches immediately prior to feeding. The time to first reproduction, time between reproductive bouts and number of reproductive bouts were determined, together with the number of cocoons produced per reproductive bout and the number of hatchlings per cocoon. Significant non-zero correlations between life history variables were fitted with simple linear regression models following Zar (1984). All statistical tests were conducted at $\alpha = 0.05$.

Results

The number of bouts of reproduction (cocoon production) ranged from two to nine (4.0 ± 0.98 , mean \pm SE) with an average time to first reproduction of 289 ± 39.44 days. As developmental time to first reproduction increased, the number of cocoons produced

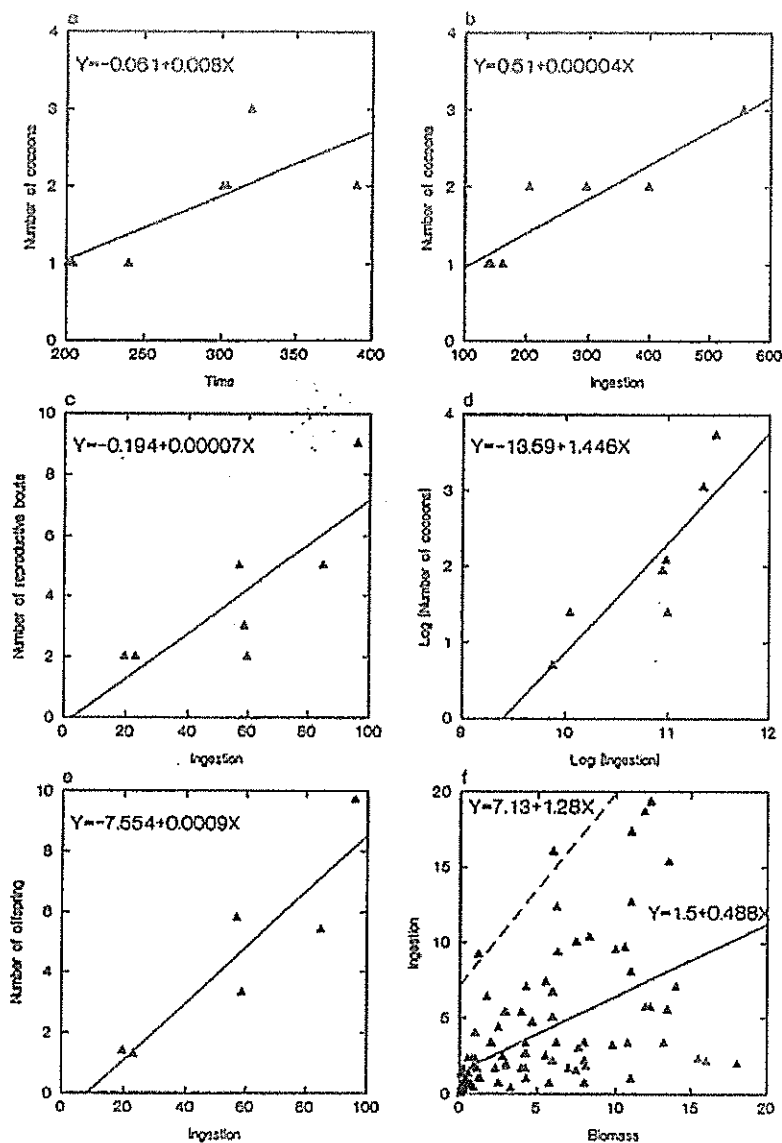


Fig. 1. Data per individual *Hirudo medicinalis*. (a) Number of cocoons produced in the first reproductive bout against time (days) to the start of cocoon production. (b) Number of cocoons produced in the first reproductive bout against the quantity of blood ingested ($\times 10^3$ mg wet weight) to the initiation of cocoon production. (c) Total number of lifetime reproductive bouts against total lifetime blood ingestion ($\times 10^3$ mg wet weight) to final reproductive bout. (d) Logarithmic transformation of total lifetime number of cocoons produced against logarithmic transformation of total lifetime ingestion of blood (mg wet weight) up to final reproductive bout. (e) Total lifetime number offspring produced ($\times 10$) against total lifetime blood ingestion ($\times 10^3$ mg wet weight) to final reproductive bout. (f) Biomass of blood ingested ($\times 10^3$ mg wet weight) plotted against initial leech biomass (mg wet weight). Dotted line represents the estimate by Lent *et al.* (1986) of blood ingestion *v* biomass. The solid line in each figure indicates a significant linear regression.

in the first bout of reproduction also increased significantly ($R^2 = 0.568$; $F_{5,1,0.05} = 6.587$; $P = 0.050$; Fig. 1a), with all individuals producing between one and three cocoons (1.71 ± 0.29). However, variance in the number of cocoons produced in subsequent bouts increased significantly with lifetime cocoon production per individual, and ranged from two to forty-one cocoons (12.43 ± 5.32 ; Table 1).

