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BEHAVIOURAL MODIFICATION OF HOST DETECTION

BY THEROMYZON RUDE

BY

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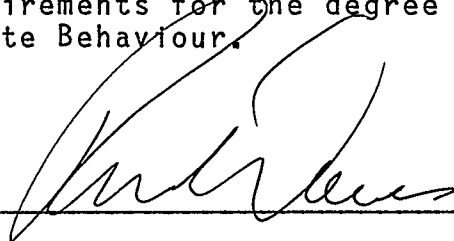
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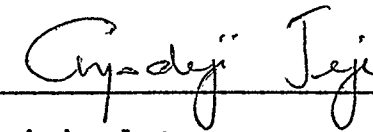
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ABSTRACT

Behavioural modification of host detection behaviour by the sanguivorous duck leech Theromyzon rude in the size range 0.17-0.21 mg, was investigated in the laboratory. In response to repetitive presentation of surface waves, illumination changes and concurrent surface waves and illumination changes, T. rude stop responding (short-term habituation) or modify the expression body waving and inchworm crawling activities.

Both surface waves and illumination changes initiate host detection activity. Increase of surface wave amplitude from 0.29-0.58 mm to 1.80-3.93 mm or illumination change from 1000 to 15634 lux proportionally increased the number of individuals active. Continuous surface waves of 3.5 min duration and 16.0 min interstimulus interval (ISI) increased the number of individuals which became active compared to surface waves of 0.25 min duration and 1.25 min ISI or 0.5 min duration and 1.0 min ISI. Short-term habituation was demonstrated for repetitive presentation of both surface wave and illumination stimuli separately, and together. For increasing post-feeding period, the rate of habituation of the number of T. rude which became active was slower at 4 months post-feeding than at 6 or 8 months post-feeding. Concurrent stimulation by surface waves (1.80-3.93 mm) and illumination change (15634 lux) increased the number of T. rude active compared to concurrent stimulation by

combinations of surface waves (0.29-0.58 mm) and illumination changes (1000 lux). Concurrent surface waves and illumination changes appear to have an antagonistic affect on initiation of activity. Different illumination changes did not alter expression of activity time. Increasing surface wave amplitude increases body waving time, while surface waves of short duration and increased ISI increase inchworming time. Concurrent surface waves and illumination changes reduce total activity time. Concurrent illumination stimuli reduce inchworming time while concurrent surface wave stimuli increase body waving time. Starvation increases initial number of T. rude which became active and the rate of short-term habituation of this response. Increasing the post-feeding period reduces total activity by T. rude in response to surface waves due to a reduction of both body waving and inchworming times.

Body waving allows for the attachment to a host while inchworming moves T. rude to new locations presumably increasing the probability of host encounter. Theromyzon rude initially respond to surface waves and illumination changes which are characteristically different from previous experiences with body waving. If T. rude do not contact a host following initiation of activity, individuals increase inchworming time or habituate to the stimulus. A trade-off between energy investment in activity vs. increasing probability of host encounter occurs.

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CHAPTER 1

INTRODUCTION

1.1. Purpose of study

Theromyzon rude (Baird 1869) is a temporary ectoparasitic sanguivorous leech which primarily feeds in the nares of waterbirds (Aves: Anatidae). In the field, T. rude receives a wide spectrum of stimuli, only some of which are generated by potential hosts. Like all temporary ectoparasites, T. rude must be able to detect and then locate a suitable host in order to feed. This raises the important question of: how T. rude discriminate between non-informative stimuli and stimuli which indicate the approach or presence of a host?

Each stimulus has differential importance, depending on such variables as the state of the organism, the context within which the stimulus is presented, and the preceeding experience of the organism (Petrinovich 1973). Non-associative learning theory provides an approach to the study of this problem and emphasizes the importance of stimulus novelty: only a stimulus which is different in some dimension (e.g. intensity or modality), acts as an efficient releaser of a behaviour which is a function of the organisms' physiology and previous experience.

The emphasis of this study is directed towards understanding the importance of the behavioural modification

of host detection by T. rude when presented with variable environmental stimuli. The information obtained for T. rude, using nonassociative learning theory, will be used to hypothesize how other sanguivorous leech species are able to discriminate between stimuli generated by a host, from those which are not.

1.2. Host detection behaviour

1.2.1. Body waving and inchworm crawling

Coulter (1933) noted that leeches are apparently forewarned of approaching hosts: "They seem to possess an uncanny instinct for detecting the distant presence of living creatures." What Coulter described, very loosely, was host detection behaviour. There are two elementary responses which proceed host detection behaviour in T. rude: (i) body waving, consisting of the lateral pivoting of the body from side to side, while attached (with the posterior sucker) to a firm substrate, and (ii) inchworm crawling, consisting of two distinct phases - extension (with the posterior sucker attached) and contraction (with the anterior sucker attached). Attachment of the anterior sucker appears to trigger the detachment of the posterior sucker, followed by an anterior to posterior wave of contraction. Likewise, posterior sucker reattachment at the end of contraction triggers the detachment of the anterior sucker and an anterior to posterior wave of extension (Stern-Tomlinson et

a1. 1986).

Each elementary behaviour of T. rude involves a co-ordinated movement of the two suckers (anterior and posterior) and the 21 midbody segments. Movement in each segment is brought about by contractions of four sets of muscles: (i) longitudinal muscles parallel to the long body axis; (ii) circular muscles ringing the long body axis; (iii) oblique muscles, which form a thin layer at 45° to the longitudinal axis, between the longitudinal and circular muscle layers; and (iv) dorsoventral muscles, bridging the body dorso-ventrally. The space between the body wall and the gut of most leeches is at least partially occluded by botryoidal tissue with coelomic sinuses ramifying throughout the body (Gratiolet 1862). Longitudinal muscle contraction shortens body segments, while contraction of either the circular or dorsoventral muscles causes a body segment to lengthen. Circular and oblique muscle contractions reduce diameter, and dorso-ventral muscle contractions flatten the body (Mann 1961; Clarke 1964).

Body waving and inchworm crawling are not mutually exclusive activities in Theromyzon as Herter (1929a) noted that body waving occurred in Theromyzon tessulatum (O.F. Muller) while inchworming.

1.2.2. Selection of test stimuli

For T. tessulatum, Herter (1929a,b) and Mann (1961) described a variety of responses to stimuli of different modality: a positive phototactic movement, an attraction to objects warmed to 33-35 °C, an attraction towards a vibration in the water, and a chemotactic movement towards objects that have been in contact with the preen gland of ducks. More recent studies have demonstrated that in the sanguivorous leech Hirudo medicinalis (Linn.), chemicals, surface waves, warmed objects and moving shadows (illumination changes) all elicit a response (Young et al. 1981; Dickinson and Lent 1984; Elliott 1986). However, H. medicinalis does not move over a distance ≥ 1.0 cm towards the source of chemical or thermal stimulus (Elliott 1986).

Lentic ecosystems generally lack directional currents to carry chemical stimuli or provide the basis for rheotactic (current-directed) response to chemical stimulation. Only small and probably insignificant thermal gradients are produced by birds because of the insulatory effects of their feathers, which reduce the body surface - water temperature differential. In addition, waterbirds can enter and leave the water quickly, giving little time to establish chemical and (or) thermal gradients. Thus, thermal and (or) chemical signals for detecting a waterbird by leeches, are unlikely to be important.

Of the potential host stimuli listed by Herter

(1929a,b) and Mann (1961), surface waves and illumination changes are likely to be the most ecologically reliable stimuli. They are propagated over relatively longer distances than chemical or thermal stimuli and are good indicators of the approach or presence of a potential host. Thus, this study concentrates on the importance of water waves and illumination changes to T. rude in eliciting host detection behaviour.

1.2.3. Sensilla and water disturbance detection

The primary function of tegumentary sensilla in H. medicinalis is the transduction of surface waves, travelling at about 0.3 ms^{-1} (Friesen and Dedwylder 1978; Friesen 1981). Sensilla are comprised of photoreceptive cells and numerous, ciliated bipolar cells which transduce water movements (Phillips and Friesen 1982). The photoreceptive and bipolar cells project their axons centrally via segmental nerves which carry the input to the segmental ganglia of the central nervous system (Friesen 1981).

Neuronal activity resulting from stimulation by water waves occurs in the ventral nerve cord of H. medicinalis. Afferent input impinging on one segmental ganglion activates neuronal activity along the entire ventral nerve cord (Friesen 1981).

Herter (1929a) demonstrated that T. tessulatum respond to surface waves with host detection behaviour and Sawyer (1986) suggested that all aquatic sanguivorous leeches rely

heavily on surface waves as a means of host detection.

Photoreception in leech sensilla is mediated by a relatively small number of photosensory cells identical to those of the leech eye (Whitman 1886,1892; Bhatia 1956). Photoreceptive cells of sensilla can distinguish the level of background illumination by giving a sustained response to constant illumination (Kretz et al. 1976). Sensilla detect long-term changes in illumination, e.g. seasonal or diurnal (Kretz et al. 1976; Sawyer 1986).

1.2.4. Eyes and illumination detection

Hirudo medicinalis possess five pairs of eyes positioned on segments II-VI (Whitman 1886; Hesse 1897; Livanow 1904, 1905). Each eye consists of a pigmented cylindrical cup containing 30 to 50 stacked, spherical photoreceptor cells covered by a thin layer of translucent epidermal cells. The axons of the photoreceptor cells join in the vertical axis of the eye cup, leave the eye at the base of the cup and project to the brain via an optic nerve (Kretz et al. 1976).

Each eye is oriented in a different direction and the deep cylindrical shape suggests that a selective response to light coming from various directions occurs. This is because only the light rays which strike an eye within a few degrees of the vertical axis would be incident at the bottom of the cup and activate the entire ensemble of receptor cells

(Kretz et al. 1976).

The ocular photoreceptors transduce changes in illumination with the amplitude of the response linearly correlated with the log of the rate of change in illumination (Kretz et al. 1976). In response to a pulse of light, the photoreceptors produce a train of impulses whose frequency first rises to an early peak and then declines to a steady state plateau at which it remains until the end of the pulse. Ocular receptors are maximally responsive to pulses of green light at a wavelength of 540 nm, adapting completely to background illumination. After the initial burst, axon firing returns to level which preceded the stimulus (Kretz et al. 1976).

During daylight, when a potential host passes over a sanguivorous leech, an illumination reduction is experienced followed by a light pulse. Behavioural responses of H. medicinalis to these stimuli include photokinetic orientation (Dickinson and Lent 1984) and cessation of ventilatory movements (Kaiser 1954).

Prolonged exposure to sunlight is lethal to all leeches (Janzen 1933). Sensitivity of H. medicinalis to light is a function of wavelength (i.e. Ultraviolet only), intensity of radiation and age of the leech (Zapkuvene 1980). Fed leeches are generally strongly photonegative seeking protection under stones or other cover (Gee 1913; Mann 1961).

Background illumination influences the activity of

leeches. Anholt and Davies (1985) and Davies and Kassera (1989) showed that the predatory leech Nephelopsis obscura (Verrill) was more active (i.e. searching, swimming and feeding) in the dark than in the light. Blinn et al. (1987) and Davies et al. (1988) found a similar nocturnal periodicity of activity in Erpobdella montezuma Davies et al. Activity levels of Erpobdella octoculata (Linn.) are controlled by diel changes in illumination and not by endogenous rhythms (Elliott 1973). Consistent with these findings, Lotz et al. (1969) found that activity of leeches was higher under alternating light:dark conditions than under either constant light or constant dark.

1.3. Physiology of host detection behaviour

1.3.1. Neuroanatomy

The central nervous system of all Hirudinoidea is comprised of two presegmental ganglia and 32 segmental ganglia. Of the 32 segmental ganglia, the anterior four are fused to form the brain; 21 separate ganglia constitute the chain of body segmental ganglia; the posterior seven ganglia are fused to form the caudal ganglion (Zipser 1982; Sawyer 1986). In H. medicinalis, apart from the ganglia located in segments XI and XII which also contain the gonads (Zipser 1979; Macagno 1980), each ganglion contains approximately 400 neurons. Adjacent ganglia are joined by paired inter-ganglionic connectives, containing approximately 2860 ($\pm$ 294

S.D.) axons (Wilkinson and Coggeshall 1975). The neuroanatomy of T. rude is undescribed, except for ganglion VI which contains 417 ($\pm$ 20 S.D.) neurons (Hagadorn 1958).

1.3.2. Neuronal circuits

In H. medicinalis it is possible to trace the pathway controlling swimming elicited by surface waves from mechanosensory neurons to motor neurons entirely through monosynaptic connections among identified interneurons (Stent et al. 1978; Kristan and Weeks 1983; Weeks 1982a,b; Brodfuehrer and Friesen 1986a,b,c,d; Nusbaum 1986; Nusbaum et al. 1987; Friesen 1985, 1987). These studies were performed by recording from pairs of neurons in semi-intact preparations or in isolated nerve cords; both preparations produce essentially the same rhythmic activity (Kristan and Calabrese 1976). All neurons can be identified by physiological and morphological criteria, e.g. Ort et al. (1974). The circuit is hierarchal: sensory neurons are connected to trigger neurons, which activate gating neurons; a constant level of firing in the trigger or gating neurons activate the pattern-generating neurons which produce oscillatory activity; pattern-generating neurons are connected to motor neurons which produce the rhythmic motor neuron bursts that constitute the motor program for swimming. The two distinct rhythmic behaviours of T. rude, body waving and inchworm crawling, are thus likely to be

controlled by two separate pattern-generators. The nature of these pattern-generators has not been examined.

