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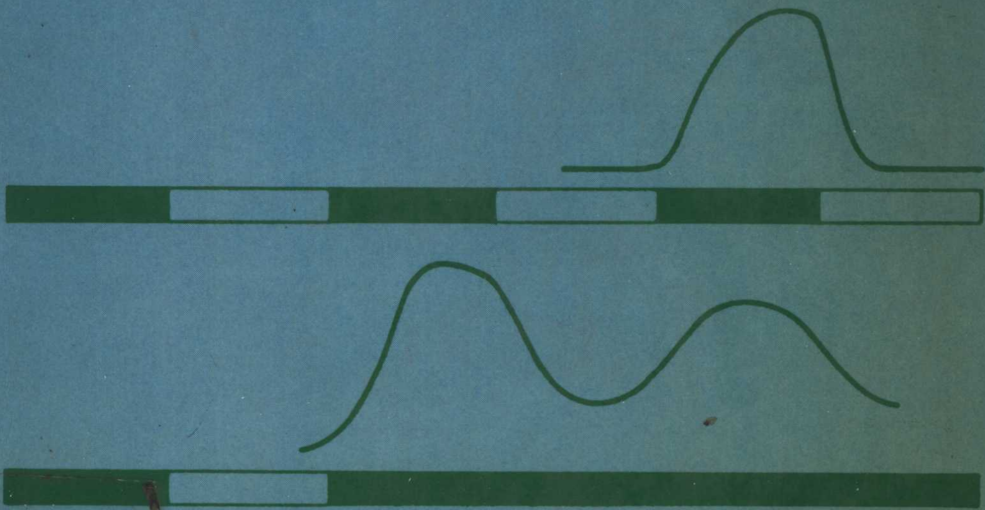
Edited by J. Brady

Biological timekeeping



BIOLOGICAL TIMEKEEPING

Edited by

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PREFACE

This book is the outcome of the first really multidisciplinary meeting on biological clocks to be held in Britain – a one-day Seminar organised by the Society for Experimental Biology at Imperial College, London, on 26 March 1980. The papers presented were mainly concerned with broad reviews and recent scientific advances. The book, however, has gathered the same authors together specifically to write a student text ranging widely over the whole subject. It is thus aimed particularly at third-year undergraduates and those embarking on research into biological clocks. The former should find the basis for an excellent specialism essay in any one of our eleven chapters; the latter, by reading the book through, should gain a clear insight into the whole field. There are other introductory books, but none at this level that provides such a balanced and well-informed coverage of both plant and animal clocks.

Aiming at this target has led to some changes in the usual format of the Society's Seminar Series. Primarily, this has meant getting all the chapters written at a level which spends time explaining the background of each topic before outlining its present 'state of the art'. A further change has been the insertion of a cross-referencing system to interlink the wide-ranging aspects of the subject. This not only saves the different chapters from having to go over some of the same ground, but also leads the reader into the several parallel avenues of research that have arisen from the subject's multidisciplinary nature: plants and animals do the same kinds of timekeeping by different mechanisms. Finally, we have included a rather full Glossary which, if read from start to finish, would provide an adequate if indigestible introduction to the formal characteristics of biological clocks.

To my co-authors, I express sincere thanks for their indulgent cooperation in the slightly unusual editorial disciplines of producing a multi-author student text-book. Without their specialist inputs the book could not possibly speak with the broad authority it does. To Louise Sanders of the Cambridge University Press, we are all indebted for a painstaking removal of many infelicities of style; scientists, even those who think they can edit, really do not write English very elegantly.

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GLOSSARY

Terms and symbols commonly used in the literature of biological timekeeping. Note that though the examples given mostly refer to daily (24-h) rhythms, they are, by analogy, usually also used in the same context for tidal (12.4-h), semilunar (14.8-day), lunar (29.5-day), and annual rhythms.