The number of meals prior to the first reproductive bout (8.86 ± 0.74) and over the entire lifetime (13.57 ± 0.95) showed a high degree of variability and was not significantly correlated with the total number

of reproductive bouts or the total number of cocoons produced. However, there were significant correlations between the quantity (mg) of blood ingested up to the first reproductive bout and the number of cocoons produced ($R^2 = 0.69$; $F_{5,1,0.05} = 11.15$; $P = 0.02$; Fig. 1b), between total quantity of blood ingested and the total number of reproductive bouts ($R^2 = 0.659$; $F_{5,1,0.05} = 9.68$; $P = 0.027$; Fig. 1c), and between \log_{10} total blood ingestion and \log_{10} total lifetime cocoon production ($R^2 = 0.729$; $F_{5,1,0.05} = 13.48$; $P = 0.014$; Fig. 1d). Total lifetime fecundity was also significantly correlated with total blood ingestion to final reproductive bout

Table 1. Life history variables for *Hirudo medicinalis* maintained at 20°C and fed to satiation exclusively on mammalian (bovine) blood every 30 days

| Life history variable | Mean \pm SE | Range |
|---|-----------------------|-----------------|
| Number of meals to first reproductive bout | 8.9 \pm 0.9 | 6.0–13.0 |
| Number of cocoons produced during first reproductive bout | 1.7 \pm 0.3 | 1.0–3.0 |
| Total blood ingested (mg) to first reproductive bout | 27095.2 \pm 5911.9 | 13833.3–55333.2 |
| Meal size (mg) to first reproductive bout | 3066.7 \pm 595.8 | 1576.9–6148.1 |
| Time (days from hatching) to first reproductive bout | 280.0 \pm 26.0 | 201.0–390.0 |
| Time (days) between reproductive bouts | 120.2 \pm 43.8 | 30.0–365.0 |
| Biomass (mg) at first reproduction | 8142.9 \pm 1615.4 | 3004.0–13800.0 |
| Blood ingested (mg) between reproductive bouts | 6213.7 \pm 1627.4 | 1011.2–11033.4 |
| Total number of meals taken prior to final reproductive bout | 13.6 \pm 1.0 | 10.0–17.0 |
| Total blood ingestion (mg) prior to final reproductive bout | 56904.7 \pm 10751.3 | 19499.8–95833.2 |
| Meal size (mg) between reproductive bouts | | |
| 1–2 | 5393.8 \pm 1769.1 | 1000.0–13791.7 |
| 2–3 | 7366.9 \pm 1812.5 | 2333.4–1072.0 |
| 3–4 | 10111.3 \pm 2377.7 | 7042.4–10683.6 |
| 4–5 | 11031.4 \pm 2757.1 | 8022.1–15742.3 |
| Total number of reproductive bouts per individual | 4.0 \pm 1.0 | 2.0–9.0 |
| Total number of cocoons produced per individual over lifetime | 12.4 \pm 5.3 | 2.0–41.0 |
| Total number offspring produced per individual over lifetime | 44.8 \pm 13.0 | 13.0–97.0 |
| Number of hatchlings per cocoon | 3.9 \pm 1.1 | 0.0–14.0 |
| Time (days) from hatching to death | 713.0 \pm 48.1 | 479.0–838.0 |

($R^2 = 0.821$; $F_{4,1,0.05} = 18.38$; $P = 0.013$; Fig. 1e; Table 1). As the biomass of *H. medicinalis* increased, a significant increase in the quantity of blood ingested at each feeding was observed ($R^2 = 0.27$; $F_{88,1,0.05} = 31.7$; $P < 0.001$), although the specific quantity of blood ingested (mg biomass blood ingested (mg leech biomass) $^{-1}$) decreased with increasing biomass of *H. medicinalis*. Although average meal size increased with each subsequent meal, the variance in meal size also increased (Table 1).

Average wet biomass of *H. medicinalis* at first reproduction was 8142.9 ± 1615.4 mg, with an average time between successive reproductive bouts of 120.2 ± 43.9 days and an average ingestion between reproductive bouts of 6213.8 ± 1627.4 mg of blood.

Discussion

Elliott & Mann (1979) and Elliott & Tullett (1986) suggested that *H. medicinalis* takes at least 2 years and could take 3–4 years to reach reproductive maturity in the field. In our study *H. medicinalis* fed to satiation on bovine blood every 30 days attained maturity after ≈ 9.5 months at an average size of 8142.8 mg. Sineva (1944) suggested that the optimum diet for *H. medicinalis* was achieved by allowing juvenile leeches to consume one or two blood meals from amphibian

hosts followed by all subsequent meals on mammalian blood, with reproductive maturity being attained within 8–10 months. Feeding on only mammalian blood, reproductive maturity was reached after 12–15 months, and on a diet solely of amphibian blood, maturity was not reached for 17–20 months (Sineva, 1944). The more rapid development to maturity in this study compared with that of Sineva (1944) is probably the result of differences in the methodology of feeding the leeches, which was not specified by Sineva (1944). In our study, mammalian blood was maintained at 37°C following Dickinson & Lent (1984), with regular intervals of 30–32 days between feedings. The effect that regularity of feeding has upon quantity of blood ingested can be seen by comparing the regression model for ingestion *v* wet biomass of *H. medicinalis* presented by Lent *et al.* (1988), based upon single feedings by leeches starved for a long period, with the ingestion rates obtained in this study (Fig. 1f). When fed regularly (every 30–32 days), the quantity of blood ingested at any feeding was significantly lower.