1.3.3. Behavioural expression

An individual leech can respond very differently to well defined and controlled stimuli presented on two different occasions (Kristan et al. 1988). Mechanical stimulation (light stroking) of the body wall of H. medicinalis and Mooreobdella microstoma (Moore) can evoke a variety of behaviours, including curling, bending, shortening and swimming (Gee 1913; Kristan et al. 1982). Gee (1913) suggested that the expression of one behavioural response over another depends upon both the intensity and site of stimulation, and this was confirmed by Kristan et al. (1982). However, response variability has also been attributed in other invertebrates to such factors as motivational state, ontogenetic development and (or) the environmental context in which the stimulus is presented. Terms used to describe variations in behavioural state include: motivation (Eibl-Eibesfeldt 1970), behavioural set (Evarts et al. 1984) or drive (von Holst 1973). Behavioural states are deduced from experiments monitoring an animal's response to a particular stimulus under different conditions (McFarland and Sibly 1975), at different times (von Holst 1973), or to simultaneous presentation of two different stimuli which when presented individually evoke different

behaviours (Davis et al. 1974, 1977; Davis 1979). If the test stimulus elicits more than one behaviour, selection among the possible behaviours is made. Expression of behaviour can be quantified as the fraction of trials that elicit each of the possible behaviours. Behavioural threshold (i.e. the minimum stimulus intensity needed to elicit a behavioural response) also measures expression, between response and lack of response.

After a stimulus is presented, behaviour can be modified by influences that vary over time, e.g. learning, reproductive state and hunger level (Davis et al. 1974). These influences are thought to affect neuronal properties through hormones or other neuro-modulatory substances which determine the magnitude of response of pattern-generating circuits (Selverston 1985). For example, hungry H. medicinalis position themselves at the water surface (Dickinson and Lent 1984) and display host detection behaviour in response to surface waves, i.e. they either orient to the source and (or) swim towards it (Young et al. 1981). Lighting does not affect the responsiveness of H. medicinalis to surface waves. However, fed individuals are less likely to swim, and orient less accurately to surface waves than starved individuals (Young et al. 1981). Similarly starved T. tessulatum and T. rude are photopositive but satiated individuals are photonegative (Denzer-Melbrandt 1935; Herter 1936; Mann 1961; Davies 1984).

1.4. Nonassociative learning

Thorpe (1963), reviewing the extensive research done on habituation with animals from different phyla, offered what has become the standard ethological definition of habituation: "The relatively permanent waning of a response as a result of repeated stimulation which is not followed by any kind of reinforcement. It is specific to the stimulus, relatively enduring, and distinct from fatigue and sensory adaptation." Thorpe considered habituation to be a primitive form of learning in the sense that is learning not to respond to stimuli which are of probable low ecological importance.

Habituation was initially conceived as a purely inhibitory process. As the development of the concept progressed it became necessary to devote increased attention to two facilitatory processes: sensitization (the facilitation of nondecremented responses) and dishabituation (the facilitation of decremented responses). The relation among these different forms of learning has been the subject of debate for several decades (Rescorla and Solomon 1967). For example, until recently (Whitlow and Wagner 1984) it has been thought that the three forms of nonassociative learning could be explained by the interactions of two opponent processes: a decremending process that gives rise to habituation and a facilitatory process that gives rise to both sensitization and dishabituation (Groves and Thompson 1970a;

Carew et al. 1971). However, Marcus et al. (1988) suggested that a multi- process view perhaps involving inhibitory, facilitatory and decrementing interactions is necessary to account for the mechanisms underlying nonassociative learning.

Habituation is a behavioural process fundamental to all organisms, which has evolved in response to environmental pressures. If a stimulus does not have importance to an organism, repetitive presentation of the stimulus results in a response decrement. If the consequences are important, the response may increase (or sensitize) with repetition. These two phenomena, habituation and sensitization, are perhaps the most elementary forms of behavioural modification (Thompson et al. 1973; Petrinovich 1984).

When studying the behaviour of a whole organism, the terms habituation and sensitization refer to inferred biological processes which are used to explain the response changes of a behaviour to a given stimulus. It is assumed that habituation and sensitization processes are independent of one another, but interact to yield the final response. Thompson et al. (1973) made it clear that hypothetical processes can be characterized and differentiated on the basis of behavioural studies, making feasible the identification and analysis of the neuronal mechanisms underlying these two processes.

Gee (1913) provided the first demonstration that

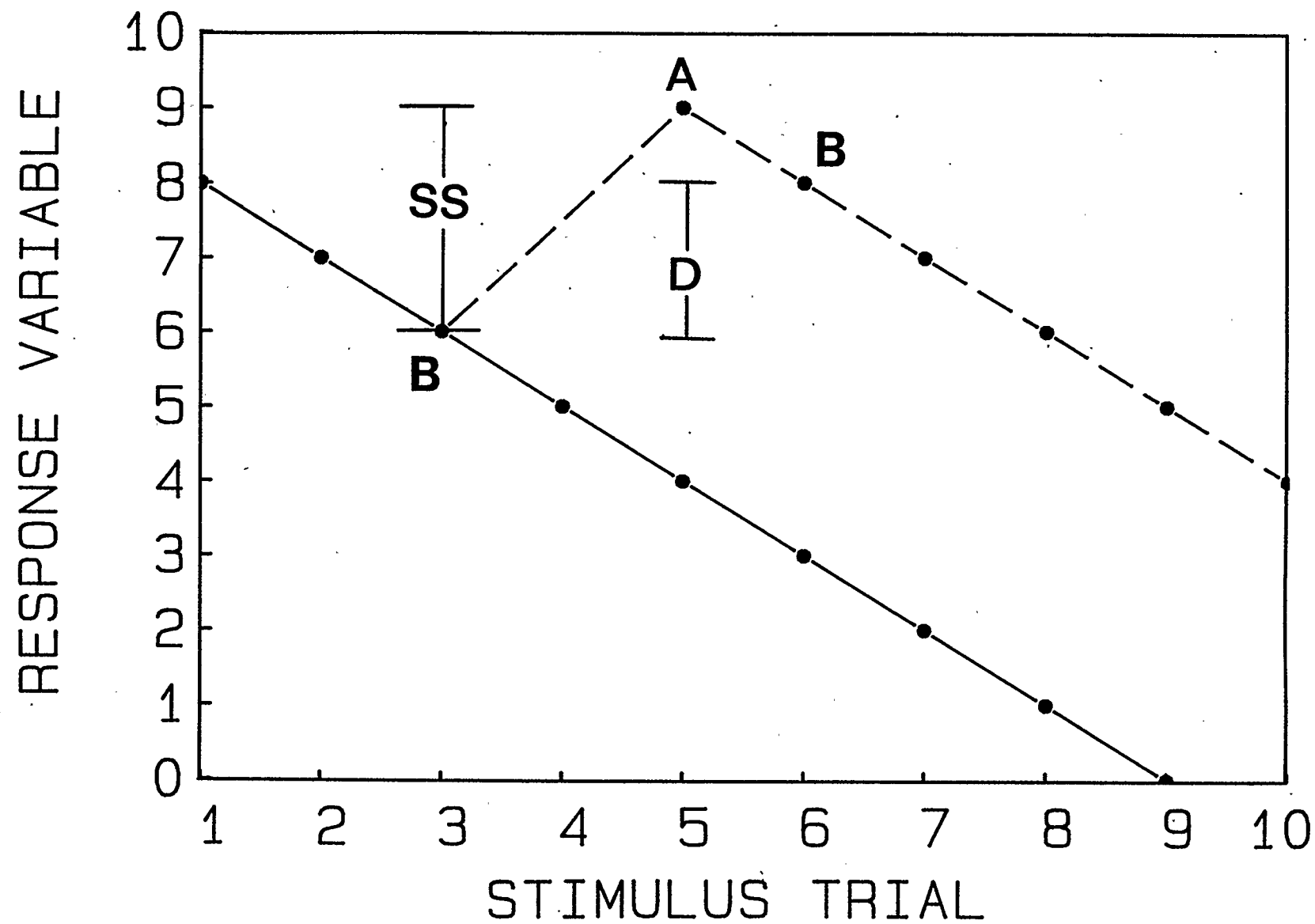
habituation occurs in leeches. He found that M. microstoma, when given an illumination change or when the substrate they were attached to was disturbed, the animals momentarily interrupted their ventilatory movements. Upon repetitive presentations of either type of stimulus, M. microstoma eventually failed to respond and the ventilatory rhythm continued despite presentation of the stimulus. When illumination changes were used as the stimulus, habituation usually occurred within 1-3 trials but with vibratory stimulation 9-11 trials were required. Kaiser (1954) later showed that the rate of habituation of the illumination reflex of H. medicinalis, Haemopsis sanguisuga (Linn.) and E. octoculata were inversely proportional to the intensity of the light used.

1.4.1. Terminology

1.4.1.1. Stimulus specificity and generalization

After a series of identical stimuli are presented and response levels recorded, if a new stimulus, differing in some dimension is presented, the change in level of response indicates the similarity of the new stimulus to the previous stimuli. A new stimulus unlike the previous stimuli should elicit a large difference in response, whereas a stimulus similar to the originals should elicit a similar response (Figure 1.1). Debski and Friesen (1985) found that following habituation to light stroking the swimming response of

Figure 1.1 : Stimulus specificity (SS) and dishabituation (D). Stimulus A increases the level of response following partial habituation to stimulus B, demonstrating specificity. Dishabituation is assessed by comparing the level of response to stimulus B before and after presentation of stimulus A.



H. medicinalis could be restored by pinching the body wall. It is the stimulus-specific nature of the response decrement that rules out sensory adaptation and fatigue as explanations of response decrement to an iterative stimulus.

Stimulus generalization of habituation is assessed after a second habituation series is done using a new stimulus (Wyers et al. 1973). Comparing the rates of habituation between the original and the new stimulus (e.g. light stroking and pinching of the leech body wall, respectively) demonstrates the amount of generalization (Figure 1.2). Faster habituation in the second series indicates generalization and similar rates between the two series demonstrating lack of generalization. Thus, while stimulus specificity is demonstrated by the response to a single different stimulus, generalization requires comparison of the habituation rates between the original and second stimulus (Wyers et al. 1973).

1.4.1.2. Recovery and retention

After presentation of an iterative series of stimuli, a rest period of variable time can be interpolated before the test species is subjected to a second series of the same stimulus. The response to the first stimulus of the second series is a measure of recovery during the rest period (Figure 1.3), e.g. recovery from ventilatory inhibition by M. microstoma occurs after 60 min (Gee 1913). The amount of

Figure 1.2: Stimulus generalization is demonstrated by comparing the habituation of a response (A1) to the habituation of a response (A2) which has been preceded by a series of different stimuli (B).

