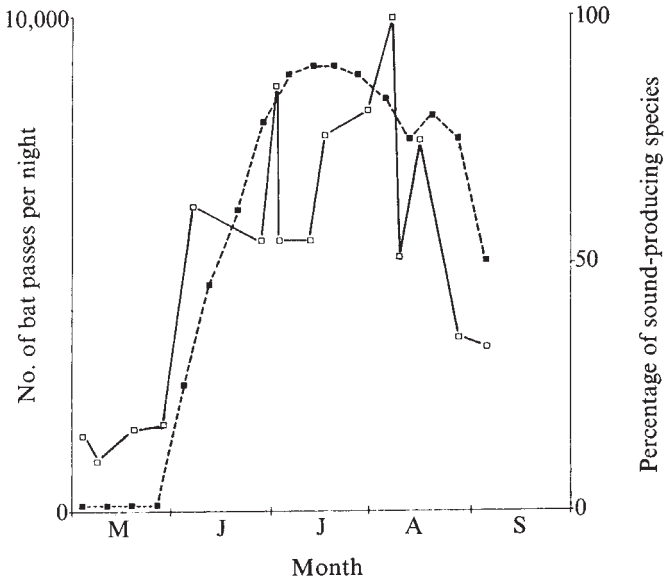
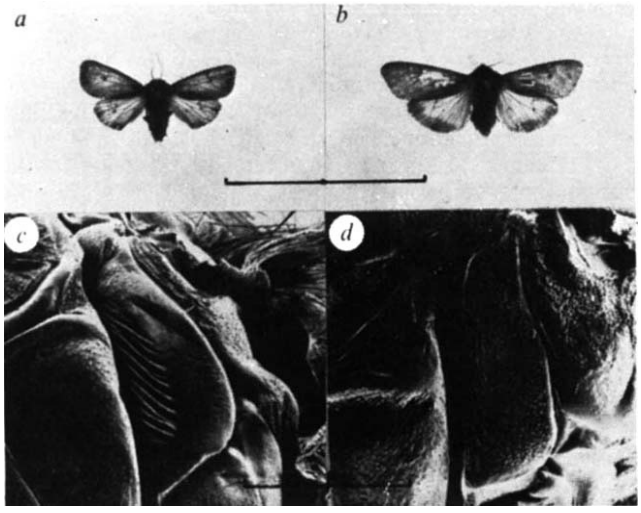


Fig. 1 Relationship between summer activity levels of insectivorous bats and phenology of sound-producing arctiid moth species. Bat activity (□) is recorded as 'bat passes'[8]; incidence of acoustically active arctiids (■) are the averages per quarter month.

signals intended for the ultrasonically sensitive ears of predators such as bats. Since the maintenance of such specific defences should be dependent on the level of threat posed by the predators concerned, species exposed to lower levels of activity of these predators should not possess the defences of individuals more severely threatened. Therefore, the high proportion of silent arctiids present in the spring may be a response to the lower levels of bat activity at these times.

Phragmatobia offers an intriguing example of this relationship. *P. assimilans* is not only a silent species, similar to its acoustically active summer relative, *P. rubicosa,* but also possesses a tymbal structure which suggests that *P. assimilans* may have been a sound-producing species at one time. By shifting its emergence time to spring and thus avoiding the time of high bat activity (although this may not have been the original reason for the shift), *P. assimilans* no longer required a functional tymbal and now retains only a vestigial form of the organ. *Hypoprepia*

Fig. 2 *a* and *c*, Acoustically active summer arctiid, *P. rubicosa*, and its tymbal. *b* and *d*, Silent spring species, *P. assimilans*, and its non-functional tymbal with what appears to be a vestigial micro-tymbal band. Upper scale indicates 40 mm; lower scale indicates 1 mm.



illustrates a similar situation, the important difference being that both species emerge during high levels of bat activity and both produce sound.

Silent species that persist into or emerge in mid-summer (for example, *Spilosoma virginica* (Fabricus) and *Estigmene* acrea (Drury)) may be less vulnerable to bats for a variety of reasons. *E. acrea* is a very large arctiid and this may afford it sufficient protection from most hunting bats. Since arctiids will perform aerial evasive tactics in the presence of echo-locating bats[12], there may be a greater adeptness at this and other anti-bat defences that have allowed certain silent arctiids to exist in times of high bat activity.

As bats are not the only ultrasonically sensitive insectivorous predators that arctiids must contend with, it is likely that the ambient summer levels of non-Chiropterans (for example, *Peromyscus*) may also exert an influence on the phenology of sound-producing arctiid species. The diverse interactions that arctiids have with their predators are poorly understood and must be clarified before some of the puzzling problems associated with comprehending this unique form of defence can be dealt with.

