

part of the marine flora, and occasionally it invades marine animals where it may become a potential human health problem.

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## Larval Trematodes: Double Infections in Common Mud-Flat Snail

**Abstract.** Larvae of the trematode *Zoogonus lasius* are involved in most double infections of *Nassarius obsoleta*. The two most common trematode parasites of this snail do not occur together in double infections. Double infections were found in 14 of 340 infected snails in a total sample of 5025 snails.

There have been a number of studies on snails infected with more than one species of larval trematode. Cort *et al.* (1) demonstrated that while some species of trematode larvae coexisted in the same host, other combinations of species were rarely or never found together. Ewers (2) suggested that infection with one species of trematode predisposes some mollusks to infection with another species. Recent evidence supports the idea that echinostome redia are antagonistic to development of another species of larval trematode in the same host (3). With some species, echinostome larvae can completely eliminate a competing species, while in other instances the echinostome remains dominant but does not completely eliminate the other species.

The common mud-flat snail *Nassarius obsoleta* has been recorded as the first intermediate host for at least eight species of larval trematodes in the area of Beaufort, North Carolina. Some of the snails serve as a host for double infections. This study was undertaken to determine the extent of such double infections in a natural population and what combinations of larvae could be found coexisting in a host.

*Nassarius obsoleta* were collected between 17 June and 25 July 1968. The snails were isolated in finger bowls and checked for shedding cercariae. Snails which were shedding cercariae were then dissected and their tissues examined. Of 5025 snails, 340 (or 6.8 percent) were infected, 326 of them with a single trematode, 14 with double infections (Table 1). In half of the double infections, both species of cercariae were being shed. The others were shedding but one species, and the

Table 1. The most commonly found species of larval trematodes as found in 5025 *Nassarius obsoleta*. There were 326 single infections, and 14 double infections.

Species in single infections	Single infect- ions (No.)	Double infect- ions (No.)
<i>Lepocreadium setiferoides</i>	103	5
<i>Himasthla quissetensis</i>	89	3
<i>Zoogonus lasius</i>	72	12
Strigeid cercaria (probably <i>Cardiocephalus brandesii</i> )	27	1
<i>Austrotilharzia variglandis</i>	20	3
<i>Stephanostomum dentatum</i>	14	2
<i>Gynaecotyla adunca</i>	1	0
Monostome cercaria	0	2

double infection was obvious only upon examination of the digestive gland of the snail. A comparison of the actual number of double infections with the expected number (assuming that the proportion of double-infected snails among the total infected snails was equal to the proportion of infected snails among the total population, and that all double-infected snails can be detected) gave a chi-square value of 2.41, which is not significant. Therefore, out of this population sample, the number of double infections did not differ significantly with the expected number.

The combinations of infections that did occur were not significantly different than would be expected on the basis of relative abundance of species found in single infections. There are, however, two points that are statistically significant. (i) One species, *Zoogonus lasius*, is involved in 12 of 14 double infections. When the actual number of *Z. lasius* involved in double infections is compared with the expected number, the chi-square value is 9.2 with a probability of .005. Thus it is highly improbable that this many larvae of *Z. lasius* would be involved in a double infection based on random selection. (ii) The most common trematodes, *Lepocreadium setiferoides* and *Himasthla quissetensis*, were never found together (chi square, 6.6;  $P < .01$ ). Again, it is quite unlikely that these two would not be found together if the double infections were the result of random selection.

It is not clear from the present study why *Z. lasius* is involved in such a high percentage of the dual infections. This species is not the most commonly found larval trematode in *N. obsoleta*.

It has been demonstrated that each of the larval trematodes alters the thermal acclimation patterns of cytochrome c oxidase in the host tissue. Furthermore the alteration in host response is distinctive for each species of trematode. That is, the thermal acclimation patterns of this enzyme in digestive gland tissue from snails infected with one species of trematode is quite different from that of the same tissue from snails infected with another species (4).

Since the larvae are carefully dissected out of the host tissue before the assays are made, these differences in response are not due to the parasite itself, but rather to the alterations in the host tissue due to the presence of the parasite. This suggests that

perhaps the biochemical alteration of the host tissue by one parasite makes it unsuitable for another species, thereby limiting the species involved in double infections.

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### Circadian Rhythm in Mammalian Body Temperature Entrained by Cyclic Pressure Changes

**Abstract.** A 24-hour cycle of pressure (1.0 to 1.09 atmospheres) can act as a zeitgeber to entrain the endogenous circadian rhythm of body temperature in pocket mice (*Perognathus longimembris*) under constant conditions of environmental temperature and light.

