

THE MECHANISM FOR CO-ORDINATING METACHRONAL LIMB MOVEMENTS BETWEEN JOINED MALE AND FEMALE *ARTEMIA SALINA* DURING PRECOPULATORY BEHAVIOUR

BY CHARLES M. LENT

*Neurobiology and Behavior, State University of New York,
Stony Brook, New York 11794*

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SUMMARY

Male and female *Artemia salina* swim in tandem pairs before copulation and during this period the metachronal rhythms of the individuals are entrained. *Artemia* entrain to pulsatile water stimulation of the head at frequencies between 4 and 6 Hz. The antennules are mechano-sensitive. Antennular responsiveness to phasic input is very probably the actual mechanism by which interorganism co-ordination is achieved, as mechano-stimulation of other *Artemia* structures, electro-stimulation and photo-stimulation are ineffective modes of entraining metachronal movements.

INTRODUCTION

Male and female brine shrimps, *Artemia salina* (Crustacea; Branchiopoda), swim in tandem pairs before copulation. During such behaviour the limb movements of the male and female are entrained, and this is a report of the mechanism of interorganismal coordination. Each adult *Artemia* has eleven pairs of extensively flattened setaceous limbs (phyllopods) which move in a cyclic pattern. This pattern consists of a sequence of power and return strokes which are initiated by the posterior phyllopods and followed, after a slight delay, by a similar sequence in the next most anterior limb pair. These sequences progress anteriorly, usually at rates of 3-6 c/s. As these sequences are incrementally out of synchrony, waves are produced along the length of the *Artemia* and these waves are an array of every phase of the limb stroke sequence. As the metachronal waves progress along the *Artemia*, alterations of the interlimb spaces produce volume changes which direct water currents between the phyllopods. Food particles are strained from these currents by specialized setae and respiratory gases are exchanged across the thin cuticle of the phyllopods. Thus, the metachronal movements of these Branchiopods generate both the locomotor thrusts and water currents necessary for filter feeding and respiration (Cannon, 1933).

Before copulation the male *Artemia* attaches himself to the posterior ovisac of the female by means of his claspers - highly modified second antennae. The pair of *Artemia*, thus joined swim in tandem for some days and copulate following moults by the female. The metachronal phyllopod movements of the joined shrimps are co-ordinated (Lent, 1971). The metachronal waves of the posteriorly located male usually precede

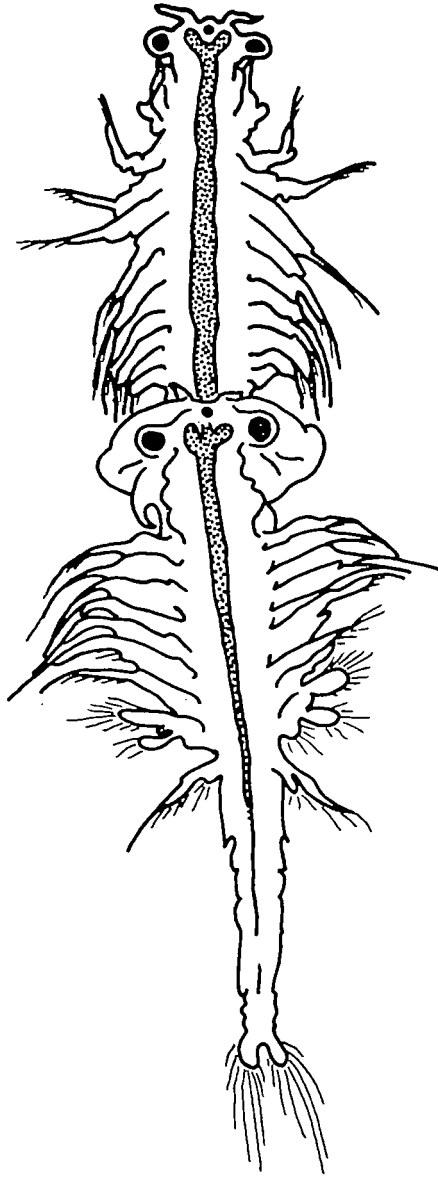


Fig. 1. The dorsal view of a male and female *Artemia salina* joined in precopulatory swimming behaviour. Note the large claspers of the posteriorly positioned male. The most anterior phyllopods of both individuals are about to initiate power strokes, and the posterior phyllopods of the male, which is the pacemaker, have begun a new metachronal sequence. Drawing taken from a stroboscopic photograph.

those of the female such that each wave appears to pass anteriorly along the joined shrimps and involves all 22 pairs of phyllopods (Fig. 1). However, the rate of movement of the metachronal wave is essentially unaffected by pair formation and there are usually two complete metachronal waves along the joined *Artemia*.