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Survival value of fever in fish

THE phenomenon of behavioural fever, manifested as an increase in preferred temperature following injection of live or killed bacteria, or other pyrogenic substances, has been demonstrated in bony fishes[1–3], amphibians[4,5], reptiles[5–10], and mammals[11,12]. Ectothermic vertebrates, including some newborn mammals[11], are unable to increase their body temperatures much above ambient by physiological means, and so are largely limited to behavioural thermoregulation by selecting favourable temperatures in their environment. The behavioural febrile response of a lizard, *Dipsosaurus dorsalis,* has been shown to have adaptive value[5,8–10] in conferring increased survival during infection by the Gram-negative bacterium *Aeromonas hydrophila. A. hydrophila* is pathogenic to bony fishes, amphibians and reptiles, causing haemorrhagic septicaemia. All three classes show similar (1–5 °C) elevations in preferred temperature in response to injection of this bacterium[1–10]. The absolute preferred temperatures, however, both normal and febrile, differ considerably among these vertebrates. The preferred temperatures of lizards such as *D. dorsalis* (about 40 °C) are quickly lethal to most fishes and amphibians. We report here that fever significantly

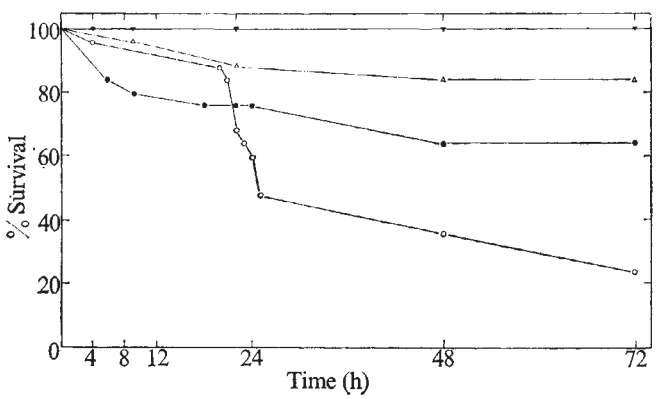
enhances survival of goldfish, *Carassius auratus*, at febrile temperatures about 10 °C lower than those of *D. dorsalis*, after injection with live *A. hydrophila*.

To study the thermoregulatory behaviour of fish we used an electronic shuttlebox device which allows a fish to control water temperatures and, thereby, its body temperature, by its movements between chambers monitored by photocells[13,14]. The behaviour of a fish in this device is equivalent to seeking out preferred temperatures in its natural environment[15]. At least some fishes have been shown to exhibit circadian changes in preferred temperature[14,15]. Specifically, the goldfish exhibits a diel rhythm of preferred temperature with a pre-dawn peak (W. W. R., M. E. Casterlin, J. K. Matthey, S. T. Millington and A. C. Ostrowski, in preparation). Accordingly, for this study we pooled data for 24-h periods[1-4], to simplify presentation of the data by showing changes in mean preferred temperature for 24 h before and after injection with bacteria.

Earlier work with largemouth blackbass (*Micropterus salmoides*) and bluegill sunfish (*Lepomis macrochirus*) indicated a mean febrile increase in preferred temperature of 2.6 °C after injection with killed *A. hydrophila*[1]. We therefore used a nominal febrile elevation of 2.5 °C to represent a typical fever in fish. We tested 10 goldfish in our thermoregulatory apparatus (Ichthyotron)[13]. These had a mean baseline (afebrile) preferred temperature of 27.9 °C, which agrees well with a previously published final preferendum value of 28.1 °C for *C. auratus*[16,17]. Using a nominal 28 °C baseline temperature and the nominal 2.5 °C febrile elevation value, we tested three groups of 25 goldfish at fixed temperatures of 25.5 °C, 28.0 °C and 30.5 °C for 72 h. These represented, respectively, hypothermic, normothermic and febrile temperatures. The 75 goldfish, weighing about 4 g each, were injected intraperitoneally with 0.1 ml of sterile pyrogen-free fish saline containing 2×10^6 live *A. hydrophila* cells.