Symbols and abbreviations

(*terms in italic are defined elsewhere in the Glossary*)

LD	Light: dark; as in LD 12:12 meaning a cycle of 12 h light: 12 h darkness.
LL	Continuous light.
DD	Continuous darkness.
τ	('tau') The 'natural' period of a biological rhythm *free-running* in constant conditions (especially in DD or LL).
T	The *period* of a *zeitgeber* cycle; most commonly used when that is other than 24 h for *circadian* rhythms.
ϕ	('phi') A *phase* of a biological rhythm; e.g. daily onset of locomotor activity, daily peak of photosynthesis; especially used as ϕ_i to indicate the inducible phase of a circadian rhythm in *photoperiodic* induction (§5.5).
$\Delta\phi$	('delta phi') A *phase shift*, expressed in hours or degrees of *phase angle* (Fig. G.1).
ψ	('psi') A *phase angle* of one rhythm relative to another, especially to a *zeitgeber*; sometimes expressed as hours, but more correctly as degrees, with one full cycle of a rhythm = 360° (Fig. G.1).
CAM	Crassulacean acid metabolism; special photosynthetic mechanism in some succulent plants, much used for studying plant rhythms (see §10.5).
PRC	See *phase-response curve* (p. xv).
Q_{10}	Temperature coefficient; the change in rate of a process (expressed as a multiple of the initial rate) produced by raising the temperature 10 °C. Usually more than 2 for most physiological processes, but characteristically near 1.0 for most biological timekeeping – see *temperature compensation*, below, and Fig. 1.1.
SCN	Suprachiasmatic nucleus in the vertebrate hypothalamus – a 'clock' (see §11.4.2).

Terms

(*terms in italic are defined elsewhere in the Glossary*)

Aestivation; summer or dry season *diapause* or *dormancy*.

Amplitude; the peak to trough difference in a biological oscillation (Fig. G.1); in ideal physical oscillators the mean to peak difference.

Azimuth; the compass-bearing to the point on the horizon vertically below the sun, or moon, etc. (§4.4).

Biological clock; a term implying an underlying physiological mechanism that times the overt measurable rhythm or other form of biological timekeeping; see also *oscillator* (§1.2, 1.3).

Biological rhythm; a cyclical variation exhibited in a biological function; especially daily, tidal, monthly and annual rhythms (§1.1).

Circadian rhythm; a cyclical variation in the intensity of a metabolic or physiological process, or of some facet of behaviour, with a *period* (τ) of about 24 h when in constant conditions (the term 'circadian' is derived from the Latin for 'about a day'). It is often incorrectly applied to rhythms that have been measured only in natural or artificial 24-h day–night cycles. These may or may not persist in constant conditions (§2.2.2), and until it is known, or can be reasonably assumed, that they do, the term *diel* is the correct label.

Circatidal, also *circasemilunar*, *circalunar* and *circannual rhythms*; biological rhythms with periods of about 12.4 h, 15 days, 29 days, and 12 months when *free-running* in constant conditions.

Crepuscular activity; an activity mainly performed at dusk and/or dawn.

Critical photoperiod; that 24-h LD ratio at which 50% of the population under study is *photoperiodically* switched from one state to another, e.g. into flowering from non-flowering, or into development from *diapause* (§5.2.1).

Diapause; a period of arrested growth or reduced physiological activity, commonly induced by a seasonal change in *photoperiod* (i.e. day-length); a term used mainly for invertebrates, especially insects. Distinguished from mere *quiescence* by lasting for a fixed minimum length of time, usually several weeks or months, and by the *dormancy* not being broken immediately upon the return of equable conditions.

Diel rhythm; a rhythm that has been measured only in natural or artificial day–night cycles, and not yet known to persist in constant conditions – cf. *circadian*.

Diurnal activity; activity performed mainly during the daytime – cf. *nocturnal*.

Dormancy; a term used either more or less synonymously with *diapause*, but especially for plants, or to mean a period of *quiescence* caused directly by adverse conditions, and lasting only while they prevail.