The male *Artemia*, which is usually smaller and has a higher rate of metachrony than the female, acts as the pacemaker for the pair (Lent, 1971). The male apparently

provides some type of stimulus to the female, causing her to respond with a higher frequency of movements than she exhibits when single. References will be made throughout this paper to a metachronal oscillator which is the causative agent controlling the frequency of metachronal behaviour and it is presumed to be of a neuronal nature. While the *Artemia* are joined, the slower metachronal oscillator of the female becomes entrained by the faster oscillator of the male. The possible mechanisms by which these two independent oscillators become entrained are limited. (1) The male generates an electrical field by his movements and the female might detect it. (2) The more rapid thrusts of the male might cause changes in light intensity which the female could detect as these movements could alter either the eye position of the female or the rate of movement of reflective particles in the water. The male could provide a mechanical stimulus to the female by any of the following methods: (3) the male might squeeze the abdomen of the female with his claspers, (4) the anterior limbs of the male might provide a phasic stimulus to the posterior phyllopods of the female and (4) the male could stimulate the anterior of the female by phasically accelerating her through the water with his metachronal thrusts. Evidence detailed in this paper suggests that the last mechanism is utilized for entraining the metachronal oscillators of the paired *Artemia*.

METHODS

Artemia were hatched from commercially available cysts in artificial sea water (Instant Ocean). The nauplii were transferred to sea water within gallon jars and periodically fed baker's yeast. The animals grew and all experiments were conducted at room temperature (18–20 °C).

The cyclical limb movements generate field potentials which can be recorded with silver wire electrodes. *Artemia* are positioned individually between two equal lengths of silver wire. The shrimp is confined within a drop of distilled water and secured in that position to a layer of transparent silicon resin by positioning a stainless-steel staple around its 'neck'. The recording chamber is flooded with distilled water, the metachronally generated potentials are differentially amplified through a low-pass filter (1/2 amplitude, 30 Hz), and the signals are displayed upon an oscillograph. The phyllopod movements generate potentials of about 100 μ V in distilled water; however, these fields shrink with increasing conductivity of the recording medium, and in sea water no potentials can be detected. *Artemia* are delicate organisms and many are injured by these necessary manipulations. Uninjured *Artemia* survive for long periods in distilled water with no discernible effects upon their behaviour. This corresponds with the euryhaline osmoregulatory capacity of *Artemia* (Croghan, 1958).

Limb movements were recorded by means of a piezo-electric transducer (phonograph crystal). For this purpose either a paddle was secured to the crystal and positioned near a freely moving shrimp in the chamber, or a glass stylus, drawn fine in a flame and secured to the crystal, was carefully positioned with the aid of a micro-manipulator behind individual phyllopods.

To test for the electro-responsiveness of the metachronal oscillator of the female, individual *Artemia* were exposed to electric fields of four shapes (i.e. the time courses of voltage change described as square, sine, ramp and triangular waves) over a broad range of amplitudes and frequencies. The output of a function generator (Krohn-Hite

5100A) impressed voltages upon a pair of longitudinally arranged silver wires while the behaviour of the shrimp between them was observed through a dissecting microscope. The output of the function generator was simultaneously monitored by means of an audio amplifier driving a speaker. To test for photoresponsiveness, *Artemia* were exposed to flashes of light as their metachronal fields were recorded. Flashes over a range of frequencies greater than twice that of metachrony were generated with a Strobotac (General Radio). The intensity of the flashes was varied by changing the distance between the subject shrimp and the Strobotac, both with and without background illumination. The wavelengths of the light flashes were varied by flashing the strobe through sheets of cellophane of primary colours.

Responsiveness to mechanical stimulation was tested by driving water 'pulses' toward the shrimp with a moving flat paddle (5×5 mm). The paddle was usually positioned 4–7 mm from the shrimp and the amplitude of the paddle excursions was adjusted to produce a maximum response from the *Artemia*. The paddle movement, which usually ranged between 1 and 2 mm, was driven through its attachment to the cone of a dynamic speaker powered by the output from a function generator. During such mechanical stimulation, the metachronal fields were recorded simultaneously with the output of the function generator. Possible squeezing by claspers was tested by securing the phonograph crystal to the clasper by means of a fine glass hook. Clasper movements were recorded simultaneously with metachronal field potentials.

RESULTS

Field potentials and phyllopod movements

The externally recorded field potentials are usually sinusoidal. Each complete sine wave appears to correspond to a full, single metachronal sequence of the eleven pairs of limbs as adjudged by the simultaneous macrocinematography of a swimming *Artemia* and the recording of its electric field (Lent, 1971). I have attempted to substantiate more fully the field technique as it provides such a simple method of measuring the metachronal frequency of *Artemia*, and one presumes many freshwater crustacea as well. By positioning a small paddle, attached to the phonograph crystal, in the recording chamber with a freely moving *Artemia*, water movements can be detected simultaneously with limb-generated field potentials.

Each discrete mechanical movement of the water in the recording chamber occurs in concert with a large electrical potential (Fig. 2A). This finding implies that strong limb contractions produce both large field potentials and detectable water movements. Thus, limb movements and field potentials are correlated phenomena. More direct evidence can be mustered by recording the individual phyllopod movements using the phonograph crystal together with the field potentials from the remote electrodes (Fig. 2B). Each phyllopod power stroke generates a discrete, detectable signal from the crystal and this signal always occurs at a constant phase of the sinusoidal field potentials. Thus, the rhythmic movement of each pair of limbs corresponds to a phase-fixed point in the field potential sine wave.