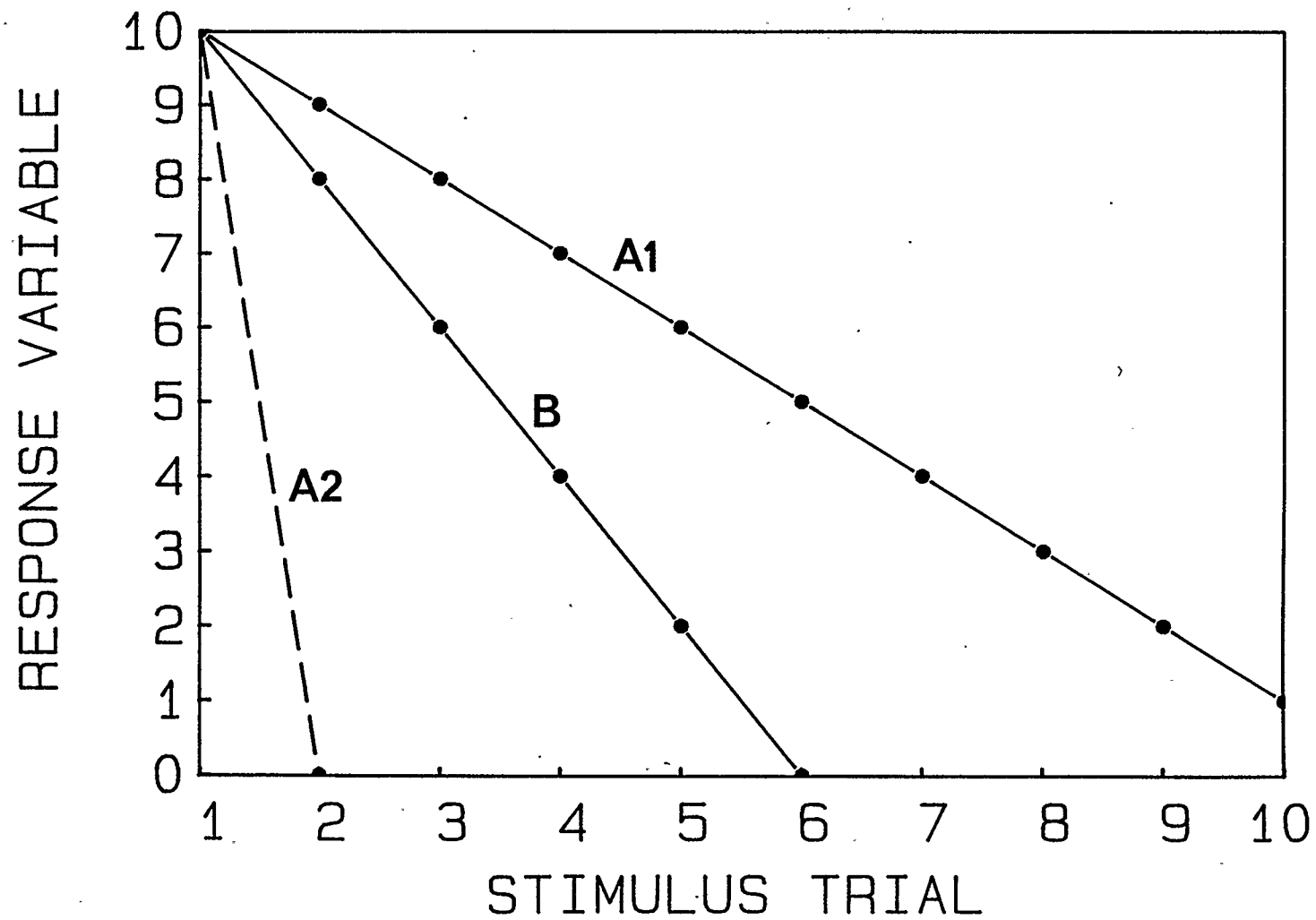
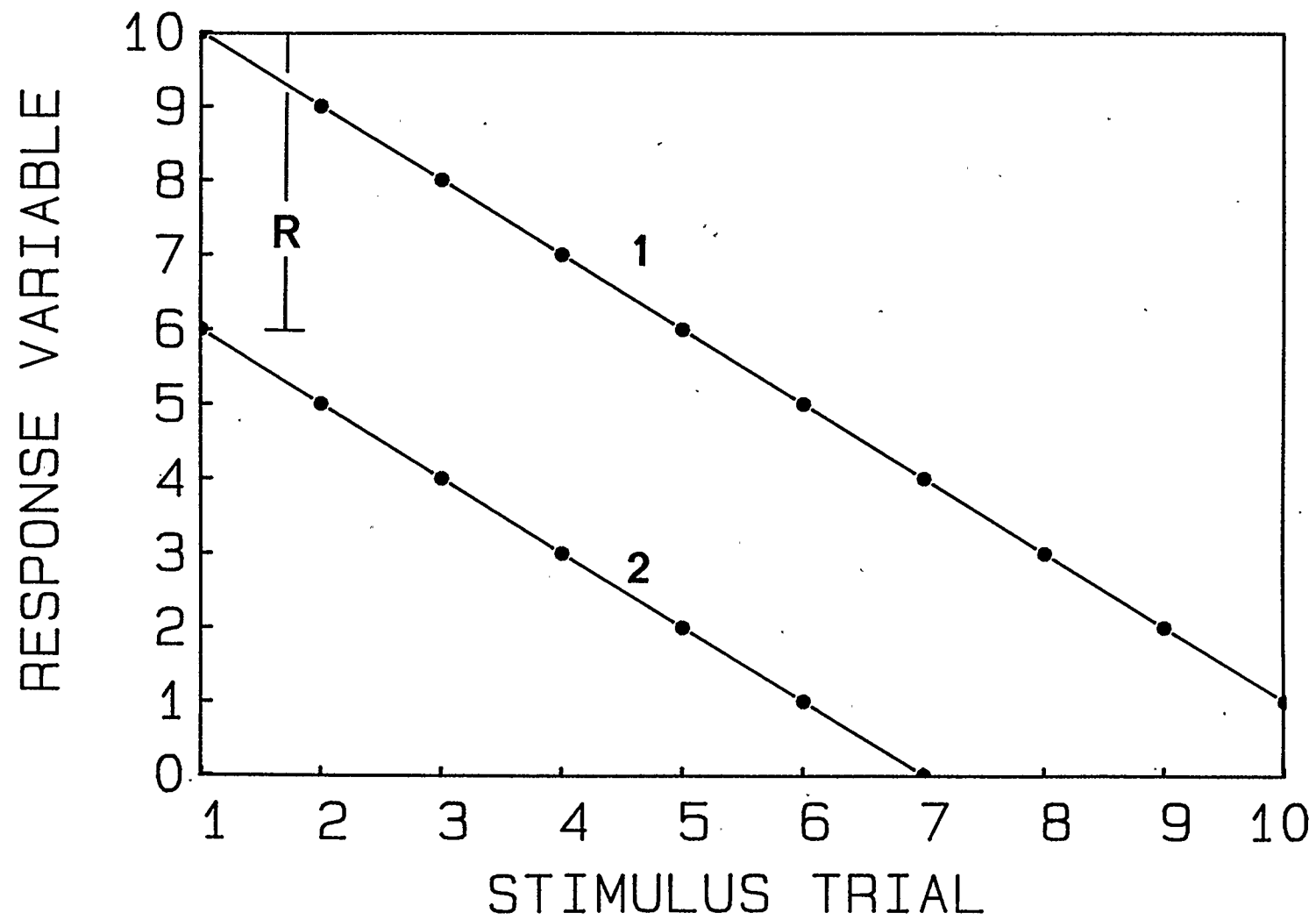


Figure 1.3: Recovery (R) is assessed by comparing the response to the first stimulus of two successive stimuli series (1, 2) after a rest period is interpolated.



habituation retained is assessed by comparing the rate of the response decrement between the first and second series of the same stimulus (Figure 1.4), e.g. repetitive illumination changes presented to Macrobdella decora increased the habituation of movement over three successive 24 hr periods (Ratner 1972).

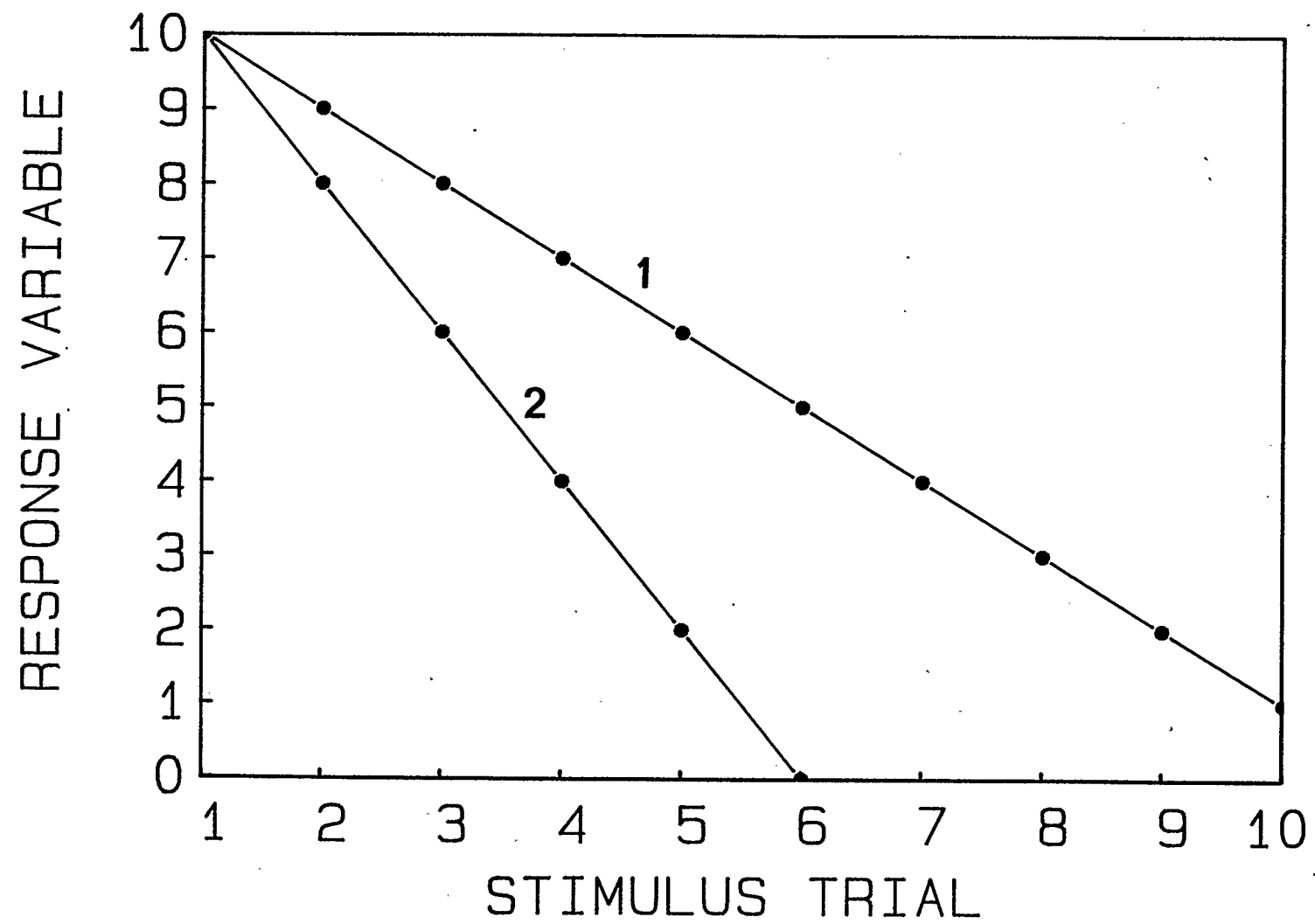
1.4.1.3. Dishabituation and sensitization

Dishabituation refers to the removal of habituation by interpolation of an extraneous stimulus differing from the habituation stimulus. Dishabituation is assessed by recording the level of response to the next iterative stimulus in the series, after a different stimulus is presented (Figure 1.1). Dishabituation of the swimming response in H. medicinalis by pinching the body wall following habituation to repetitive light stroking of the body wall could be assessed by comparing the levels of response to light stroking of the body wall, before and after the pinch.

Sensitization refers to the blocking of habituation by the interpolation of a extraneous stimulus. Sensitization is assessed by comparing the response to the next iterative stimulus after a different stimulus is presented with the response if no initial different stimulus is presented.

Groves and Thompson (1970b) and Thompson et al. (1973) delineated a sensitization process independent of

Figure 1.4: Retention of habituation is assessed by comparing the rate of response decrement between two successive series (1, 2) of the same stimulus. Faster habituation of a response to the second series indicates retention.



habituation of the hind limb reflex in cats. If a relatively strong stimulus is given following habituation of a response (to some other stimulus) the response typically increases or dishabituates. Thompson and Spencer (1966) first presented evidence that dishabituation is in fact a superimposed sensitization rather than a specific disruption of the habituated response. They also suggested that the amplitude of a response depends on the balance existing between these two processes.

1.4.2. Two-factor dual-process theory

All hypotheses tested concerning the details of host detection behaviour of T. rude were conducted within the theoretical framework of the two-factor dual-process theory of non associative learning. Thompson and Spencer (1966), Thompson et al. (1973) and Groves and Thompson (1970b) studying habituation of the hind-limb reflex responses of decorticate, spinal-cats, laid the foundation for current conceptual models of nonassociative learning by emphasizing the underlying physiological mechanisms involved. Petrinovich (1984) studying habituation of white-crowned sparrows (Zonotrichia leucophrys nuttalli L.) to playback of territorial songs, developed the neurophysiological model of Thompson and his associates, and proposed a two-factor dual-process theory which incorporates physiological, ethological and evolutionary components.

The two factors which affect the behaviour are stimulus specificity and relative permanence which act in concert to affect two antagonistic processes: habituation and sensitization. This theory assumes that every stimulus has three properties: that it can (i) elicit a response through a stimulus-response (S-R) sensory motor pathway, (ii) influence the physiological state of the organism, and (iii) that habituation and sensitization occur independently but interact to yield the final behaviour.

Habituation is an inferred decremental process hypothesized to occur in the S-R pathway. There are five assumptions regarding habituation:

1. Habituation develops exponentially and reaches an asymptotic level.
2. The rate of development and degree of relative habituation is directly related to stimulus frequency and inversely related to stimulus intensity. Frequency has a strong effect, and intensity has a weak effect.
3. Upon cessation of stimulation, habituation decays spontaneously.
4. A repeated series of habituation trials and spontaneous recovery periods results in progressively more habituation.
5. Response habituation exhibits generalization to a test stimulus.

Presentation of different stimuli results in an inferred incremental process or an increased tendency to respond. This is the sensitization component. There are eight assumptions regarding sensitization:

1. Sensitization occurs within the central nervous system, not in S-R pathways.
2. During habituation, sensitization first increases and then decays.
3. The amount and duration of sensitization is directly related to stimulus intensity. At high intensities, sensitization is directly related to stimulus frequency. At low intensities there may be no sensitization.
4. Sensitization decays spontaneously.
5. Repeated presentations of a stimulus result in progressively less sensitization.
6. Response sensitization will exhibit generalization.
7. Dishabituation is an instance of sensitization.
8. Temporal conditioning of sensitization may occur.

The assumptions of habituation and sensitization are clearly conceptualized and sufficiently explicit to allow qualitative predictions of host detection behaviour by T. rude under a particular stimulus regime. Examination of host detection behaviour by T. rude within this theoretical

framework will allow the testing, and possibly refinement of Petrinovich's theory and provide important information concerning the nature of the parasite-host relationship.

It has been suggested that habituation and sensitization are basic processes found at all phylogenetic levels (Harris 1943; Thorpe 1963; Wyers et al. 1973). This does not imply that all nonassociative learning has the same properties (e.g. on a cellular and (or) biochemical level) (Sharpless and Jasper 1956). For host detection behaviour in leeches, the relative strengths of habituation and sensitization could vary inter-specifically, according to the dictates of the ecological pressures to which each leech species have adapted.

1.4.3. Proximate and ultimate explanations

Biological explanations of a specific behaviour can be made in terms of proximate causes, i.e. stimuli, sensory organs, neuronal circuits, behavioural state and functional morphology. Thus, behaviour is explained in terms of stimulus-response and processes that immediately influence the response of an organism. Ultimate explanations of a behaviour are at the evolutionary level and refer to the genotype acquired through natural selection over evolutionary time.

To contrast these two approaches, Davies and Wilkialis (1980) showed a seasonal migration of T. rude into the

shallow water zone during the ice-free period and from the shallow water zone into the deep water zone during the period of ice-cover. A proximate explanation would suggest that T. rude migrate because food becomes less available and the days become shorter and colder. This results in an alteration of hormonal activity which triggers a restlessness (behavioural state) that finally results in migration to deeper water. An ultimate explanation would suggest that with shortening days, only those leeches which migrate into the deeper water zone survive. Leeches that do not migrate, starve and die during winter. Those leeches migrating to deeper water zones are able to survive and remain there until the days lengthen in the spring and return to the shallow water zone to feed, reproduce and rear young. The adaptive value of the migration is of primary importance, and the currency is fitness as indexed by reproductive success.