Endogenous; of rhythms or other biological timekeeping controlled from within the organism by some kind of physiological '*biological clock*' (§1.2, 2.2, 3.3.1).

Entrainment; the synchronisation of one biological rhythm to another of closely similar frequency, especially to a *zeitgeber* cycle, e.g. a circadian rhythm entrained to exactly 24 h per cycle by LD 12:12 (§2.3).

Equinox; the day when the sun passes directly over the equator so that there is a 12-h day and 12-h night over the entire earth. The *vernal equinox* occurs on about 21 March and the *autumnal equinox* on about 21 September – cf. *solstice.*

Exogenous; of rhythms or other biological timekeeping that arise solely, or even mainly, as direct responses to environmental signals (e.g. *diel* 24-h rhythms) – cf. *endogenous.*

Free-running; of rhythms running at their own 'natural' frequency (see τ) in constant conditions, and which are therefore not *entrained* to a *zeitgeber* such as a day–night cycle.

Frequency; the reciprocal of the *period* of a rhythm, e.g. once per 24 h.

Gating; the timing of a biological function so that it occurs only during a limited space of time (the 'gate'); especially refers to the circadian timing of once-in-a-lifetime events such as hatching, and used most commonly for insect adult emergence (§8.4.3).

Hibernation; winter or cold season *dormancy* or *diapause*; especially in vertebrates – cf. *aestivation.*

Hour-glass; an interval-timer which does not oscillate (i.e. repeat its timing cycle) in constant conditions. It is used especially in relation to *photoperiod* measurement (§5.4).

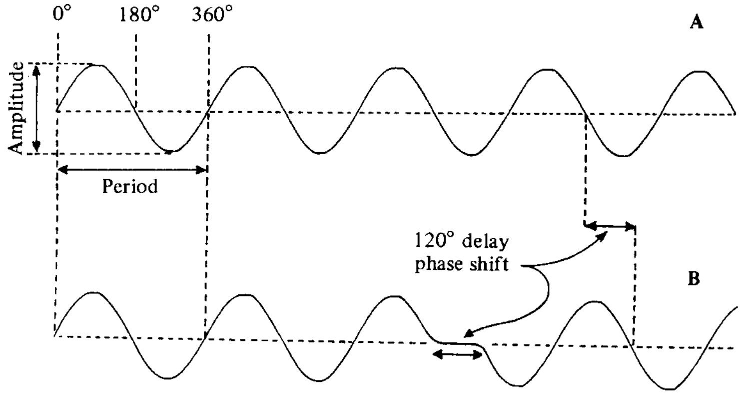
Neap tides; the smallest tides of the 15-day, semilunar cycle (Fig. 3.1).

Nocturnal activity; activity performed mainly at night – cf. *diurnal.*

Oscillator; usually applied to the unseen, *endogenous* 'driving' oscillator (the *biological clock*) whose influence from within the organism causes the measurable changes seen as the overt rhythm.

Period; the length of one complete cycle of a rhythm (Fig. G.1); reciprocal of *frequency*; τ for a *free-running* rhythm.

Fig. G.1. (**A**) Some oscillator terminology illustrated with a pure sine wave. (**B**) The same oscillation illustrating a phase shift relative to **A**. See also §2.2.1.



Phase; a particular reference point in the cycle of a rhythm, e.g. the daily onset of locomotor activity, or the light-to-dark transition in a *zeitgeber* cycle – see ϕ, above.

Phase angle; see ψ above (Fig. G.1).

Phase–response curve; the 24-h profile of an organism's *phase shifts* in response to environmental signals (Fig. 2.6).

Phase shift; a re-setting of a rhythm (Fig. G.1); either as an *advance shift* (i.e. forward, or earlier = $+\Delta\phi$), or as a *delay shift* (i.e. backward, or later = $-\Delta\phi$).