If several pairs of phyllopods along the length of a single animal are sampled, it becomes evident that the contractions of the more posterior limbs precede those of the anterior limbs within the time course of the sine-like field potential. The power

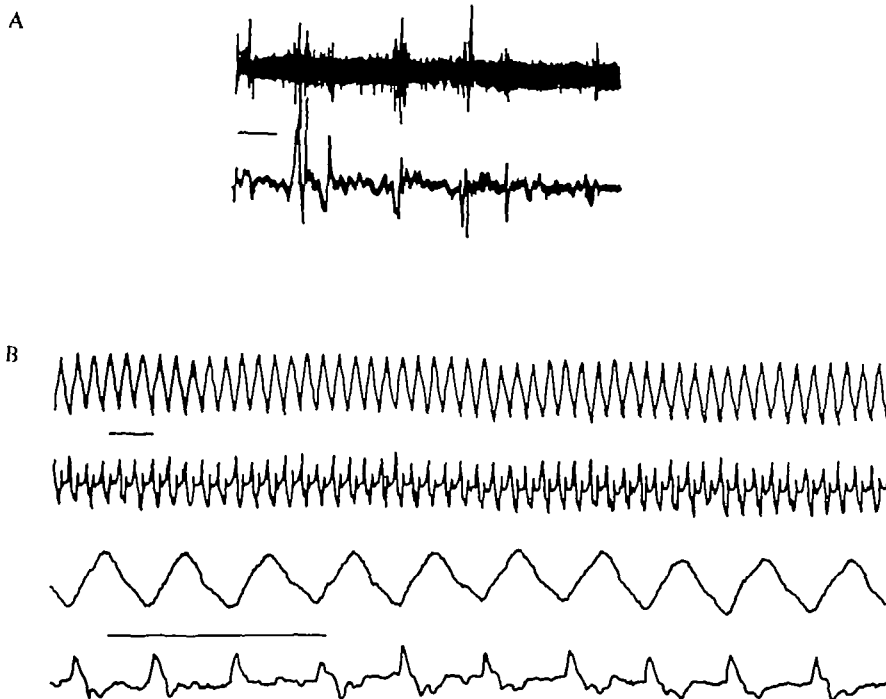


Fig. 2. The temporal relationships between the phyllopod movements of *Artemia* and the externally recorded electrical fields. (A) Mechanical movements (top trace, from crystal) and the electrical fields (bottom trace) generated by a freely moving *Artemia*. Time mark = 1 s. (B) Simultaneous traces of the electrical fields (top) and limb power strokes (bottom). The top pair of traces are at slow speed and the bottom pair at higher speed. Time marks = 1 s. Data are from the sixth phyllopod of a male *Artemia* with a metachronal frequency of 2.6 c/s. Note that the phyllopod contraction always occurs at a phase-fixed point with respect to the electrical record.

strokes of six pairs of the eleven phyllopods of a single female *Artemia* are related to their phase-fixed points within the sine wave potential by Fig. 3. Further, the 11 pairs of phyllopods generate their entire sequence of metachronal movements within every complete sinusoidal oscillation. The last observation which argues that limb movements are responsible for the potentials is that more vigorous limb movements, or even violent thrashing, generate larger potentials than do quiet, metachronous movements. Thus, the weight of the combined evidence demonstrates that the metachronal limb movements cause the potential oscillations and that each full sine wave recorded corresponds to one full set of eleven phyllopod movements. Therefore this is a valid technique for measuring metachronal frequency.

Responses to stimulation

The results presented below are from those experiments designed to test the possible mechanisms by which the metachronal oscillators of the two joined *Artemia* could be coupled.

Electric fields

Electric fields of various frequencies and waveform are ineffective in altering the frequency of limb movements, as long as the stimulus voltages are below certain

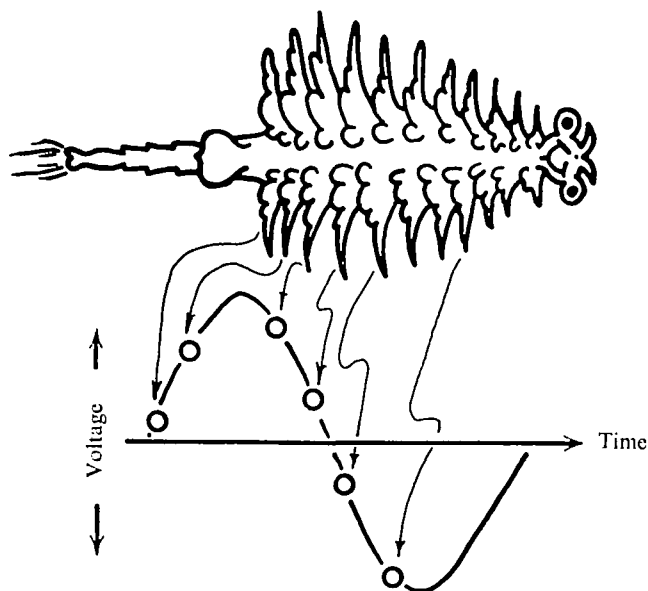


Fig. 3. The relation between the movements of six phyllopods (11, 10, 9, 8, 7 and 4) and the electrical field. Each limb generates a particular phase of the sine wave. While the voltage increase begins with the contraction of the 11th phyllopods of this particular individual *Artemia*, the relationship between phyllopod contractions and phase of the field varies from one shrimp to another.

levels. When the voltage exceeds a threshold for a particular *Artemia*, it responds with uncoordinated, convulsive twitches. Although the frequency of these twitches can be modulated by changing the frequency of the function generator, clearly this does not constitute a co-ordinated response. It is rather the phasic, spastic response of an organism exposed to excessive current. Eight individuals were tested and the results were uniformly negative. Thus, these experiments do not reveal any capacity by *Artemia* to utilize electro-reception as a mechanism for entraining individual meta-chronal oscillators.