Goldfish maintained at a febrile temperature of 30.5 °C (Fig. 1) had the highest survival rate (84%), while only 64% survived at 28.0 °C and 24% at 25.5 °C. A control group of 10 fish, injected only with sterile saline, all survived at 25.5 °C. Another 10 fish injected with live *A. hydrophila* and allowed to thermoregulate in the shuttlebox attained an unexpectedly high mean febrile preferendum of 32.7 °C, a mean increase of 4.8 °C. The circadian rhythm of preferred temperature was maintained around the higher set-point. The high 4.8 °C febrile increase may have been due to multiplication of the live bacteria, resulting in a higher weight-specific dosage in these goldfish, which were smaller than the bluegills and bass tested previously[1] with killed bacteria. It may be relevant that the ultimate upper incipient lethal temperature of goldfish is unusually high[16] (about 41 °C) for fish—about 6 °C higher than that of bluegill and bass. None of the 10 febrile thermoregulating goldfish died, so the 32.7 °C temperature afforded even greater protection (Fig. 1) than did the nominal febrile temperature of 30.5 °C.

Fig. 1 Survival curves for goldfish at four temperatures after intraperitoneal injection with live *A. hydrophila*. Three groups of 25 fish each were held at fixed temperatures of 25.5 °C (○), 28.0 °C (●), and 30.5 °C (△); 10 fish (▼) were allowed to behaviorally thermoregulate at a mean temperature of 32.7 °C.



To examine the effect of temperature on growth rate *in vitro* of *A. hydrophila*, we grew cultures at 28, 30 and 33 °C (Fig. 2). We determined growth curves by inoculating 100 ml of glucose nutrient broth or brain–heart infusion broth with 0.1 ml of a 14 h broth culture; at 1–2 h intervals the cultures were plated and turbidometric readings made. There was no significant difference among the growth curves at the three test temperatures (Fig. 2), so the survival value of the fever apparently cannot be attributed to an effect of temperature on bacterial growth within the range of temperatures selected by goldfish. This is also true for lizards (*D. dorsalis*) at still higher temperatures[8].

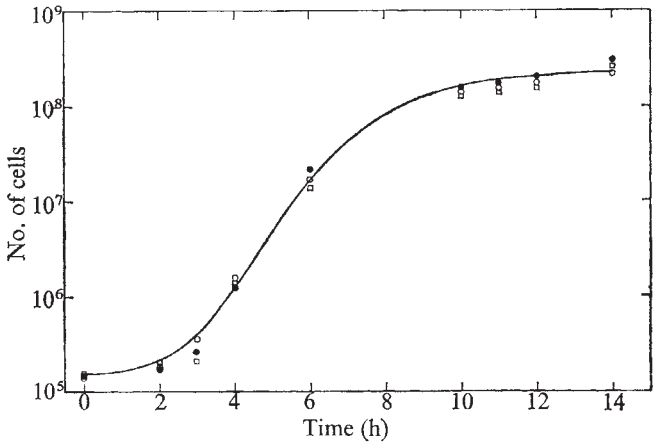


Fig. 2 Growth curves for three *A. hydrophila* cultures *in vitro* at 28 °C (□), 30 °C (○) and 33 °C (●).

Immunological responses of fishes and other poikilothermic animals have been shown to be significantly affected by temperature[18,19]. The critical temperature levels are species-specific[18], and vary considerably among classes[19]. For example, the normal body temperature of the lizard *D. dorsalis*[5-10] exceeds even the febrile temperatures of the fish species studied, and indeed would be lethal to most fishes and amphibians. It thus seems that the absolute febrile temperature *per se* is less important than the febrile elevation above temperatures normal for the species.

Similar enhancement of survival during a viral disease by elevation of the water temperature has been reported for sockeye salmon, *Oncorhynchus nerka*[20]. Mortality was prevented at 18 °C, which is 3.5 °C above the 14.5 °C normal preferred temperature of this cold-water salmonid species[17]. Amend[20] did not study behavioural responses to the virus infection. The virus was not destroyed by the 18 °C temperature, and enhancement of some critical hormone or enzyme activity was postulated as the cause of the increased survival[20]. Another possibility is that increased phagocytosis could result from increased vascular permeability leading to greater mobility of the antigen-processing cells in the early stages of infection. It has also been suggested[21] that there may be a relationship between temperature-dependent toxic effects of various substances and their effects on preferred temperatures of fishes, which might serve to reduce mortality.

Survival of infection can apparently be enhanced by fairly short-term increases in temperature, as the heliothermic *D. dorsalis* is able to thermoregulate only during the day, with body temperatures dropping to ambient

(12 °C) at night[10]; the daytime elevation of body temperature is apparently sufficient to enhance survival of infection[8–10]. Similarly, our febrile infected goldfish experienced no mortality following removal to holding tanks at room temperature (23 °C) after thermoregulating for several days at elevated temperatures. Avtalion[18] has shown that after fish are allowed to develop a positive immunological memory at a high temperature, they are then able to produce antibodies even at low temperatures. It thus seems likely that some initial step in the immune response is enhanced by a febrile increase above the normal preferred temperature of a species, and that this is effective even if the febrile temperature is not constantly or indefinitely maintained.