Photoperiodism; the seasonal day-length responses that cause altered physiological states such as flowering or non-flowering, diapause or development; the photoperiod is usually taken as the time between lights-on and lights-out in an artificial LD cycle (though many photoperiodic responses are in reality to changes in *night* length) – see *critical photoperiod* (§5.2.1).

Photophase; the light part of a LD cycle, usually used in a non-photoperiodic context.

Phytochrome; the plant pigment which is red/far-red light reversible and is involved in *photoperiodic* responses (§7.2).

Pulvinus; a specialised leaf or petiole joint which causes the so-called 'sleep' movements of leaves and leaflets in plants, especially in Leguminosae.

Rhythm; a function which oscillates at a regular *frequency*; biological rhythms (*circadian, circatidal,* etc.) are the overt, measurable activities whose periodicity is taken to reflect that of some internal *oscillator* (or 'clock') driving them.

Skeleton photoperiod; experimental photoperiod cycle consisting of one long light phase followed by darkness interrupted by a short light break, with the organism responding as if the interval between the first light-on and the second light-off marked a single photophase (§5.3).

Skotophase (or *scotophase*); the dark part of a LD cycle.

Solstice; the day when the sun reaches its furthest declination north or south, on about 21 June and 21 December, i.e. half-way between the *equinoxes*.

Spring tides; the largest tides of the 15-day, semilunar cycle (Fig. 3.1).

Synodic month; the lunar month of 29.5 days between new moons; it includes two complete *spring-tide* cycles of 14.8 days (Fig. 3.1).

Temperature coefficient; see Q_{10}.

Temperature compensation; the phenomenon (best known for τ in *circadian* rhythms) whereby biological timekeeping typically does not respond to different levels of constant ambient temperature in the way that would be expected if it obeyed normal physiological Q_{10} principles; it has Q_{10}s of around 1 instead of 2 or more (see Table 2.1).

Transients; unstable cycles which occur in a rhythm that has been subject to a large *phase shift*; for example, a *circadian* rhythm subjected to a reversal of its previous LD 12:12 *zeitgeber* cycle, usually takes a few days to stabilise at its new setting.

Zeitgeber; from the German for 'time-giver'. A periodic environmental signal that *entrains* some *biological rhythm*, for example a natural or artificial day–night cycle for a *circadian* rhythm (may also be a temperature cycle, or a social cycle) (see §2.2, 3.3.2, 9.4).

Zeitgedächtnis; from the German for 'time-memory'. Used mainly in reference to the time-sense in honey bees by which they can be trained to come to feed at particular times of day (§4.2, 4.4).

Zugunruhe; from the German for 'migration restlessness'. Describes the continually repeated, directionally biased hopping movements towards the migratory course that birds in a migratory 'mood' make when confined in a cage (§4.3).

CONTENTS

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1 Introduction to biological timekeeping

1.1 Biological timekeeping phenomena

Many of the pressures placed on organisms by the environment arrive more or less arbitrarily – sudden death, meeting a rival, infection, cloud cover – but many more arrive quite predictably, in association with the appearance of daylight, low tide, full moon, onset of winter, and so on. The ability to anticipate these events in order to avoid their dangers and exploit their benefits must clearly be of great adaptive advantage (see e.g. §2.4, 3.3.1, 4.2, 6.1). It comes as no surprise, therefore, to find that apparently all organisms or at least all eukaryotes, have evolved various forms of timekeeping to gear their lives to fit this time-tabling of the environment. Nor is it a surprise that such timekeeping ability is usually built into the organisms' genome, and is inherited just like any other physiological characteristic (§11.5.1).

The ability to keep time demands the possession of some mechanism to measure it – a clock. What man-made clocks do is mark the passage of time against equal segments of some process occurring at a constant rate, for example the governed unwinding of a spring, the fall of a weight on a chain, the cycling of mains a.c. electricity, etc. For living organisms there are in principle two different ways of measuring time: either they can rely on the exogenous periodicity of the environment, using that as their clock so that they get up at sunrise, flower at mid-summer, hide under stones at low tide, and so on; *or* they can use some internal process to measure time, rather in the sense that a man-made clock does. There are examples of the former type of timekeeping in this book, a particularly clear one being discussed in §3.3.1, but for the most part the book concerns the much more intriguing second type. The basis for believing that these latter do represent endogenous physiological chronometry is argued in §2.2.