1.5. The species studied

1.5.1. Taxonomy

The first description of Theromyzon rude Baird (1869) was based on a single contracted specimen collected by Sir John Richardson M.D. from Great Bear Lake (Northwest Territories) and was mistakenly assigned to the genus Glossiphonia. Baird made the following morphological description: "body of an ovate form, and of uniform olive-

color; roughly annulated, each ring armed with a series of tubercles along its surface, and having a number of larger, reddish-coloured tubercles scattered irregularly over the surface of the back. Head and body continuous. Dorsum rather convex, ventrally concave. Oral sucker smaller than ventral, which is round, hollow and smooth internally. Eyes ? six in number (as far as could be made out). Sexual orifice about the twenty-third ring. Length about 1 inch, greatest breadth nearly one-half inch." Two of these characters; the parenthetical implication of more than six eyes and the tuberculated, reddish spotted annuli, led to the suspicion that the species may belong to the genus Theromyzon Philippi. The classification remained unchanged until Moore and Meyer (1951) and Meyer and Moore (1954) using four specimens collected from Murray Lake (Saskatchewan) ascertained that there were four eye pairs in two parallel rows on body segments II, III, IVa and Va, the gonopores are separated by three annuli and recommended the transfer of the species to the genus Theromyzon. This classification has been accepted by subsequent workers (Davies 1971; Klemm 1972, 1977; Sawyer 1986).

Taxonomically, T. rude belongs to the order Rhynchobdellida (aquatic; protrusible proboscis; true vascular system separate from coelom); family Glossiphoniidae (eggs yolky and brooded; cocoons attached directly to venter of parent); subfamily Theromyzinae (four pairs of eyes in

stereotyped position, viz. in two parallel rows on II, III, IVa2, and Va2; mating by copulation through female gonopore) (Sawyer, 1986). Species within the genus Theromyzon have undergone considerable synonymy (Soos 1969; Davies 1971, 1972, 1973; Klemm 1972) and some confusion still exists. Currently, differentiation between species is based primarily on the number of annuli (two, three or four) between the male and female gonopores. However, Klemm (1977) suggested that the validity of separating species on the basis of the number of annuli between gonopores is questionable especially as Meyer and Moore (1954) recorded variation in the annular separation of gonopores in T. rude.

In addition to T. rude, three additional species of Theromyzon have been reported in North America: T. tessulatum, with four annuli between the gonopores; Theromyzon biannulatum Klemm, with two annuli between the gonopores; and Theromyzon maculosum (Rathke) also with two annuli separating the male and female gonopores. Because T. maculosum is primarily a European species with only a few reports from North America (Moore 1964; Davies 1973) and because of differences in size and colour, Klemm (1977) proposed that all North American species with two annuli separating the gonopores be placed in a new species T. biannulatum.

1.5.2. Geographic Distribution

Theromyzon rude is restricted to the nearctic and is characteristically found in lakes, ponds, and sloughs distributed along the Rocky Mountain duck migration routes of the Provinces, Territories and States west of the Great Lakes (Herrmann 1970; Davies 1973) and the American midwest (Klemm 1972, 1977). Theromyzon rude is almost exclusively lentic (Meyer and Moore 1954; Herrmann 1970) with only a single lotic record (Moore 1966).

Theromyzon tessulatum is holarctic in distribution and in North America has been recorded from Alberta and the Yukon (Davies, 1973), Saskatchewan (Oliver 1958), Colorado (Herrmann 1970), Newfoundland and Nova Scotia (Pawlowski 1958). Theromyzon biannulatum has been recorded from Michigan, Illinois, Minnesota and Pennsylvania (Klemm 1977) and T. maculosum from Alberta (Moore 1964; Davies 1973).

1.5.3. General biology and ecology

In Alberta, T. rude produce cocoons from May through September (Davies and Wilkialis 1980). Young first appear in the population in July and continue to form a large numerical proportion of the population through October. Seasonal migrations into the shallow water zone during the ice-free period and from the shallow water zone into the deep water zone during the period of ice cover have been observed (Davies and Wilkialis 1980).

During the ice-free periods, starved T. rude are found primarily in shallow waters at a depth of 1 cm where the probability of meeting a host is high (Davies and Wilkialis 1980). Starved T. rude not digesting a blood meal, readily display host detection behaviour in response to the presentation of appropriate stimuli, and are found on the upper exposed surfaces of rocks, stones and plants. After a blood meal, or when carrying eggs or young, T. rude occur in sheltered sites, e.g. under rocks and in deeper waters and do not respond to host stimuli.

Davies (1984) determined the effects of the timing of blood meals by T. rude on its population dynamics and life cycle. A minimum of three full blood meals are necessary before T. rude reaches sexual maturity. The majority (>80%) of the population take all three meals in the first six months after hatching. The remainder of the population overwinters after two meals and take their third meal in the spring so that all the population reaches sexual maturity within approximately 12 months of hatching. After each blood meal there is a further increase in body weight as a result of water uptake. It has been suggested that this dilution of the blood facilitates the faster digestive rates shown by T. rude compared with other sanguivorous species which rapidly eliminate water from their guts after feeding (Davies 1984).

In the laboratory, mature T. rude brood young for a minimum of 5 to 10 d. In the field brooding occurs for up to

35-40 d with a mean of 319.3 young on each adult (Wilkialis and Davies 1980). Release of young from the parent can occur without the stimulus of the presence of a potential host. Death of the parent occurs within 3-19 d of the release of the young (Davies and Wilkialis 1980).

Mortality of T. rude during feeding varies with each meal and is primarily due to ingestion by ducks (Davies et al. 1982a). In Alberta, the majority of the lentic ecosystems in which T. rude occur do not contain fish, and predation by other invertebrates has not been recorded. The inability of T. rude to find a suitable host and mortality from ingestion by ducks are thus the principal determinants of changes in population size (Davies 1984).

1.5.4. Parasitism of waterfowl

Of the four species of Theromyzon endemic to North America, T. rude is the principal species infesting waterfowl (Meyer and Moore 1954; Moore 1964, 1966; Bartonek and Trauger 1975). Trauger and Bartonek (1977) recorded twenty species of waterbirds as hosts of T. rude, which feeds from the soft epithelial surfaces of the nasal chambers (nares), trachea, bronchi and eyes. The nasal chambers are the most common sites for feeding (Trauger and Bartonek 1977; Davies and Wilkialis 1981; Davies 1984).

There has been speculation that heavy infestations of T. rude can suffocate waterfowl by blockage of the upper

respiratory passages (Scooter 1937; Butler 1940; Meyer and Moore 1954; Mendall 1958; Banko 1960; Smith et al. 1964; Moore 1966). Kuznetsova (1955) characterized severe leech infestations of the upper respiratory system as causing short, laboured breathing, terminating in death from asphyxiation. More recently, Davies and Wilkialis (1981) showed that parasitism of domestic ducklings by T. rude induces stress (sensu Selye 1950) resulting from a combination of blood loss, immunological reaction and irritation of the nares mucosa. The potential for induced stress to result in mortality, or reduced fecundity has not been determined.

Waterfowl stressed or weakened by disease are more susceptible to the debilitating effects of leech parasitism than healthy birds (Kalmbach and Gunderson 1934; Scooter 1937; Quortrup and Shillinger 1941; Meyer and Moore 1954; Banko 1960; Sawyer 1972; Bartonek and Trauger 1975). However, the evidence presented has been invariably speculative, inferential, and (or) circumstantial (Davies and Wilkialis 1981). Kalmbach and Gunderson (1934) and Quortrup and Shillinger (1941) reported that T. rude aggravated cases of botulism, and that verminous pneumonia developed in ducks where leeches had infested the bronchi. T. rude infestation has also been associated with toxoplasmosis (Roever-Bonnet 1974) and Pseudomonas sp. infections (Tuggle 1986).

1.6. Objectives

The intent of this study was to examine host detection behaviour by T. rude, with respect to the external stimuli which bring about changes in the level of response. A probabilistic approach is used to describe how T. rude behaviourally interacts with its environment and with a potential host. At this level, host detection behaviour is examined in terms of stimulus-response and the inferred processes influencing host detection behaviour. Thus, the objectives of this study were:

1. To examine the significance and role played by water surface waves and changes in illumination in eliciting host detection behaviour in T. rude.
2. To examine the dynamics of host detection behaviour by T. rude within the context of the two-factor dual-process theory, and to test its assumptions.
3. To attempt to provide biological and evolutionary explanations of the host detection behaviour of T. rude within the context of environmental variability of surface waves and illumination.
4. To provide an understanding of behavioural modification in leeches.

CHAPTER 2

EXPERIMENTAL DESIGN

2.1. Experimental animals and acclimation

Theromyzon rude were collected in May-July 1988 from Stephenson's Pond located 4.5 km west of Calgary, Alberta and described in Davies et al. (1987). The leeches were maintained in the laboratory at 20 °C with a 12:12 h light-dark cycle in aerated pond water.

In the laboratory, T. rude hatchlings were collected from the ventral surface of brooding parents and allowed to feed on domestic ducks (Anas platyrhynchos L.) using the techniques described by Davies (1984). After a minimum of four months post-feeding, starved individuals (0.19 ± 0.02 mg) were selected for experimentation by visual inspection using two criteria; (i) the absence of blood from the digestive tract and (ii) a display of host detection behaviour (i.e. body waving and inchworm crawling) in response to surface waves and illumination changes.

Theromyzon rude were acclimated in still pond water at 20°C for 24 h prior to stimulation. At the onset of stimulation, individuals were randomly distributed within the experimental arena.

2.2. The production and measurement of stimuli

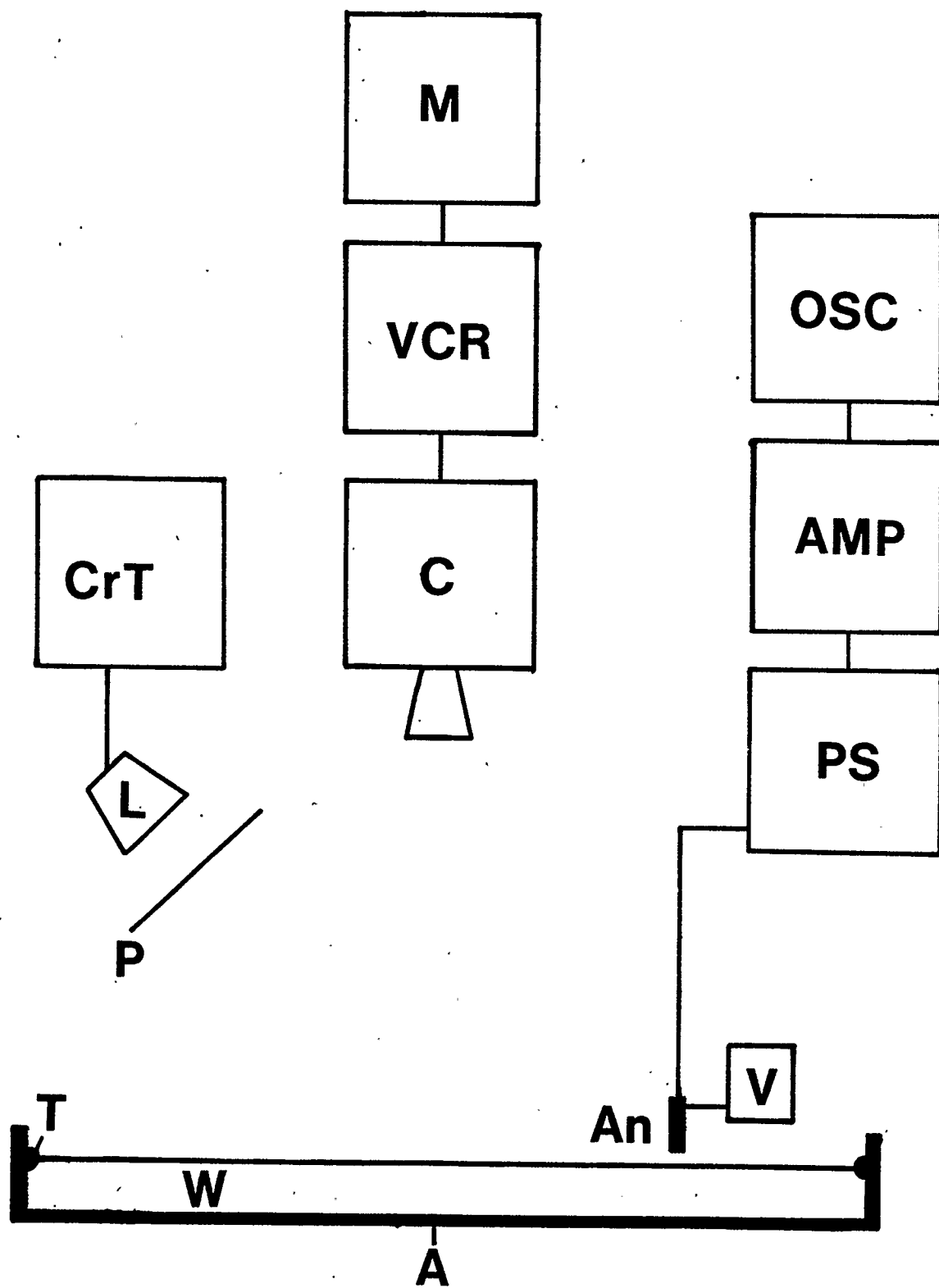
2.2.1. Surface water waves

All experiments were conducted within a circular plexiglass arena (21.5 cm inside diameter) filled to a depth of 1.0 cm with filtered pond water at 20°C. To minimize surface wave reflection, the arena walls were lined with tygon tubing at the water surface. To maintain constant water depth, five holes (3.0 mm diameter) were drilled into the arena walls at a 1.0 cm depth. The arena was set within a sand filled box (10.0 cm) to dampen accidental floor vibrations.

Water waves were produced by allowing water drops ($0.04 \pm 9.0 \times 10^{-3}$ (S.E.) ml) to fall at a drip rate of 24.6 ± 6.1 (S.E.) drops min^{-1} from a Travenol 2C004S solution administration set into the center of the arena from three heights (2.0, 20.0 and 45.0 cm) above the water surface.