Many biological rhythms involve events which recur at intervals of approximately a day and have been termed circadian (1). Although it has been suggested that external environmental cues are responsible for the persistence of these rhythms (2), the bulk of the available evidence supports the usually accepted interpretation that these rhythms are the manifestation of innate oscillations maintained by yet undescribed physiological mechanisms (3). Under normal circumstances, circadian rhythms are synchronized and have their phase set by environmental cycles of light and, in some cases, by cycles of temperature (4).

Under constant conditions of light and temperature, environmental cycles of sound can also act as weak entraining agents for the circadian rhythms of birds (5). Other environmental factors, such as feeding regimes, social interactions, barometric pressure, and magnetic fields have been proposed but not

rigorously tested as potential synchronizers (6).

We consider the following criteria essential for the demonstration that an environmental cycle can entrain a circadian rhythm: (i) the free-running period of the experimental individuals must be demonstrated to differ from that of the imposed cycle; (ii) the rhythm examined must assume the same period as that of the stimulus cycle; and (iii) it must be shown that the phase of the rhythm, in a subsequent free run, differs from a simple extrapolation of the preentrainment free run (5). On the basis of these criteria, we have now demonstrated that large changes in ambient pressure can induce entrainment of a circadian rhythm, when light and temperature are held constant.

The metabolic rate and body temperature of the little pocket mouse have been shown to oscillate with a circadian period (7). A manifestation of the daily temperature cycle in isolated animals is a daily torpor during which body temperature drops to near ambient. The duration of the torpor is variable between individuals and ranges from hours to days, depending upon the experiment conditions. The time of arousal from torpor is an easily distinguishable event which is clearly under the control of the circadian system, because the time of arousal is predictable as a function of the circadian period even for animals which may remain in continuous torpor for more than 1 day. Infrequently, some animals will enter torpor and arouse more than once each day. This usually occurs during the transient period in disturbed

animals that have not yet stabilized to a new environment. Even less frequently an individual animal may skip a torpor period. For experiments of several weeks' duration, neither of these latter two occurrences detract from the use of the interval between successive times of arousal as a measure of the circadian period in pocket mice.

Our data are based upon continuous monitoring of body temperature of eight pocket mice for an interval of 6 weeks. Temperature monitoring telemeters were implanted within the abdominal cavity several months prior to the experiment. The animals used included both males and females and all had previously shown a clear tendency for daily torpor when housed under normal laboratory conditions.

Four animals were housed individually in separate sealed chambers provided with incurrent and excurrent airflow systems. A timer and a series of solenoid valves were located outside the chambers and were used to route the excurrent air through a water manometer to pressurize the system. Four control animals were housed in similar chambers except for the excurrent flow control system. Constant conditions of 20°C and complete darkness were provided by an incubator which was sealed for the duration of the experiment. The animals were provided in advance with an adequate supply of mixed seeds. No drinking water is necessary for this species.

The pressure cycle consisted of 12 hours at ambient pressure and 12 hours at ambient plus an additional 67 mm-Hg (1.3 pounds per square inch); the transition to increased pressure took about 30 seconds, and the decrease took about 5 seconds. The pressure cycle began 8 days after the start of the experiment and continued for 22 days, followed by 12 days without the imposed cycle. Both the experimental and the control animals were equally exposed to any residual periodic variables (for example, laboratory noise and normal atmospheric pressure).

With the time of the midpoint of arousal from torpor as a marker in the circadian cycle of body temperature, the results show that two of the four animals receiving cyclic pressure change did entrain to the stimulus (Fig. 1, A and B), as judged by the entrainment criteria described above. The third experimental animal showed a rhythm that was probably entrained for about the first 12 days of the pressure cycle

Table 1. Summary of circadian periods ( $\tau$ ) of time of arousal from torpor measured in *P. longimembris* maintained continuously in conditions of constant dark and constant temperature (20°C) for 41 days. Four of eight mice were exposed to 22 days of atmospheric pressure change, administered at 12 hours at ambient pressure and 12 hours at ambient pressure plus 67 mm-Hg.

Animal No.	Circadian period (hours : min)		
	Day 1-7	Day 8-29*	Day 30-41
<i>Cyclic pressure change days 8-29</i>			
A	22 : 54	Entrained	23 : 32
B	23 : 22	Entrained	23 : 12
C	23 : 6	Disturbed	23 : 21
D	23 : 10	Disturbed	22 : 52
<i>No pressure change</i>			
E	23 : 46	22 : 54	23 : 4
F	23 : 26	22 : 41	22 : 41
G	No torpor	23 : 6	21 : 28
H	22 : 38	23 : 14	25 : 8

\* See Fig. 1.