A clock can be used to do three kinds of timing.

(1) *Time-set*; set something to be 'on time' relative to environmental time, for example to be awake during daylight hours, lay eggs in spring, or hide under stones before low tide.

(2) *Measure duration*; time the length of something, for example the number of hours of light in a day in order to gauge the season, or, in a slightly different sense, time the duration of a longer period by adding up numbers of days.

(3) *Find local time*; for example in order to be able to navigate by celestial cues.

Biological systems can evidently do all of these things, and variants of them, by one means or another. The actual timekeeping occurs in five general forms.

(a) *High frequency physiological rhythms*; as in heart-beat, leaf fluttering, neurone spiking, etc.

(b) *Environmentally related rhythms*; that is those that follow the five basic environmental cycles of the 24-h day (Chapter 2), 12.4-h tides, 14.8-day spring tides, 29.5-day lunar month (Chapter 3), and the year (§4.7, 5.6).

(c) *Continuously consulted clocks*; of the kind needed to know what the time is at any given time of day, as used by bees visiting different species of flower that open at different times, or as used in sun-compass navigation by bees and migrating birds (Chapter 4).

(d) *Photoperiodism*; responses to seasonally changing day-length (Chapter 5).

(e) *Dormancy duration*; when seed dormancy, hibernation or diapause (§6.3.1) have a fixed minimum period of quiescence.

1.2 Clock concepts

The prime characteristic of a good clock is that it shall continue to measure time at the same rate under all conditions. For this reason man has expended considerable ingenuity in devising means to make mechanical clocks temperature-compensated, by using bimetallic curves on balance wheels, mercury columns in pendulum staffs, and so on. The point is that if circumstances change the basic rate constant of the process being used in the clock as a model of time (e.g. the unwinding spring, etc.), then the duration of the measured units of the process will vary relative to 'real', astronomical time.

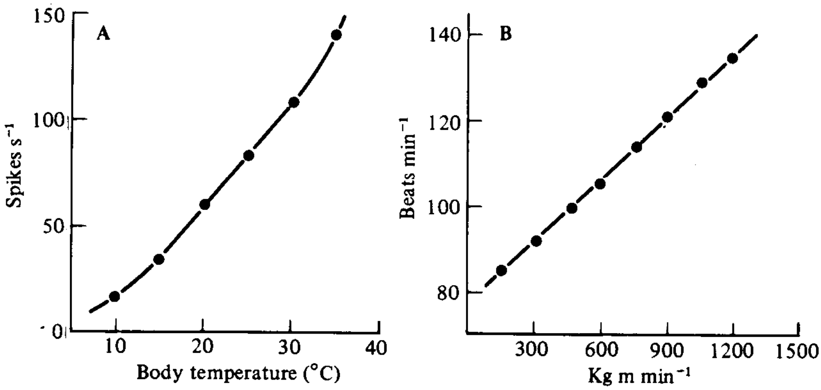
It may be that biological clocks use high frequency physiological oscillations of the type (a) above as their basic time-dependent process, and some hypotheses for biological clock mechanisms explicitly invoke that possibility (§10.2.1), but there are real difficulties for this idea. The most important is that the rates at which these high frequency rhythms run is much affected by irrelevant physiological conditions, especially temperature (Fig. 1.1). The function of these rhythms, of course, is not to measure time; it is just that they happen to work by oscillating, so they do measure time in the sense of marking it out in units of equal length, but the speed at which they oscillate is in practice regulated not against external time but in response to internal physiological demand. They are not, therefore, true biological clocks any more than the clockwork motor of a toy car is a true mechanical clock.