Light flashes

Light flashes of all the frequencies, intensities and wavelengths examined were uniformly ineffective in altering the metachronal frequency of *Artemia*. Twelve individuals were tested over the entire range of photo-stimulation parameters. Even though these repetitive changes in light intensity are ineffective stimuli for the metachronal oscillator, a shadowlike decrease in light intensity evokes a very strong albeit transient, response. *Artemia* respond to shadows by a rapid and very nearly synchronous contraction of all 11 pairs of phyllopods. In the recording chamber these responses have a latency of 100–250 ms and produce potentials well in excess of $100\ \mu\text{V}$ (Lent, 1971). Freely behaving *Artemia* often swim near the surface and shadows from above evoke the sequence of strong limb contractions, propelling the animal downward and away from the source of the shadow. Such behaviour appears to be adaptive as shadows could well be cast by potential predators. The shadow response habituates rapidly, and in order to evoke a second response the interstimulus interval

must be at least 15 s. Pairs of *Artemia* also exhibit a distinct, synchronous shadow response; however, the rapid habituation to shadows and the ineffectiveness of the light flashes argue strongly that changes in light are not used as the coupling medium for entraining the two metachronal oscillators during tandem swimming.

Mechanical stimulation

This was employed to determine whether movements by the male *Artemia* might provide a source of phasic inputs to the female. The phonograph crystal recordings, which are sufficiently sensitive to detect the movements of the small posterior phyllopods do not discern any movements of the claspers which are phasically related to the metachronal rhythms. Microscopic examination of a restrained, but metachronous, male does not reveal any such phasic clasper movements either. In some arthropods, such as *Limulus* (Fourtner, Drewes, & Pax, 1971; Page, 1973), the anteriorad metachronal movements can involve segmentally iterated appendages with different functions such as the book gills and the walking legs. If the phyllopods and claspers were metachronally co-ordinated, one could expect metachronal movements of those appendages (e.g. mouthparts) which are segmentally interposed between them. The mouthparts of *Artemia* move with rhythmic frequencies which differ obviously from the metachronal frequencies of the phyllopods. Thus, these data strongly suggest that the male *Artemia* does not entrain the female by means of phasic contractions of his antennal-derived claspers.

The metachrony of the phyllopods by the male generates water movements and this could provide a mechanism for entraining the metachronal oscillator of the female brine shrimp. Those forward movements of the most anterior phyllopods of the male could generate water movements to which the many setae on the posterior phyllopods of the female might be responsive. Pulses of water from the speaker-driven paddle are not effective in altering the metachronal frequency of female *Artemia* when directed toward the rear, or sides, of the specimen. In fact, such pulses often disrupt the otherwise, smooth metachronal rhythms of the female shrimp.

The power strokes of the limbs of the male accelerate both joined *Artemia*, and therefore the head of the female is exposed to water velocities whose rate of change is controlled by the metachronal movements of the male. Attempts to simulate this situation by directing water pulses on to the head of a restricted female are successful. Female *Artemia* respond to such pulses by adjusting their metachronal frequency in the direction of the stimulating pulse rate.

The female shrimp both responds quickly to the onset of water pulses (within 2 cycles) and returns equally quickly to pre-stimulation levels of metachronal output after the pulses stop (Fig. 4A). Not only do most *Artemia* increase the frequency of their metachronal oscillator toward the frequency of the water pulses, but they also increase the 'vigour' of their movements during stimulation. These more vigorous movements can be detected by the larger peak-to-peak field potentials which the stimulated *Artemia* generate (Fig. 4B). Those paddle movements which are most effective in entraining metachronal rhythms are generated by ramp voltages driving the speaker. The constant voltage change of the ramp moves the paddle slowly away from the specimen, while the rapid voltage change drives the paddle toward the shrimp and provides a pulse onto its head. Such non-linear paddle velocities effectively drive