Measurements were made to determine the wave frequency and the maximum amplitude (Table 2.1) of the water waves for each drip height, at three lateral distances (2.0, 6.0 and 10.0 cm) from the stimulus source. The instrumentation consisted of a TSI 1210-20 anemometer probe attached to a Summit SD1156 vernier caliper connected to a Grass P15 amplifier and a Tektronix T912 oscilloscope (Figure 2.1). A power supply provided a current which was passed through the probe and contact of the probe the water was detected on the oscilloscope. Maximum wave amplitude was measured by

Figure 2.1: Schematic of instrumentation used for the measurement of surface waves, production of illumination changes and the recording of Theromyzon rude behaviour. A, arena; AMP, amplifier; An, anemometer; C, Camera; CrT, Chron Trol timer; L, flood lamp; M, monitor; OSC, oscilloscope; P, plastic sheet; PS, power supply; T, tygon tubing; V, vernier caliper; VCR, video cassette recorder; W, pond water.



lowering the probe until it just contacted with the stationary water surface. The caliper was zeroed and the probe raised. Wave activity was initiated and the probe lowered until it just recontacted the water. The difference between the probe settings gave the half maximum wave amplitude. Wave frequency was recorded directly from the oscilloscope.

2.2.2. Illumination

Three different illumination changes were used (Table 2.2) to elicit host detection behaviour in T. rude within the circular arena. Four 150 W Westinghouse flood lamps were spaced 40.0 cm from each other and positioned 1.0 m directly above the circular arena. The flood lamps were connected to a Chron Trol ® timer which controlled the stimulus presentation, duration, and the number of lamps turned on to produce the different levels of illumination (Table 2.2; Figure 2.1). A 100 W light provided continuous ambient lighting conditions. Illumination was measured using a Protomatic light meter. To prevent warming of the arena, sheets of transparent plastic were placed between the lamps and the arena.

Table 2.1: Surface wave frequency (mean $\pm$ S.E.) and amplitude (mean $\pm$ S.E.) generated by water drops falling from three heights (2.0, 20.0 and 45.0 cm). Recording positions distance from the stimulus source (2.0, 6.0 and 10.0 cm). N = number of recordings.

Drip Height(cm)	Distance from Source (cm)	N	Frequency (Hz) ($\pm$ SE)	Amplitude (mm) ($\pm$ SE)
2.0	2.0	10	2.0 (1.4×10^{-1})	0.58 (3.2×10^{-2})
	6.0	10	2.5 (1.5×10^{-1})	0.46 (2.8×10^{-2})
	10.0	10	2.7 (3.1×10^{-2})	0.29 (1.9×10^{-2})
20.0	2.0	10	3.3 (1.1×10^{-1})	1.70 (1.3×10^{-1})
	6.0	10	2.3 (3.2×10^{-4})	1.34 (7.9×10^{-2})
	10.0	10	2.4 (1.2×10^{-2})	0.92 (4.4×10^{-2})
45.0	2.0	10	2.0 (2.6×10^{-2})	3.93 (1.2×10^{-1})
	6.0	10	1.5 (1.5×10^{-1})	2.20 (1.4×10^{-1})
	10.0	10	1.5 (1.9×10^{-1})	1.80 (1.5×10^{-1})

Table 2.2: Illumination (lux) changes used in behavioural experiments, showing the ambient and post-ambient light illuminations, the change in light illumination and the number of lamps used. Irradiation ($1 \text{ lux} = 2.323 \times 10^{-2} \text{ uEm}^{-2}\text{s}^{-1}$) in parentheses.

Level	No. lamps	Illumination		
		Post-Ambient	Ambient	Change
1	4	16140 (375.00)	506 (11.76)	15634 (363.24)
2	2	8070 (187.50)	506 (11.76)	7564 (175.74)
3	1	1506 (35.00)	506 (11.76)	1000 (23.23)

2.3. Filming process and response variables

Host detection and response activity of T. rude triggered by water surface waves and (or) changes in illumination were videotaped using a Panasonic 6010 VCR, a WV-5410 video monitor and a WV-1850 TV camera mounted vertically 1.5 m above the experimental arena (Figure 2.1). Surface waves and (or) changes in illumination and behaviour of T. rude were recorded simultaneously.

Four response variables by T. rude were measured for each active individual over 5 successive 19.5 min stimulation blocks for each experiment: (i) the body waving activity time (sec) of an individual (T_{wave}), was measured from the act of anterior sucker detachment through the pivoting of the body from left to right (or vice versa) while attached to the substrate with the posterior sucker, to the time of anterior sucker reattachment (ii) the inchworm crawling activity time (sec) of an individual (T_{inch}), was measured from the act of anterior sucker detachment through the sequential extension (with the posterior sucker attached) and contraction (with the anterior sucker attached) to the time of anterior and posterior sucker attachment or the beginning of the act of body waving, (iii) the total activity time (sec) of an individual (the sum of the body waving and inchworm crawling activity times = T_{time}) and (iv) the number of individuals responding to presentation of a stimulus (n).

2.4. Statistical analysis

Repeated measures analysis of variance (ANOVA) (O'Brien and Kaiser 1985) was performed individually on the response variables n , T_{time} , T_{wave} and T_{inch} testing for regime (R), block (B) and R X B interaction effects. Individuals which did not respond to stimulation were not included in the analyses. When significant regime and (or) block effects were observed, Tukey's studentized range test (Zar 1984) was used to determine where significant differences occurred.

All statistically significant results are indicated by one, two or three asterisks which indicate the level of significance: a significant result was designated by one asterisk (*) = $P < 0.05$; a very significant result was designated by two asterisks (**) = $P < 0.01$; and a highly significant result was designated by three asterisks (***) = $P < 0.001$.

CHAPTER 3

HABITUATION AND EXPRESSION OF BODY WAVING AND INCHWORM CRAWLING ACTIVITIES TO SURFACE WAVES

3.1. Effect of surface wave ISI and duration

The expression of body waving and inchworm crawling activities by Theromyzon rude, initiated by surface waves was examined, and the following assumptions concerning habituation (Petrinovich 1984) (Chapter 1) tested: (i) the response decreases exponentially before reaching an asymptote, (ii) the rate of development and degree of relative habituation, is directly related to the stimulus frequency, (iii) with cessation of stimulation, habituation decays spontaneously, and (iv) a repeated series of habituation trials and spontaneous recovery periods results in progressively more habituation.

Kandel (1976) made a distinction between short- and long-term habituation. Short-term habituation occurs with interstimulus intervals (ISI) in the order of seconds or minutes, while long-term habituation occurs in the order of hours or days. Leech activity habituates to mechanical (Gee 1913; Ratner 1972), tactile (Debski and Friesen 1985), and illumination (Gee 1913; Kaiser 1954; Ratner 1972; Lockery et al. 1985) stimuli using short-term intervals and to long-term light intervals (Ratner 1972).

Stimulation of leeches can initiate a variety of

behavioural responses (Gee 1913). Each activity may occur alone or in combination with other activities as part of a complex behavioural sequence. Gee (1913) suggested that expression of one behavioural response over another depends on both stimulus intensity and site of stimulation. The importance of both parameters has been confirmed by Kristan et al. (1982) who showed that moderate to high intensity electrical stimulation of the anterior body wall of Hirudo medicinalis evoked a curling activity, while similar stimulation applied to the posterior body wall frequently caused swimming. A low intensity stimulus at either site elicited local bending of the body. However, no study has investigated the effect of presenting different stimulus patterns on the expression of leech activity when more than one behavioural response is possible.

This study examined the effect of repetitive presentation of surface waves of different ISI and duration (three regimes) on the short-term habituation and expression of the total activity time (Ttime), the activity times spent inchworm crawling (Tinch) and body waving (Twave), and the numbers (n) of T. rude initiating host detection behaviour.

3.1.1. Methods

Three groups of T. rude (30 individuals per group) were exposed to three different surface wave regimes differing in frequency of stimulus presentation and duration of

stimulation. Surface waves were generated from a drip height of 20.0 cm. The mean surface wave ($\pm$ S.E.) amplitudes (mm) and frequencies (Hz) used to initiate body waving and inchworm crawling activities are listed in Table 2.1 (Chapter 2). Surface wave stimuli were administered systematically in five consecutive blocks for each regime. In regime 1, a block consisted of one continuous 3.5 min stimulation period (CSP), followed by a 16.0 min no stimulus (recovery) period. In regime 2, a block consisted of seven CSPs of 0.5 min each separated by a 1.0 min (no stimulus, CSP-ISI) interval followed by a 10.0 min no stimulus (recovery) period. In regime 3, a block consisted of seven CSPs of 0.25 min each separated by a 10.25 min no stimuli (recovery) period. The total time of successive blocks in each regime was thus 19.5 min (Figure 3.1).

3.1.2 Statistical analysis

As the total number of stimulation periods within blocks was not constant between regimes, n for each block within regime was divided by the sum of the continuous stimulation period times (regime 1 = 3.5 min, regime 2 = 3.5 min, regime 3 = 1.75 min) and the time period over which stimulation occurred (regime 1 = 3.5 min, regime 2 = 9.5 min, regime 3 = 9.75 min) and a repeated measures ANOVA performed on each data set (Table 3.1).

Figure 3.1: Schematic of the three surface wave regimes of different ISI and duration presented to Theromyzon rude. (-) denotes surface wave stimulation. 1, regime 1; 2, regime 2; regime 3. Not drawn to scale.

BLOCK = 19.5 min

1

3.5 min 16.0 min ISI

2

— a 30 sec a — a — a — a — 10.0 min
a = 1.0 min ISI

3

— b 15 sec b — b — b — b — 10.0 min
b = 1.25 min ISI

Table 3.1: The number of Theromyzon rude active for each regime which differ in surface wave ISI and duration. a, number active (n); b, n corrected for stimulus duration; c, n corrected for stimulus period.

Regime	Number active	Block				
		1	2	3	4	5
1	a	14	10	8	7	5
	b	4	3	2	2	1
	c	4	3	2	2	1
2	a	14	3	3	1	1
	b	4	0	0	0	0
	c	2	0	0	0	0
3	a	8	2	1	0	0
	b	4	1	0	0	0
	c	1	0	0	0	0

3.1.3. Results

3.1.3.1. Repeated measures ANOVA of individuals active

Significantly more individuals responded to repeated continuous stimulation periods of 3.5 min duration across blocks in regime 1, than to discontinuous stimulation periods of 0.5 min and 0.25 min duration in regimes 2 and 3, respectively (Table 3.2, Figure 3.2). Tukey's test showed that the mean number of individuals initiating host detection was significantly larger in regime 1 than in regimes 2 and 3, which were not significantly different from each other (Table 3.3).

Short-term habituation of T. rude was indicated by the decline in the number of individuals (n) responding to repeated surface wave stimulation for each regime (Table 3.2, Figure 3.2). In addition, a significant decrease in mean number of individuals responding to stimulation and the mean number of individuals responding corrected for stimulus duration, occurred between blocks 1 and 2 but not between blocks 2, 3, 4 and 5 (Table 3.3). However, when n was corrected for stimulation time period, blocks 1, 2 and 3 were significantly different from blocks 4 and 5 (Table 3.3).

3.1.3.2. Repeated measures ANOVA of activity times

Body waving was the first activity exhibited by all T. rude responding to surface waves.

Table 3.2: Repeated measures ANOVA of the number of Theromyzon rude active in response to surface waves of different ISI and duration. a, number active (n); b, n corrected for stimulus duration; c, n corrected for stimulus period; R, regime; B, block.

Number active	Source of variation	DF	SS	MS	F	Pr>F
a	R	2	101.73	50.87	19.44	0.0008
	B	4	182.27	45.57	17.41	0.0005
	B(R)	8	20.96	2.62		
b	R	2	103.77	51.89	19.54	0.0008
	B	4	181.56	45.39	17.09	0.0006
	B(R)	8	21.25	2.66		
c	R	2	13.35	6.67	44.14	0.0001
	B	4	3.52	0.88	5.82	0.0170
	B(R)	8	1.21	0.15		

Figure 3.2: Frequency polygon of the number of Theromyzon
rude active for each regime which differ in surface wave ISI
and duration. A, number active (n); B, n corrected for
stimulus duration; C, n corrected for stimulus period; 1,
regime 1; 2, regime 2; 3, regime 3.