These high frequency rhythms, type (a), are therefore not further considered as real clocks in this book, which concerns itself only with those biological processes that (1) measure time by some means, (2) do so in relation to environmental time-cues such as sunset, high tide, day-length, etc., and (3) use this temporal information to control the *timing* of the organism's biochemistry, physiology or behaviour. The dormancy duration type of biological clock, (e) above, falls within this definition, but its time-measuring mechanisms have been little studied and it is here only briefly touched upon (in Chapters 5, 6 and 7). That leaves us essentially covering environmentally-related rhythms (b), sun-compass type clocks (c), and photoperiodism (d).

Any clock, whether man-made or biological, consists in principle of five notional components.

(1) '*Hands*'; the overt, measurable processes that are regulated by the escapement.
(2) '*Mainspring*'; the source of energy that drives the system.
(3) '*Escapement*' (plus balance wheel or pendulum); the primary regulating oscillator, or pacemaker.
(4) '*Cogs*'; the coupling that links the 'escapement' to the 'hands'.
(5) '*Adjuster*'; the mechanism for shifting the 'hands' to entrain them to external astronomical time.

Fig. 1.1. The lack of constancy in high-frequency biological rhythms. **(A)** The effect of temperature on the rate of generation of spontaneous spikes in a nerve running to the eye muscle of the blowfly; between 15° and 30 °C the relationship is linear, with a Q_{10} of nearly 2. **(B)** The relationship between the heart rate of a fit middle-aged man and his rate of energy expenditure measured as Kg lifted 1 m min^{-1}. (After Brady, 1979.)



In terms of biological clocks, the 'hands' are all those rhythmic and other time-tabled phenomena that the organism exhibits overtly to the observer, such as locomotor activity, leaf movement, flowering, and dormancy. It is through these phenomena that the researcher must draw his inferences about the unseen driving oscillator (the 'escapement') within. They thus provide the same kind of information to him as does the sound of the heart-beat in a stethoscope to a doctor studying heart disease.

The 'mainspring' energy demands of biological clocks are apparently exceedingly small. Circadian (that is, about 24-h) rhythms, for example, though temporarily stopped by deep anoxia, re-emerge unchanged in phase when the animal or plant recovers. Virtually nothing else is known about the energy demands of clocks, though there are models which invoke energy metabolism as a basic component in plant circadian timing (§10.2.2).

The ultimate objective of much research is to discover the 'escapement' mechanism, that is, the underlying driving oscillator. So far, no such mechanism has been unequivocally identified, though there are certain strong hints as to the kinds of process that may be involved (Chapters 10 and 11). The much better understood component is the 'cogs' coupling the driving oscillator to the overt rhythm. For animals, at least, both hormonal and neural links occur (Chapter 11). The ability to synchronise with environmental periodicities is, of course, the vital adaptive function of biological clocks. The 'adjuster' which permits this re-setting, and thence entrainment of the clock to the environment, must operate at two levels, both as a receptor mechanism to detect the environmental time-signal (sunset, low tide, etc.), and as a system which couples that information to the driving pacemaker and shifts its oscillation appropriately towards the environment's (§2.3, 3.3.2).

It is common jargon to talk of '*the* clock' in discussions of biological timekeeping, but this short-hand is misleading. It disguises the fact that in all multicellular organisms there are probably as many clocks as there are cells. Since both protista and individual metazoan cells in culture keep time (§11.5.2), it is assumed that all five of the above components reside in each and every eukaryotic cell. 'The clock' in the metaphytan, and even more so in the metazoan, is thus in reality a unified complex of cellular and tissue-organised timekeeping, a hierarchy of clocks (§8.2, 11.3).

1.3 Biological clocks as oscillators

Three points are worth noting here. First, the evidence increasingly suggests that tidal rhythms, sun-compass clocks, and photoperiodism are all, or nearly all, based on the fundamental 24-h (circadian) rhythmicity of organisms (see also §5.7). It thus appears that the environmental effects of the earth's rotation about its axis have produced, by natural selection, a