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Soluble fragments from fungal cell walls elicit defence reactions in crayfish

ALTHOUGH most fungi that parasitise arthropods are relatively harmless, many can penetrate different types of cuticle[1]. Thus active defence mechanisms presumably operate *in vivo*. In crayfish, for example, resistance to fungal penetration is associated with melanisation of the cell wall of the fungus by host phenols and phenoloxidase[2]. This constitutes a system for recognising the invading organism at different levels, from the cuticle surface inwards[3]. Melanisation in insect blood, due to phenoloxidase activity, is also induced by the presence of cells or cell walls of fungi[4,5]. Crayfish phenoloxidase in cuticle and blood is highly and specifically activated in contact with the purified hyphal cell walls of most fungi but not with other plant cell walls[6]. We have now found that soluble β-1,3-glucans from fungal cell walls activate the enzyme and may serve as specific elicitors of recognition in crayfish of invading fungal parasites.

Haemolymph was bled from the abdominal haemocoel of crayfish, *Astacus astacus*, using a hypodermic needle (0.8 mm diameter, 21 gauge). Because phenoloxidase is released by blood cells during clotting, cell free serum from pooled, clotted blood was used as a source of enzyme[6]. The clot was homogenised and centrifuged for 10 min at 3,000*g*. The phenoloxidase activity of the serum obtained was determined in a mixture of 0.2 ml serum, 0.1 ml dihydroxyphenyl alanine (dopa, 4 g l^{-1}), and 0.2 ml 0.01 M sodium acetate buffer, *p*H 5.2, with or without dissolved cell wall extract. The mixtures were incubated at room temperature, about 22 °C, in an open microscope slide cuvette[7] for about 30 min. Before addition to the serum mixture the cell wall extract was preheated to 100 °C for 20 min to disperse aggregates. After covering the slide cuvette with a coverslip the activity of phenoloxidase on dopa was calculated from the increase in absorbance at 480 nm using a Shimadzu MPS-50 L photometer[7].

Zymosan (purified cell walls from *Saccharomyces cerevisiae*) activated crayfish phenoloxidase, especially in regions close to the cell wall surface (Fig. 1). This effect is similar to that seen in fungal cell walls purified in alcoholic KOH[6]. Furthermore, the supernatant from centrifuged suspensions of Zymosan or purified cell walls (even after six washings) of different fungi strongly activated phenoloxidase. Apparently, water-soluble fragments with activating capacity were released in very small concentrations from the solid cell walls.

In the present work we used the supernatant (containing the yeast cell wall activator) from a 1% suspension of Zymosan suspended in 0.01 M sodium acetate, *p*H 5.2. The molecular weight of the activator was estimated on a column (26 mm × 550 mm) of Sephadex G 200, using acetate buffer at *p*H 5.2. Blue dextran 2000 (Pharmacia), bovine γ globulin and trypsin (both Sigma) were used as standards. When compared with the standards, the apparent molecular weight of the activator was between 10^5 and 10^6; small amounts of material with a molecular weight between 3×10^4 and 10^5 were also present. It seems that the elicitor is not a distinct molecule but consists of macromolecules or molecular aggregates of varying size.

Most elicitor activity was not bound to a cation (SP-Sephadex C 25), nor to an anion exchange resin (DEAE-Sephadex A 25) when the material was dissolved in and eluted with 0.01 M sodium acetate buffer, *p*H 5.2, and 0.01 M Tris-HCl buffer, *p*H 8.2, respectively and was apparrently not charged to any detectable extent at these *p*H values.

The activator solution (from 1% Zymosan) contained about 0.4 g of dry material per l (desiccation at 100 °C for 3 h). It activated phenoloxidase even when diluted 10,000-fold. Elicitor activity could therefore be measured at least down to a concentration of about 10^{-11} M.

The yeast cell wall elicitor was also pretreated at 22 °C for 1–24 h with different hydrolytic enzymes (0.2 mg ml^{-1}) to investigate the possibility that the elicitor molecules

Fig. 1 Zymosan activation of phenoloxidase of the cell free serum of crayfish. In the presence of a substrate, dopa, the cell walls thereby become melanised (dark). *a*, Dopa added; *b*, dopa not added. (×1,000).