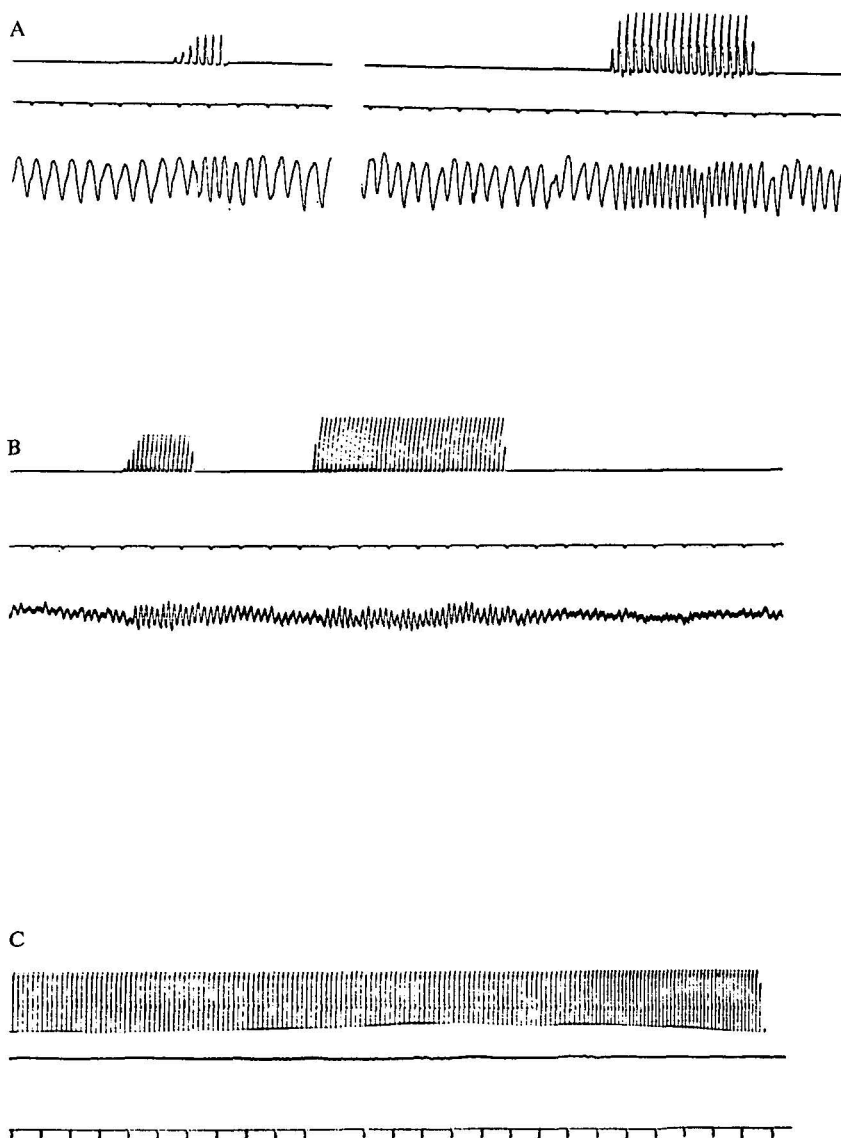


Fig. 4. The metachronal responsiveness of female *Artemia* to water pulses directed onto the head. (A) Top traces are the voltages which drive the stimulating paddle and the bottom metachronal field traces show the responses of a 10.5 mm female shrimp. (B) The response of a 9 mm *Artemia* showing the increased vigour of metachrony during stimulation. (C) A control experiment without a shrimp. Time marks = 1 s for all records.

water movements toward the *Artemia* but do not usually move water in the opposite direction. Sine wave movements of the paddle are less effective as stimuli; while square and triangular movements are without effect. Fig. 4 C is an important control for these experiments as it shows that the electrical field recorded during stimulation is generated by the *Artemia* and not induced by the voltages which drive the speaker. No voltage signals are detected by differential recordings from the experimental chamber without a brine shrimp, even during large, voltage-driven paddle deflexions.

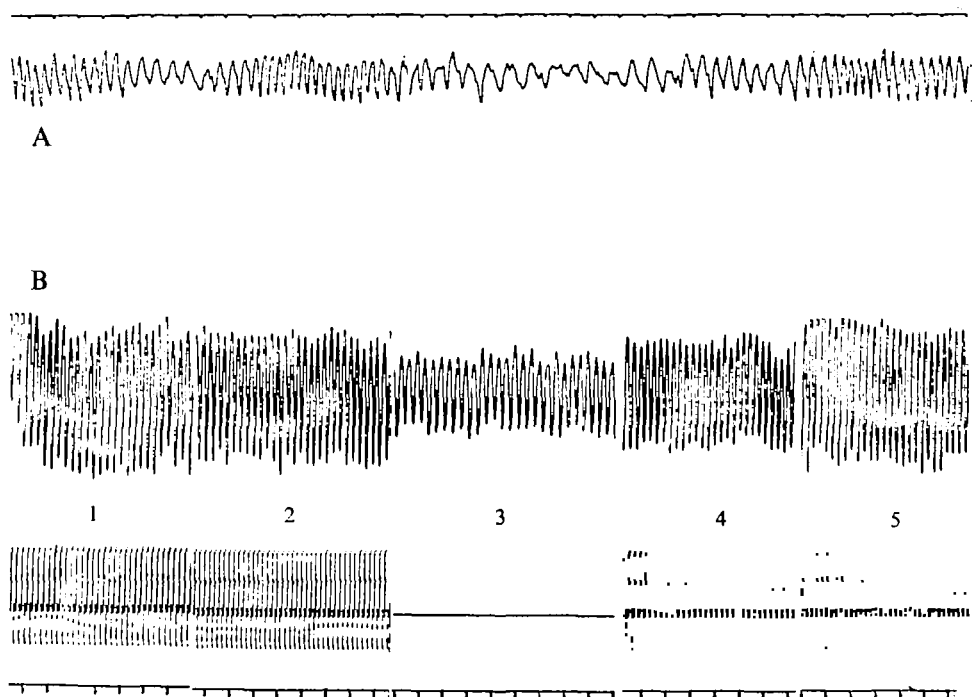


Fig. 5. The metachronal responses of *Artemia* to various frequencies of water pulse stimulation. (A) The response of a 9.5 mm female to continuously modulated stimulation frequency. Each pulse is depicted by a dot in the top trace. (B) Responses of a 10 mm female to constant frequencies of pulsatile stimulation. The data depict five panels with the stimulus (bottom) and response (top). The stimulus : response for each of these panels is (1) 4.4:3.8; (2) 5.0:4.0; (3) 0.3:2; (4) 4.4:4.0; (5) 5.0:4.2. Time marks = 1 s.