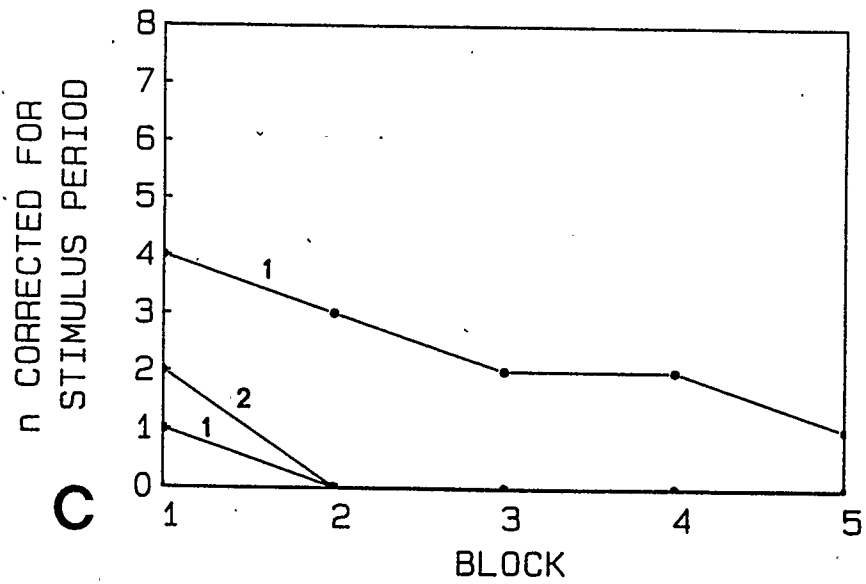
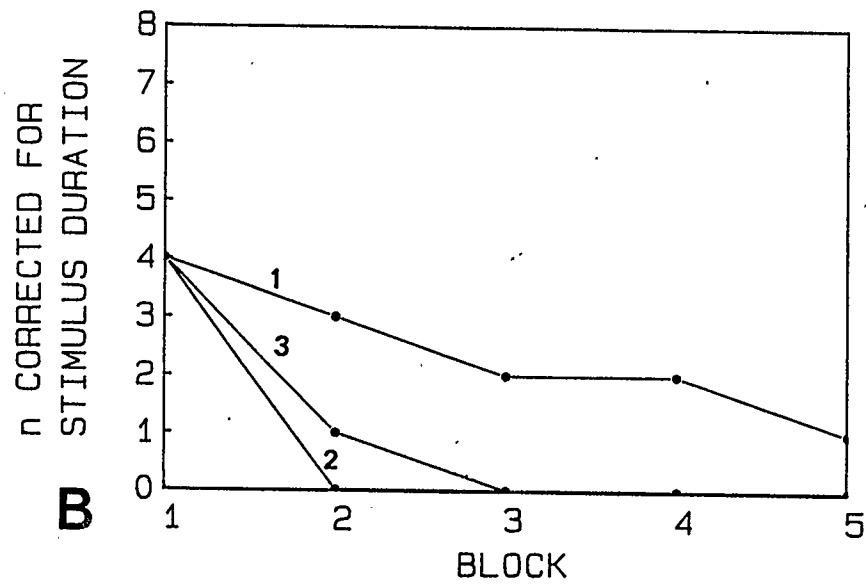
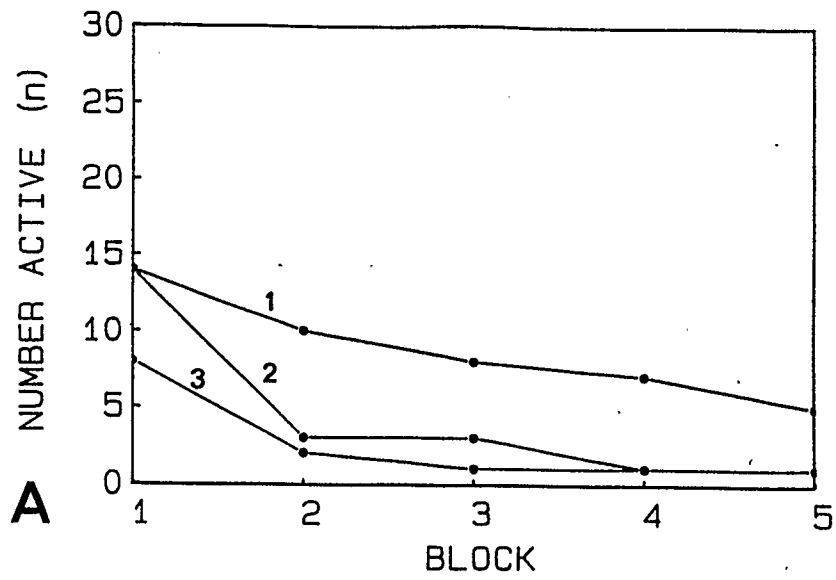


Table 3.3: Tukey's analyses of number active and activity time (Ttime and Tinch) by Theromyzon rude in response to surface waves of different ISI and duration. a, number active (n); b, number active (n) corrected for stimulus duration; c, number active (n) corrected for stimulus period. Ranked sample means in parentheses.

Dependent variable	Regime	Block
a	1 2 3 _____ (8.8)(4.4)(2.6)	1 2 3 4 5 _____ (12.0)(5.0)(4.0)(3.0)(2.3)
b	1 2 3 _____ (2.5)(0.9)(0.3)	1 2 3 4 5 _____ (2.1)(1.4)(1.1)(1.0)(0.7)
c	1 3 2 _____ (2.5)(1.5)(1.3)	1 2 3 4 5 _____ (4.1)(1.6)(1.2)(1.0)(0.8)
Ttime	3 1 2 _____ (590.0)(394.3)(231.8)	
Tinch	3 1 2 _____ (410.1)(234.1)(148.7)	

Following initiation of body waving activity, T. rude would alternate between inchworm crawling and body waving. The series of seven short duration (0.25 min) surface waves of regime 3 resulted in significantly more inchworm crawling (410.11 ± 117.90 sec) by T. rude than occurred in regimes 1 (234.14 ± 35.32 sec) and 2 (148.74 ± 53.81 sec). The total mean body waving activity times of T. rude were not significantly different from each other: regime 1 = 160.16 ± 12.75 sec; regime 2 = 83.09 ± 19.06 sec; regime 3 = 179.94 ± 20.46 sec. Thus, the difference in the total mean activity time (Ttime) of T. rude between regimes was due to changes in Tinch. The mean body waving activity times were less than the total duration of surface wave stimulation (210 sec) for regimes 1 and 2 but in regime 3 the mean body waving activity time exceeded the surface wave duration (105 sec).

There was a significant difference in Ttime ($F_{0.05,2,34} = 4.43^*$) between stimulus regimes (Table 3.4). Tukey's test showed that while the mean individual activity time was highest in regime 3, this was not significantly different from regime 1, but was significantly different from regime 2. Regimes 1 and 2 were not significantly different from each other (Table 3.3). Short-term habituation of total individual activity time (Ttime) across blocks did not occur ($F_{0.05,4,30} = 0.65$) in any of the three stimulus regimes (Table 3.4) and R X B interactions similarly did not occur ($F_{0.05,8,30} = 1.64$).

Table 3.4: Repeated measures ANOVA of activity (Ttime, Twave and Tinch) times by Theromyzon rude in response to surface waves of different ISI and duration. R, regime; B, block; I, individual.

Activity	Source of variation	DF	SS	MS	F	Pr>F
Ttime	R	2	958628.36	479314.18	4.43	0.0195
	I(R)	34	3677403.63	108158.93		
	B	4	109240.28	27310.07	0.65	0.6326
	R*B	8	552110.17	69013.77	1.64	0.1558
	B*I(R)	30	1264267.69	42142.26		
Twave	R	2	53532.53	26766.27	2.75	0.0794
	I(R)	34	331514.23	9750.42		
	B	4	39421.35	9855.34	2.17	0.0929
	R*B	8	14350.63	1793.83	0.39	0.9160
	B*I(R)	30	136352.01	4545.07		
Tinch	R	2	1009889.43	504944.72	8.12	0.0014
	I(R)	34	2113377.75	62158.17		
	B	4	220429.43	55107.36	0.90	0.4746
	R*B	8	306617.18	38327.15	0.63	0.7307
	B*I(R)	30	1836219.30	61207.31		

Individual body waving activity times (Twave) were not significantly different between stimulus regimes or across blocks and there were no significant R X B interactions (Table 3.4).

There was a significant difference in individual inchworm crawling activity time (Tinch) between regimes (Table 3.4). Tukey's test showed that regime 3 was significantly different from regimes 1 and 2 which were not significantly different from each other (Table 3.3). Tinch did not change significantly across blocks and no significant R X B interaction occurred (Table 3.4).

3.1.4. Discussion

Low-amplitude surface waves (≤ 5.0 mm), have been shown (Herter 1929) to initiate host detection behaviour in Theromyzon tessulatum and in Hirudo medicinalis (Young et al. 1981). While there have been several studies on habituation of leech activity (Gee 1913; Kaiser 1954; Ratner 1972; Debski and Friesen 1985; Lockery et al. 1985), no one has examined habituation of host detection to surface waves by a sanguivorous leech. This study shows that surface waves initiate body waving and inchworm crawling by T. rude and that repetitive stimulation using different combinations of surface wave duration and ISI results in differences in the number of individuals responding, the time spent inchworm crawling and the total activity time, but not in the time

spent body waving.

For short-term habituation of host detection by T. rude the operative ISI (Kandel 1976) in regime 1, could be the interval between surface waves generated by each water drop impact or the interval between recovery periods or in regimes 2 and 3, the interval between surface waves, between continuous stimulation periods or the interval between recovery periods. This study shows that as the number of continuous stimulation periods within a fixed time period increases, the rate of habituation in terms of numbers active increases (i.e. the response asymptotes more quickly). Thus, the interval between continuous stimulation periods is the operative ISI influencing habituation of numbers of T. rude responding to stimulation. In addition, increasing the continuous stimulation period duration raises the level of T. rude responsiveness (i.e. increases n within blocks).

Debski and Friesen (1985) demonstrated habituation of swim initiation by H. medicinalis to tactile stimulation (six trials of 5 to 10 sec duration) with 30 sec intervals, while Lockery et al. (1985) showed habituation of initiation of the shortening reflex (forty trials of 4 sec duration) by H. medicinalis to light flashes delivered at 20 sec intervals. Ratner (1972) showed that different ISI's between water current stimuli had different effects on the habituation of activity by Macrobdella decora (Say). With 30

sec intervals between stimulation periods (2 sec duration), habituation in M. decora was faster than with 60 sec intervals between stimuli. Gee (1913) compared the effects of short (15 min) and long (120 min) ISIs on habituation to shadow and mechanical stimuli in Mooreobdella microstoma and showed that short ISIs resulted in faster habituation. Increased habituation by T. rude in response to increased ISI between stimuli, is thus consistent with the findings of Gee (1913) and Ratner (1972).

Although the numbers of T. rude responding exhibited habituation to repetitive surface wave stimulation, no evidence of habituation of total activity time of individuals, time spent body waving or inchworming was recorded. Similar results were recorded by Debski and Friesen (1985) who showed no habituation by H. medicinalis to light stroking of the body wall and Gee (1913) who showed no habituation by M. microstoma to 40 repeated tactile stimulations. However, stimulus duration and the interval between continuous stimulation periods set different levels of total mean activity time. With T. rude, a series of short duration stimulation periods resulted in longer activity times than a series of longer duration stimulation periods. These increases in total activity time resulted from either an increase or decrease in inchworm crawling activity time since the time spent body waving was not significantly different between regimes.

After a blood meal, T. rude is photonegative but when the meal is digested or partially digested, individuals become positively phototactic moving from sheltered locations (Davies and Wilkialis 1980) and deeper waters, to the upper submerged surfaces of rocks, stones and macrophytes (Sawyer 1986). At these locations, T. rude are exposed to surface waves (and illumination changes) and the probability of host encounter is higher. The reversal of light avoidance behaviour between starved (positively phototactic) and fed (negatively phototactic) Theromyzon tessulatum also occurs (Herter 1929).

As waterbirds swim and (or) dabble (drinking and feeding) they produce surface waves. Activity by waterbirds in shallow waters where T. rude are most abundant (Davies and Wilkialis 1980) will produce continuous surface waves. In contrast, waterbirds which visit temporarily, skirt and (or) move in and out of this location, will produce short discontinuous surface waves. To successfully contact a potential host, T. rude must respond appropriately to the stimuli received in terms of time spent body waving and inchworming.

Body waving and inchworm crawling serve different functions, with respect to successful host contact. Body waving frees the anterior sucker from the substrate, and allows for the attachment to a host, while inchworm crawling serves to move the leech to new locations perhaps with a

higher probability of host contact. Body waving time for the three regimes were not significantly different while inchworm crawling times were significantly different ranging from 150 to 410 sec. Continuous surface wave stimulation of T. rude results in relatively higher numbers of individuals initiating a response and a decrease of inchworming time when compared to the response of individuals administered a series of shorter duration surface waves. Continuous surface waves in the field increase the T. rude which become active and perhaps act as a indicator of the close proximity of a host. Theromyzon rude respond to discontinuous surface waves by increasing inchworming. Inchworm activity when a host is in close proximity could be disadvantageous: (i) because it may move T. rude away from a location with high host contact probability and (or) (ii) T. rude may be inchworming when body waving is required for attachment to the host.

A series of shorter duration surface waves results in fewer T. rude initiating host detection behaviour but these individuals show increased inchworming activity time. Thus, discontinuous short duration surface waves in the field would simulate the presence of a potential host not in close proximity, to which inchworming movements might increase the probability of host contact.

Five characteristics have been ascribed to habituating behaviour (Petrinovich 1984). This study demonstrated three of these when examining the number of individuals responding

to repetitive stimulation: the response decreased exponentially, stimulus ISI affected the rate and degree of relative habituation and a repetitive series of habituation trials resulted in progressively more habituation.

Two-factor dual-process theory assumes that the observed behavioural output is a function of two opposing processes: habituation and sensitization. Habituation is more permanent than sensitization and accumulates more gradually. The observed increase in numbers of T. rude responding to a single continuous stimulation period versus discontinuous stimulation, could be the result of an increase in general sensitization offsetting an increase in habituation. If habituation offset sensitization the numbers of individuals responding to stimulation would not increase. This suggests that sensitization increases more with presentation of a single continuous stimulation period, because sensitization does not dissipate between stimulus presentations.

This study shows that stimulus duration and ISI affect the number of active T. rude by altering relative sensitization. Specifically, initiation of body waving and inchworming by individuals tested with a single continuous stimulation period (regime 1) habituated more slowly than the groups administered with seven continuous stimulation periods within the same regime period because sensitization did not dissipate at the same rate. Two-factor dual proces

theory (Petrinovich 1984) accounts for the effects of a variable ISI on habituation (i.e. an increase of the stimulus ISI results in a decrease in the relative habituation rate, whereas an increase in stimulus ISI results in an increase of the relative habituation rate) and agrees with the results from other annelids, e.g. Lumbricus terrestris (Linn.) response to light stimuli (Ratner and Stein 1965). While habituation of total, body waving and inchworming activity times were not demonstrated, surface wave ISI and duration did influence the level of inchworming time by T. rude. Specifically, inch- worming time was increased with discontinuous surface waves and decreased with continuous surface waves. Both modifications of the expression of body waving and inchworming increase the probability of host contact.

3.2. Effect of surface wave amplitude

The following assumptions concerning habituation (Petrinovich 1984) of T. rude by surface wave spectra of different amplitude were tested: (i) the response decreases exponentially before reaching an asymptote, (ii) the rate of development and degree of relative habituation, is inversely related to the stimulus intensity, (iii) with cessation of stimulation, habituation decays spontaneously, and (iv) a repeated series of habituation trials and spontaneous recovery periods results in progressively more habituation.