The higher energetic content of mammalian blood compared with amphibian blood (Andrew, 1965) should, all else being equal, provide leeches with more energy available for somatic and reproductive growth. An increase in the proportion of amphibian blood in the diet will result in less energy available for growth

by *H. medicinalis*, with longer developmental times to sexual maturity, more frequent feedings and possibly a smaller size at maturity with lower fecundity. This is supported by the observation that *H. medicinalis* requires longer to reach maturity when fed exclusively on amphibian blood, and does so at a smaller size (Sineva, 1944). The shorter times to maturity reported for *H. medicinalis* fed on mixed amphibian and mammalian blood diets (Sineva, 1944) cannot be attributed to an energetic advantage gained from ingesting amphibian blood compared with mammalian blood, but may reflect differences in foraging ability by juvenile *H. medicinalis* on amphibians and mammals. The conclusion reached by many researchers, that the optimal diet for *H. medicinalis* must include amphibian blood (Sineva, 1944; Sawyer, 1986; Wilkin & Scofield, 1990), is not substantiated by this study.

Wilkin & Scofield (1991b) reported a maximum biomass for *H. medicinalis* fed bovine blood of 29 000 mg after 300 days, compared with a biomass of 23 000 mg attained after 322 days in this study. Previous studies of *H. medicinalis* fed exclusively on bovine blood reported significantly lower growth rates, ranging from 8000–15 000 mg wet biomass within 12–18 months (Sawyer, 1986) to 2400 mg wet biomass after 36 months (Dresscher & Engel, 1946). Inconsistencies between studies of reported growth rates are probably the result of differences in feeding frequency and method of feeding (i.e. treatment, storage and temperature of blood).

In addition to the lower energetic value of amphibian compared with mammalian blood, the potential maximum size of a blood meal from an amphibian is also lower. In the laboratory, adult *H. medicinalis* provided with a mature live *Xenopus* often kill the amphibian and still remain unsatiated (i.e. will continue to feed if provided with another host). The smaller maximum potential meal size provided by amphibians suggests that feeding frequency should be higher in *H. medicinalis* populations feeding predominantly on frogs and newts rather than mammals. This is supported by Wilkin & Scofield (1990), who reported that foraging activity and feeding frequency were high in two field populations of *H. medicinalis* feeding extensively on amphibians. Increased foraging activity and feeding frequency would reduce the energetic efficiency of the leeches and potentially lower growth rates and fecundity.

Serological analysis of the gut contents of *H. medi-*

cinialis collected from two field sites in Great Britain showed that mammals were rarely fed on and that frogs were the most important hosts, with smaller dietary contributions from birds and fishes (Wilkin & Scofield, 1990), reflecting host availability. The population size distribution of *H. medicinalis* was skewed towards the smaller size classes, with sexual maturity attained after 24 months (Wilkin & Scofield, 1991a,b), compared with estimates of 2–4 years by Elliott & Mann (1979) and Elliott & Tullett (1986).

If *H. medicinalis* feeding on energetically inferior hosts (amphibians) has lower fecundity, leech abundance will decrease even if mortality remains constant (Begon & Mortimer, 1986). In addition, longer developmental rates (i.e. increased time lags) will probably decrease population stability (May, 1975) and reduce populations resilience to environmental perturbations.

In conclusion, previous studies (Sineva, 1944; Dresscher & Engel, 1946; Sawyer, 1986; Wilkin & Scofield, 1991b) demonstrated inconsistencies in the growth rates of *H. medicinalis* fed diets containing different proportions of amphibian and mammalian blood. These inconsistencies could result from differences in the preparation, storage and presentation of blood among studies. Mammalian blood has a greater energy content than amphibian blood, resulting in more energy available for growth. In our study developmental and fecundity rates were higher for *H. medicinalis* fed exclusively on mammalian blood than for populations feeding predominantly on amphibian blood. A greater reliance on amphibian hosts by *H. medicinalis* populations will result in lower available energy for growth (somatic and reproductive) and, hence, fecundity and fitness should decline. Recent changes in cattle and deer management have decreased their availability to *H. medicinalis* resulting in amphibians becoming the primary hosts (Wilkin & Scofield, 1990). This change in dietary composition is perhaps the primary reason for the observed decline in abundance and distribution of *H. medicinalis*.

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