If an *Artemia* is exposed to a range of water pulse frequencies, it will respond with a range of metachronal frequencies. As the frequency of stimulation is modulated continuously between 1.5 and 15 Hz, the subject *Artemia* has a higher frequency of metachrony at high stimulation rates and a lower frequency of metachrony at low stimulation rates (Fig. 5 A). Although this shrimp is incapable of faithfully entraining to such a wide range of frequencies of stimulation, the basic stimulus-response pattern leads to the inference that female *Artemia* have the capacity to entrain their metachronal oscillator with certain frequencies of water pulses upon their heads. When an *Artemia* is stimulated at discrete frequencies, data such as that shown by Fig. 5 B are produced. Similar experiments were conducted on a total of nine individual *Artemia*, both male and female. The metachronal frequency (response) of these *Artemia* is shown as a function of the stimulating pulse frequency (ranging from 3 to 7 Hz) (Fig. 6.). These data are akin to a population stimulus-response curve and show that both female and male *Artemia* with an average intrinsic rate of metachrony of 3.6 c/s will entrain to pulsatile stimuli at frequencies of 3.5–5.5 Hz. Thus *Artemia* both respond to and match their metachronal oscillations to water pulses delivered over a limited range of frequencies. Several points require emphasis.

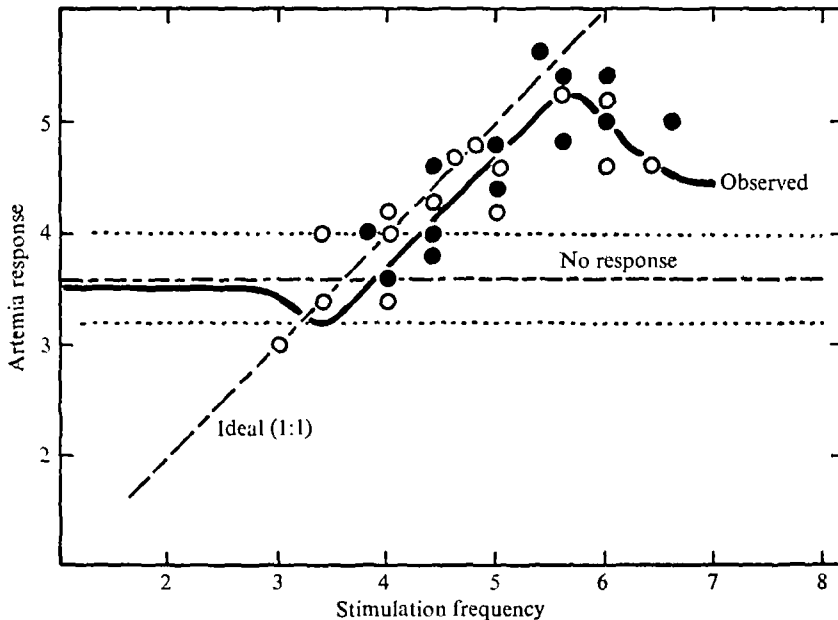


Fig. 6. The metachronal frequency responses of nine individual *Artemia* (●, males; ○, females) to water pulse frequency. The horizontal line represents no response to stimulation by *Artemia* ($3.6 \text{ c/s} \pm 1$ standard deviation is the average metachronal frequency of the unstimulated individuals). The line at 45° represents an ideal 1:1 entrainment between the pulse frequency and the metachronal frequency.

First, not only are female *Artemia* responsive to pulsatile stimuli, but so are males and there is no apparent difference, either qualitatively or quantitatively, in their response. Secondly, not only do *Artemia* increase their metachronal frequency when stimulated, but some individuals will slow down slightly when stimulated at rates lower than their inherent frequency. Third, at stimulation frequencies greater than 5.5 Hz, *Artemia* are unable to follow the pulses in a 1:1 manner and they lower the rate of their metachronal output. However, the *Artemia* obviously detect these high-frequency stimuli as they maintain a metachronal frequency higher than that exhibited in the absence of any stimulation.

An elevated metachronal response which does not follow stimulation in a 1:1 manner is called relative co-ordination (Von Holst, 1939). The relative co-ordination can be seen clearly by the response of a single *Artemia* to stimulation at frequencies between 5 and 9 Hz (Fig. 7). These data are comprised of pairs of points with the stimulating paddle on and off. During stimulation between 5 and 9 Hz, the *Artemia* elevates its metachronal frequency by about 0.8 cycles regardless of the absolute value of the stimulating pulse frequency. It is interesting to note that as the stimulation frequency rises from 9 to 11 Hz, the *Artemia* increases its metachronal frequency from 4.8 to 5.6 apparently entraining to every other water pulse. That the shrimp follows every other water pulse is not a fortuitous observation for these data showing such a 2:1 following were not collected in a single experimental sequence. Rather, half of the data points were collected during a series of increasing stimulation frequencies and the other half of the data points, which are interposed equally between those of the first half, were collected during decreasing stimulation frequencies. As the