To increase the probability of host encounter, T. rude modifies inchworming time by discriminating between continuous and discontinuous surface waves (Section 3.1). However, water waves are strongly attenuated with transmission distance (Bleckmann 1986). When waterbirds move towards or away from T. rude, changes in surface wave amplitude will occur with surface wave amplitude increasing as the attenuation distance is decreased and vice versa. The question that arises is: do T. rude discriminate surface wave amplitudes and modify their behavioural responses?

This study investigated the effect of repetitive presentation of three surface wave spectra of different amplitudes, on the expression and habituation of the total activity time (Ttime) and the activity times spent body waving (Twave) inchworm crawling (Tinch), and the numbers (n) of T. rude initiating host detection behaviour.

3.2.1 Methods

Three groups of T. rude (30 individuals per group) were administered surface waves of different amplitude. Surface waves were generated from drip heights of 2.0, 20.0, 45.0 cm, which produced mean amplitudes ranging from 0.29-0.58 mm in regime 1, 0.92-1.70 mm in regime 2, and 1.80-3.93 mm in regime 3 with the mean ($\pm$ S.E.) surface wave amplitudes (mm) and frequencies (Hz) shown in Table 2.1 (Chapter 2). For each regime, surface waves were systematically presented in

five consecutive blocks. A block consisted of seven continuous stimulation periods (CSP) of 0.5 min, each separated by a 1.0 min (no stimulus) interval followed by a 10.0 min no stimulus (recovery) period. The time interval between successive blocks was 19.5 min.

3.2.2. Results

3.2.2.1. Repeated measures ANOVA of individuals active

Increasing surface wave amplitude, increased the relative initial (block 1) responsiveness of host detection by T. rude (Figure 3.3) within block 1, 83%, 36% and 20% of the individuals responding for regimes 3, 2 and 1, respectively. The total mean number of individuals responding between regimes was not significantly different ($F_{0.05,2,8} = 2.61$; Table 3.5).

Significant short-term habituation of T. rude responsiveness (n) to stimulation across blocks was observed ($F_{0.05,4,8} = 9.23^{**}$; Table 3.5) and Tukey's test showed that block 1 was significantly different from blocks 2, 3, 4 and 5 which were not significantly different from each other (Table 3.6).

3.2.2.2. Repeated measures ANOVA of activity times

Body waving was the first activity exhibited by all T. rude initiating a response to surface wave stimulation. Following initiation of body waving activity, T. rude would

Figure 3.3: Frequency polygon showing the number of Theromyzon rude active for each surface wave regime which differ in surface wave amplitude. 1, regime 1; 2, regime 2; 3, regime 3.

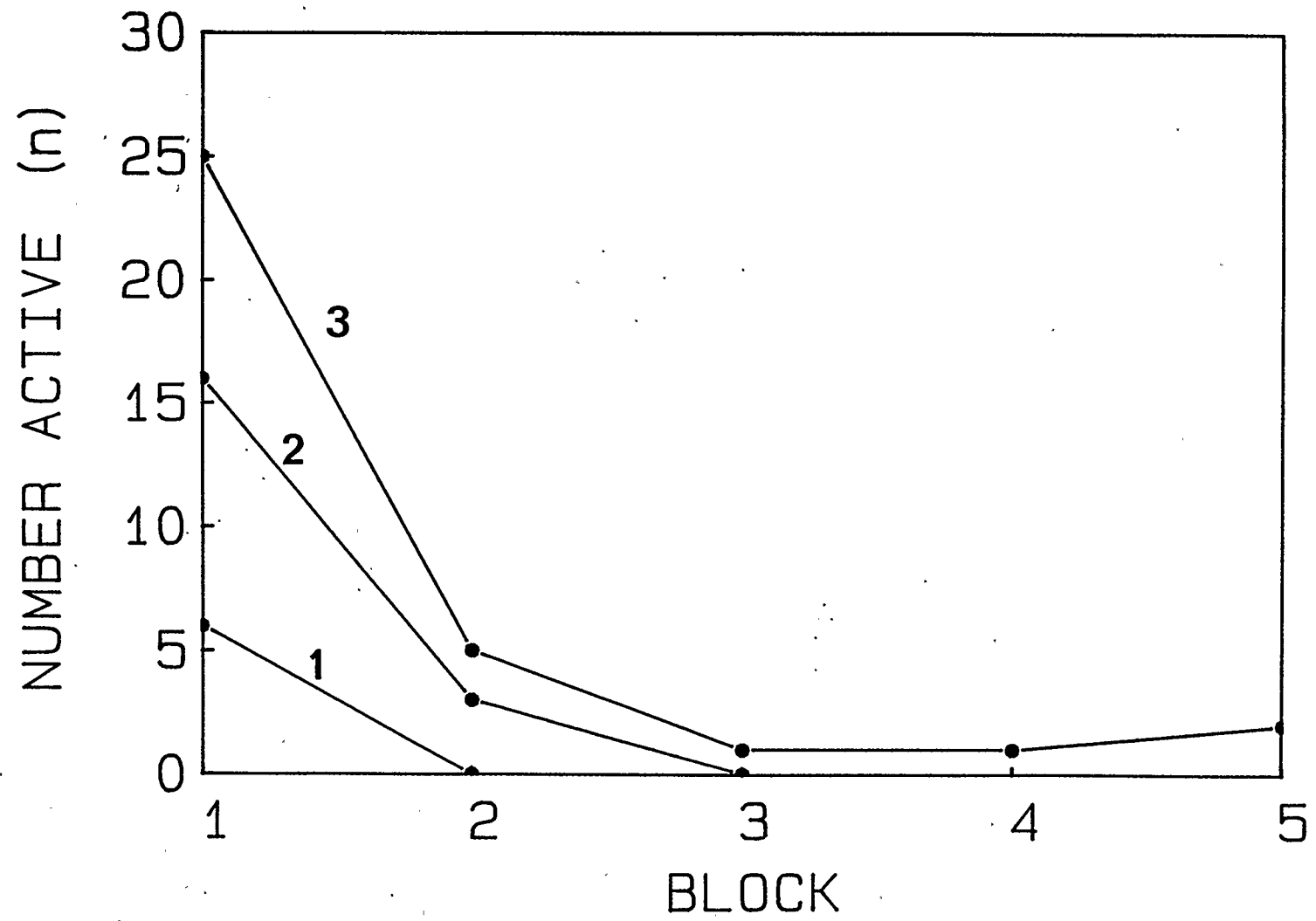


Table 3.5: Repeated measures ANOVA of the number of Theromyzon rude active in response to surface waves of different amplitude. R, regime; B, block.

Source of variation	DF	SS	MS	F	Pr>F
R	2	78.53	39.27	2.61	0.1337
B	4	554.27	138.47	9.23	0.0043
B(R)	8	120.13	15.02		

Table 3.6: Tukey's analyses of number active (n) and activity time (Ttime and Twave) by Theromyzon rude in response to surface waves of different amplitude. Ranked sample means in parentheses.

Dependent variable	Regime	Block				
n		<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
		(16.0)	(2.3)	(0.7)	(0.3)	(0.3)
Ttime	<u>3</u>	<u>2</u>	<u>1</u>			
	(304.3)	(195.8)	(194.7)			
Twave	<u>3</u>	<u>1</u>	<u>2</u>			
	(158.8)	(113.5)	(88.3)			

alternate between inchworm crawling and body waving. The high surface wave amplitude spectrum (regime 3) resulted in significantly higher total activity and body waving times by T. rude than in regimes 1 and 2. The total mean activity times ($\pm$ S.E.) (Ttime) for each regime were: regime 1 = 194.67 ± 66.95 sec; regime 2 = 195.84 ± 50.47 sec; regime 3 = 304.29 ± 41.43 sec. The total mean body waving times ($\pm$ S.E.) (Twave) for each regime were: regime 1 = 113.50 ± 21.25 sec; regime 2 = 88.26 ± 18.05 sec; regime 3 = 158.82 ± 23.07 sec. The total mean inchworming times ($\pm$ S.E.) (Tinch) for each regime were: regime 1 = 81.17 ± 36.94 sec; regime 2 = 107.57 ± 16.92 sec; regime 3 = 145.47 ± 27.02 sec. Thus, the increase of Ttime in regime 3 was due to an increase in Twave. Separately, the sum of Twave and Tinch times were less than the total duration of surface wave stimulation (210 sec) in each regime.

Surface wave amplitude significantly affected both individual Ttime ($F_{0.05,2,45} = 3.87^*$) and Twave ($F_{0.05,2,45} = 3.33^*$) between regimes (Table 3.7) and Tukey's test showed that mean Ttime and Twave was highest in regime 3, which was significantly different from regimes 1 and 2 which were not significantly different from each other (Table 3.6).

No evidence of habituation of Ttime and Twave was observed across blocks nor were R X B interactions recorded (Table 3.7). R, B and R X B interactions were not recorded for Tinch (Tables 3.7).

Table 3.7: Repeated measures ANOVA of activity (Ttime, Twave and Tinch) times by Theromyzon rude in response to surface waves of different amplitude. R, regime; B, block; I, individual.

Activity	Source of variation	DF	SS	MS	F	Pr>F
Ttime	R	2	333037.22	166518.61	3.87	0.0282
	I(R)	45	1937380.04	43052.89		
	B	4	272392.95	68098.24	2.30	0.1728
	R*B	1	1857.86	1857.86	0.06	0.8104
	B*I(R)	6	177377.11	29562.85		
Twave	R	2	71228.77	35614.38	3.33	0.0375
	I(R)	45	485650.68	10792.24		
	B	4	119472.67	29868.17	1.84	0.2410
	R*B	1	162.86	162.86	0.01	0.9236
	B*I(R)	6	97607.25	16267.88		
Tinch	R	2	98348.32	49174.16	1.47	0.2403
	I(R)	45	1503257.59	33405.72		
	B	4	123160.29	30790.07	0.89	0.5254
	R*B	1	920.58	920.58	0.03	0.8761
	B*I(R)	6	208601.36	34766.89		

3.2.3. Discussion

Two-factor dual-process theory (Petrinovich 1984) assumes that the weaker the stimulus, the more rapid and (or) more pronounced is (response) habituation. The only investigation on the effects of stimulus intensity on leech activity showed that the rate of habituation of the inhibition of respiratory movements (undulations of the body while attached to the substrate with both suckers) by Haemopsis sanguisuga to light, was an inverse function of the percent decrease in illumination (Kaiser 1954), i.e. presentation of reduced illumination changes, required fewer trials before the stimulus failed to inhibit respiratory movements. Similarly, Kuenser (1958) demonstrating habituation of the twitch reactions of the oligochaete Lumbricus terrestris to electrical shock applied every 10 sec at intensities of 150, 200, 250, and 300 mV showed that the number of trials required for complete habituation (asymptoting of the response) increased as a direct function of the intensity of the stimulation.

Different surface wave amplitudes affected the relative numbers of active T. rude. An increase in surface wave amplitude spectra, increased the block 1 number of individuals active. Habituation of initiation for each regime was indicated by the decrease in the numbers of individuals active with repetitive stimulation. The rate of habituation was greater in regime 3 than in regimes 1 and 2

since the number responding (n) asymptotized equally with repetitive stimulation between regimes.

It is not possible to rule out muscle fatigue or sensory adaptation as factors responsible for the observed response decrement. However, for the T. rude responding to stimulation, no difference in activity times between blocks occurred, suggesting that the decline of response is unlikely to be attributable to muscle fatigue.

The observed increase in numbers of T. rude responding to stimulation by larger surface wave amplitudes suggests that the sensitization of initiation of activity is influenced by surface wave amplitude. More sensitization occurs at higher surface wave amplitudes, resulting in a relatively greater offset of initial habituation and higher T. rude responsiveness. Sensitization appears to dissipate quickly (between blocks), resulting in the faster habituation rate observed in regime 3. Recovery of sensitization between continuous stimulation periods (10.0 min) was not observed.

No evidence for habituation of total activity time of individuals or the times spent body waving and inchworm crawling was observed. However, increasing surface wave amplitude increased the total activity time of T. rude by increasing body waving time.

In the field, waterbirds moving towards T. rude produce surface waves which increase in amplitude as the attenuation

distance is reduced. Stimulation of T. rude by surface waves of increasing amplitude would increase the numbers of active individuals. These active individuals modify their behaviour by increasing body waving time (and thus total activity time). Body waving by T. rude frees the anterior sucker for attachment to a potential host. Thus, surface waves of increasing amplitude indicate the high probability of the approach of a potential host.

This study demonstrates two of the five characteristics of habituation (Petrinovich 1984) when examining the number of T. rude responding to repetitive stimulation by surface waves of different amplitude: the response decreased exponentially and a repetitive series of habituation trials resulted in progressively more habituation. Contrary to the results of Kaiser (1954) and Kuenser (1958) and the hypothesized effect of stimulus intensity on habituation (Petrinovich 1984), an increase of surface wave amplitude resulted in an increase of the rate of habituation by T. rude.

In summary, increasing surface wave amplitude increased the number of active T. rude by altering the block 1 level of sensitization. The initial sensitization observed at high surface wave amplitude dissipated quickly, resulting in a faster rate of habituation. This result contradicts the assumption made by two-factor dual-process theory (Petrinovich 1984) that the rate of development and degree

of habituation is inversely related to stimulus intensity. While habituation of total, body waving and inchworming times was not observed, increasing surface wave amplitude increased body waving time (and total activity time) by T. rude, a strategy which potentially increases the probability of host encounter.