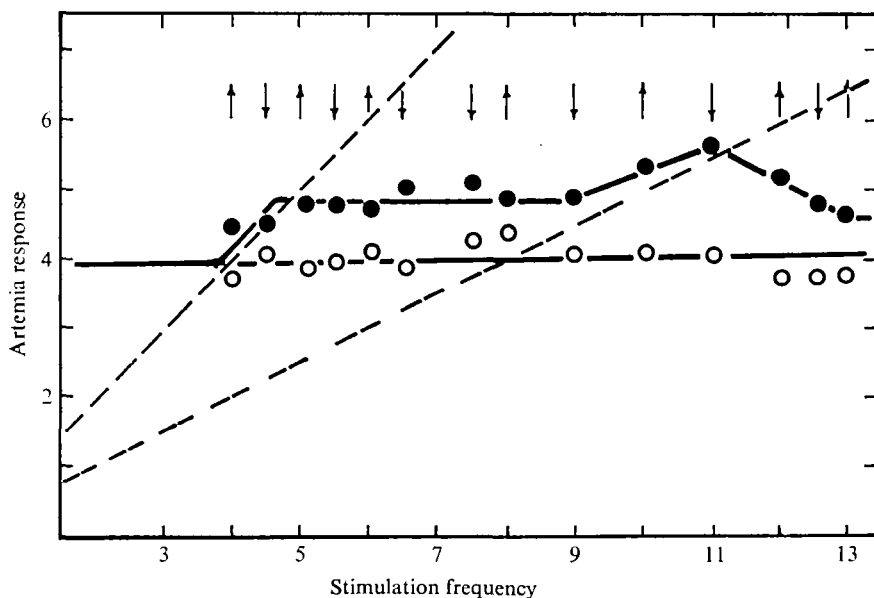


Fig. 7. The response of an 8.5 mm male to pulses from 4 to 13 Hz. The data are composed of paired observations (●, stimulus on; ○, stimulus off). The arrows indicate whether data were collected within an increasing (up) or decreasing (down) series of stimulation frequencies. ---, 1:1 entrainment; ----, 2:1 entrainment.

frequency of stimulation rises above 11 Hz, the frequency of the *Artemia* metachrony decreases once more to the level seen when stimulated between 5 and 9 Hz. The relative co-ordination of the *Artemia* metachronal oscillator can also be seen at the high stimulus frequencies in Fig. 5 A.

These experimental procedures produce many non-responsive *Artemia* which directly result from injuries to the phyllopods. I have recorded from five apparently uninjured and rhythmically metachronous juvenile *Artemia* (i.e. those without either claspers or ovisacs). In no case has any of these juveniles demonstrated the capacity to synchronize its metachronal oscillations with the frequency of the stimulating water pulses. Such stimulation may produce an increase in metachronal frequency; however, this increase resembles relative co-ordination and the response is independent of the stimulation frequency. The largest *Artemia* from which recordings were made was a 12 mm female which was unpaired and laying eggs. She was totally unresponsive to water pulses and in this way differs distinctly from other large females. Thus, the capacity of *Artemia* to entrain its metachronal frequency to phasic water pulses may be limited to the period of sexual maturity and activity.

I have attempted to localize the morphological site of mechanical responsiveness by *Artemia* with more precision. As both female and male shrimps are pulse responsive, the structure should be common to both sexes and located on the anterior end of the animal. The antennules are the most anteriorly located appendages and are similar in both sexes. The ablation of one antennule from a pulse-responsive *Artemia* does not usually interfere with the capacity to synchronize its metachrony to the frequency of

paddle movements. When both antennules are removed, however, pulse responsiveness is irrevocably lost. Control removal of small portions of the anterior pair of phyllopods does not affect the capacity of *Artemia* to entrain as long as the post-operative shrimp continues with rhythmic metachrony. Limb injuries often expose the continuous hydroskeleton of the coelom to the bathing solution, and the *Artemia* twitches briefly and becomes permanently immobile. Operations are conducted in sea water to minimize osmotic shock. Finally, the metachrony of an *Artemia* can sometimes be entrained by direct stimulation of an antennule through a carefully positioned suction electrode. Apparently this procedure directly stimulates the mechanically sensitive receptors of the antennules. Such receptors probably transmit impulses to the metachronal oscillator and entrain it to a higher frequency.

DISCUSSION

The primary focus of this research was to examine the possible sources of signals between the physically joined *Artemia* in order to describe the mechanism(s) involved in maintaining the interorganismal co-ordination of metachronal limb movements. The only type of stimulus to which *Artemia* demonstrate any capacity to entrain their metachrony is the headward direction of pulsatile water movements. Preliminary results suggest that the morphological site of the mechano-responsiveness is the anteriorly positioned antennules. Mechano-stimulation of the posterior of *Artemia*, as well as stimulation by light and electricity are ineffective in entraining metachronal rhythms.

The typical behavioural interaction between paired *Artemia* follows this sequence: a metachronal wave is initiated by the power strokes of the 11th pair of phyllopods on the posteriorly positioned male member. This newly initiated wave of power strokes progresses anteriorly, accelerating both *Artemia* through water. Such an increase in velocity stimulates the antennules of the female. This information is undoubtedly impulse coded and conducted posteriorly in the nervous system of the female. As a result, a metachronal sequence is initiated by the female earlier than it would have been without antennular stimulation. The newly initiated metachronal wave of the female begins at about the instant the wave of the male reaches his anterior pair of phyllopods. The overall appearance of such a co-ordinated behaviour is that each single wave of metachrony passes smoothly along the 22 pairs of limbs of the joined male and female *Artemia salina*. The male initiates another sequence of power strokes at the same instant as the female begins to follow his first sequence. Thus, two metachronal sequences can usually be seen in each pair of shrimp. Otherwise, the rate of movement of the metachrony along the body of any individual would have to change and this condition is not observed.

That both males and females are receptive to mechano-stimulation is a distinct advantage in maintaining metachronal co-ordination. The range of possible metachronal frequencies for any individual is so large that a male could easily be the slower oscillating member of some pairs. Such a slower male would have his antennules phasically stimulated by the more rapid movements of the female. He would therefore increase his metachronal frequency to the level of phasic stimulation and produce the same overall behavioural output for the pair as if he had been the pacemaker and not

the follower. Thus, these results indicate that either member of a joined pair can set the metachronal frequency of both individuals as long as it has the higher rate of limb movements. However, as the males are usually smaller, they have inherently higher rates (Lent, 1971), and are usually pacemakers.

Unusual pairings between brine shrimps can occur in the crowded conditions existing in the culture jars as a result of males claspings on to inappropriate structures (e.g. other males). Rarely, a male will clasp the abdomen of a male which already has an appropriate grip upon the ovisac of a female, producing a triple set of *Artemia*. Such triple sets of swimming shrimps have co-ordinated metachronal waves, and so for that matter do pairs consisting of two males. It is difficult to envisage a mechanism for co-ordinating metachronal oscillations, other than the antennular responsiveness described in this report, by which three joined individual *Artemia* could co-ordinate their behavioural outputs. Both slower individuals will entrain to the phasic inputs generated by the movements of the fastest individual, regardless of its position within the chain of three shrimps. Further, this triple co-ordination, as well as that between male-male pairs, argues strongly against the existence of any special structures in the female which could respond to some signal emanating from the male. Thus, these anecdotal observations not only support the evidence presented on antennular responsiveness, but they also argue against most other types of mechanisms of metachronal co-ordination which might be proposed.

Many other arthropods have oscillatory behaviours which are unaffected by the phasic properties of the sensory input (Wilson, 1961), and such properties contrast sharply with the metachronal oscillator of *Artemia*. An example of such a 'Wilsonian' oscillator is the flight generator of the locust. While the flight frequency is increased with sensory input, the central flight generator depends only upon the quantity of input and not upon its phasic relationship to the output. However, most oscillators of this type do not require co-ordination with another oscillator. The two *Artemia* oscillators are obviously capable of independent action, but are entrained during precopulatory behaviour and this entrainment is a direct result of the oscillators being responsive to the phasic properties of the sensory input. With such characteristics, the individual *Artemia* oscillators resemble more closely in a formal sense, the segmental oscillators controlling swimmeret movements in the abdomen of the crayfish (Stein, 1971). The abdominal swimmerets move in a metachronal sequence and are coupled by means of co-ordinating fibres within the nerve connectives. Cutting these fibres causes each pair of swimmerets to oscillate independently of its segmentally arranged neighbours. Since *Artemia* will respond to and follow every other pulse at stimulation frequencies of 9–11 Hz, the oscillator appears to have two frequency domains: a narrow one within which it can be entrained and a broader one within which it cannot be entrained.

The biological advantage of metachronal co-ordination between joined brine shrimps is obvious. Such a co-ordinated behaviour maintains smooth water currents assuring both efficient swimming and a reliable source of food and oxygen during the extensive period of precopulatory tandem swimming.

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REFERENCES

- CANNON, H. G. (1933). On the feeding mechanism of the Branchiopoda. *Phil. Trans. R. Soc. Lond.* **222**, 267-352.
- CROGHAN, P. C. (1958). The osmotic and ionic regulation in *Artemia salina* (L.). *J. exp. Biol.* **35**, 219-33.
- FOURTNER, C. R., DREWES, C. D. & PAX, R. A. (1971). Rhythmic motor outputs coordinating the respiratory movement of the gill plates of *Limulus polyphemus*. *Comp. Biochem Physiol.* **38**, 751-762.
- LENT, C. M. (1971). Metachronal limb movements by *Artemia salina*: Synchrony of male and female during coupling. *Science, N.Y.* **173**, 1247-8.
- PAGE, C. H. (1973). Localization of *Limulus polyphemus* oxygen sensitivity. *Biol. Bull. mar. biol. Lab., Wood's Hole* **144**, 383-390.
- STEIN, P. G. (1971). Intersegmental coordination of swimmeret motoneuron activity in crayfish. *J. Neurophysiol.* **34**, 310-18.
- WILSON, D. M. (1961). The central nervous control of flight in a locust. *J. exp. Biol.* **38**, 471-90.
- VON HOLST, E. (1939). Über die nervöse Funktionsstruktur des rhythmisch tätigen Fischrückemarks. *Pflügers Arch. ges. Physiol.* **241**, 569-611.