CHAPTER 4

HABITUATION AND EXPRESSION OF BODY WAVING AND INCHWORM CRAWLING ACTIVITIES TO CHANGES OF ILLUMINATION

4.1. Introduction

The first evidence that repetitive stimulation with light produced habituation in leeches was presented by Gee (1913) who showed that a momentary shadow passed over Mooreobdella microstoma, interrupted ventilatory movements. Repetitive presentation of shadows showed no response, i.e. M. microstoma ventilatory rhythm continued despite presentation of the stimulus. Kaiser (1954) later showed that, for Haemopsis sanguisuga, the rate of habituation of the shadow reflex was inversely proportional to the illumination change used. Ratner (1972) showed that light stimuli also produced habituation of the movement response of Macrobdella decora. Lockery et al. (1985) recorded that forty light flashes delivered at 20 sec intervals to Hirudo medicinalis produced short-term habituation of the shortening reflex.

During daylight, waterbirds cast shadows while moving about shallow waters where starved T. rude might occur. The magnitude of illumination change produced by a potential host, will vary on a diel and seasonal basis. Several studies have shown that sanguivorous leeches (e.g. Theromyzon tessulatum and Hemiclepsis marginata (O.F.

Muller) by Herter (1929a,b,c), Calliobdella vivida (Verrill) by Sawyer and (Hammond 1973), Haemadipsa zeylanica (Moquin-Tandon) and Haemadipsa picta (Moore) by Sawyer (1986)) respond to changes of illumination (shadows) in a similar manner to their response to surface waves, i.e. by displaying body waving. It is not known if changes of illumination initiate activity by T. rude, and if so, which response will occur.

Thus, this study investigated the effect of repetitive presentation of changes of illumination at three different levels on the short-term habituation and expression of the total activity time (Ttime), the time spent body waving (Twave) and inchworm crawling (Tinch) and the numbers (n) of T. rude initiating activity.

4.2. Methods

Three groups of T. rude (30 individuals per group) were exposed to one of three stimulus regimes, differing only in the level of illumination change. In regimes 1, 2 and 3, changes of illumination (1000, 7564, 15634 lux) were produced using one, two and four 150 W flood lamps, respectively (Table 2.2). For each regime, changes in illumination were administered systematically in five consecutive blocks. A block consisted of three stimulation periods of 0.5 min each separated by a 4.0 min no stimulus (flood lamp(s) off) interval followed by a 10.0 min no

stimulus period. Each 0.5 min stimulation period consisted of two 10 sec high illumination (flood lamp(s) on) period, separated by a 10 sec low illumination (flood lamp(s) off) period (Figure 4.1). The time interval between successive blocks for each illumination regime was 19.5 min.

4.3. Results

4.3.1. Repeated measures ANOVA of individuals active

The level of illumination change did not significantly effect the total number of individuals responding to stimulation ($F_{0.05,2,8} = 1.12$; Table 4.1). Increasing the change in illumination increased the block 1 responsiveness (n) of T. rude within block 1, which as percentages (illumination changes given in parentheses) were; 17% (1000 lux), 40% (7564 lux) and 50% (15634 lux) (Figure 4.2).

Short-term habituation of the number of T. rude responding to changes of illumination was indicated by the significant block effect (Table 4.1, Figure 4.2). Tukey's test showed that n in block 1 was significantly different from blocks 2, 3, 4 and 5 which were not significantly different from each other (Table 4.2).

4.3.2. Repeated measures ANOVA of activity times

Body waving was the first activity exhibited by all T. rude responding to illumination change. Following initial body waving activity, T. rude alternated between inchworm

Figure 4.1: Schematic of the illumination regime presented to Theromyzon rude. (-) denotes high illumination. Not drawn to scale.

BLOCK = 19.5 min

— a — ^{10 sec} b — a — b — a — c

a = 10 sec ISI

b = 4.0 min ISI

c = 10.0 min

Table 4.1: Repeated measures ANOVA of the number of Theromyzon rude active (n) in response to different levels of illumination change. R, regime; B, block.

Source of variation	DF	SS	MS	F	Pr>F
R	2	12.13	6.06	1.12	0.3715
B	4	265.60	66.40	12.30	0.0017
B(R)	8	43.20	5.40		

Figure 4.2: Frequency polygon showing the number of Theromyzon rude active for each illumination regime which differ in level of change. 1, regime 1; 2, regime 2; 3, regime 3.

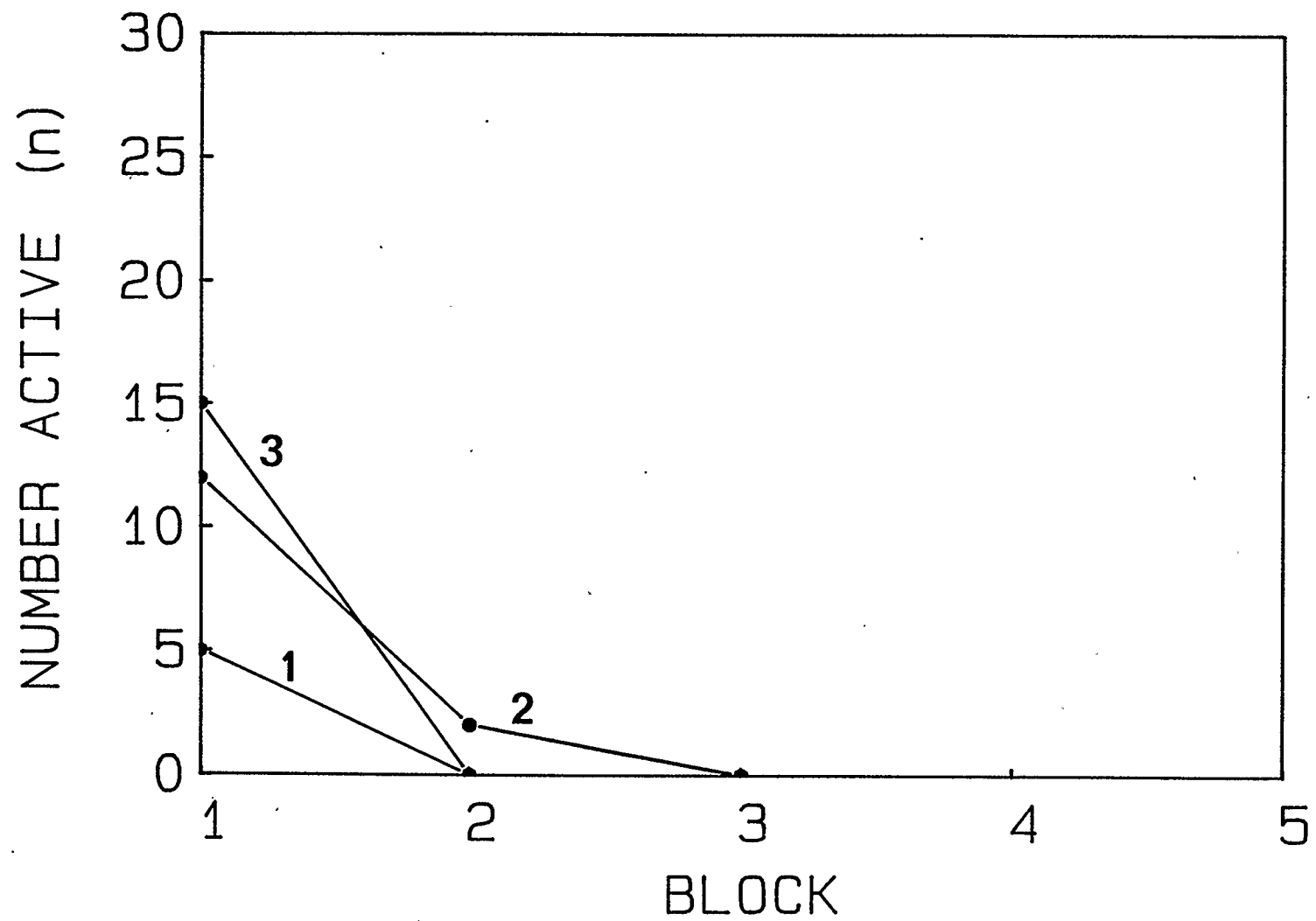


Table 4.2: Tukey's analysis of number of Theromyzon rude active (n) in response to different levels of illumination change, across blocks. Ranked sample means given in parentheses.

Dependent variable	Block				
	1	2	3	4	5
n	(10.7)	(0.7)	(0.0)	(0.0)	(0.0)

crawling and body waving. Level of illumination change between regimes did not significantly affect the mean total, body waving or inchworming times by T. rude. The total mean activity times (Ttime) ($\pm$ S.E.) for each regime were: regime 1 = 43.60 ± 17.33 sec; regime 2 = 40.21 ± 11.64 sec; regime 3 = 60.93 ± 13.43 sec. The total mean body waving times ($\pm$ S.E.) (Twave) for each regime were: regime 1 = 31.80 ± 12.80 sec; regime 2 = 33.14 ± 10.09 sec; regime 3 = 50.06 ± 11.77 sec. The total mean inchworming times ($\pm$ S.E.) (Tinch) for each regime were: regime 1 = 11.80 ± 5.07 sec; regime 2 = 7.07 ± 2.11 sec; regime 3 = 10.86 ± 2.16 sec.

Illumination did not significantly affect total individual activity time individual activity time (Ttime) body waving time (Twave) or inchworming time (Tinch) by T. rude, between regimes or across blocks (Table 4.3).

4.5. Discussion

Little information is available concerning the manner in which photic stimuli received by the eyes and segmental sensilla of leeches affect behaviour (Kretz et al. 1976). Some species are known to be phototropic when hungry and photophobic when satiated (Gee 1913; Herter 1936). Sudden changes in light intensity can initiate a variety of behaviours, e.g. shadows passing over the body of Mooreobdella microstoma, Hirudo medicinalis and Haemopis sanguisuga, produce longitudinal contraction of the body

Table 4.3: Repeated measures ANOVA of activity (Ttime, Twave and Tinch) times by Theromyzon rude in response to different levels of illumination change. R, regime; B, block; I, individual.

Activity	Source of variation	DF	SS	MS	F	Pr>F
Ttime	R	2	1703.18	851.59	0.36	0.6980
	I(R)	29	67832.80	2339.06		
	B	1	10302.25	10302.25	5.70	0.2524
	R*B	0	0.00	0.00	0.00	
	B*I(R)	1	1806.25	1806.25		
Twave	R	2	1500.35	750.18	0.43	0.6522
	I(R)	29	50162.65	1729.75		
	B	1	6642.25	6642.25	3.51	0.3121
	R*B	0	0.00	0.00	0.00	
	B*I(R)	1	1892.25	1892.25		
Tinch	R	2	70.97	35.48	0.45	0.6398
	I(R)	29	2268.78	78.23		
	B	1	400.00	400.00	4.00	0.5675
	R*B	0	0.00	0.00	0.00	
	B*I(R)	1	100.00	100.00		

wall or movement and arrest of ventilatory movements (Gee 1913; Kaiser 1954; Dickinson and Lent 1984; Lockery et al. 1985), while in M. decóra light flashes produce body movements (Ratner 1972).

Changes in the level of illumination at first initiate body waving activity in some T. rude, then an alternation between inchworming and body waving. Repetitive presentation of this stimulus produces short-term habituation of initiation of activity but not of total, body waving or inchworming times. An increase in illumination change, increases the block 1 numbers of individuals responding and the rate of habituation was greater for changes of illumination > 1000 lux, since the response (n) asymptoted equally with repetitive stimulation between regimes.

It is unlikely that the decline in number of individuals responding to illumination change can be attributed to sensory adaptation because even at a much shorter ISI (2.0 sec), there is no decrease in photoreceptor response (Kretz et al. 1976). Also, those individuals responding to stimulation across blocks showed no difference in total, body waving or inchworming times.

The increase in numbers of T. rude responding to stimulation by increased illumination changes suggests that sensitization of initiation of activity occurs. More sensitization occurs at increased illumination changes, resulting in a relatively greater offset of initial

habituation and higher T. rude responsiveness (n). Sensitization appears to dissipate quickly (between blocks), resulting in faster habituation rates seen at increased illumination changes. Recovery of the sensitization process between continuous stimulation periods (10.0 min) was not observed.

Although the numbers of T. rude responding to illumination stimulation exhibited habituation, no evidence of habituation of total, body waving or inchworm crawling times was observed. Thus, none of the assumptions of habituation and sensitization outlined by Petrinovich (1984) were demonstrated for these three response variables. While T. rude increased total activity time (by increasing body waving time) in response to surface waves of increasing amplitude (Section 3.2), individuals do not modify the expression of body waving and inchworming times in response to increased illumination changes.

In the field, waterbirds passing by T. rude produce illumination changes, the level of which will vary on a diel and seasonal basis. Stimulation of T. rude by increasing illumination changes, increases the numbers of individuals that are active but does not modify activity time. Thus, T. rude respond to high illumination changes as an indicator of higher probability of the presence of a host, than lower illumination changes.

This study demonstrated two of the five characteristics

of habituation (Petrinovich 1984) when examining the number of T. rude responding to repetitive stimulation by illumination change of different levels: the response decreased exponentially and a repetitive series of habituation trials resulted in progressively more habituation. An increase in stimulus intensity (i.e. level of illumination change) was expected to result in a slower rate of relative habituation. Kaiser (1954) showed that the rate of habituation of H. sanguisuga was faster at reduced illumination changes (25% reduction in light intensity) than at increased illumination changes (100% reduction in light intensity). However, increasing the magnitude of illumination change to T. rude, resulted in an increase of habituation rate.

CHAPTER 5

THE EFFECT OF STARVATION ON THE HABITUATION AND EXPRESSION OF BODY WAVING AND INCHWORM CRAWLING ACTIVITIES

5.1. Introduction

Two-factor dual-process theory assumes that every stimulus that initiates a behaviour has three properties: (i) it elicits a response through a stimulus-response sensory motor pathway; (ii) it influences the physiological state of the organism; and (iii) habituation and sensitization occur independently but interact to yield the final behaviour. A question that arises is: does the physiological state of T. rude modify the behavioural output in response to repetitive surface wave stimulation? Thus, the effects of starvation on the numbers responding and the expression of body waving and inchworm crawling by Theromyzon rude exposed to surface waves were examined.

In sanguivorous leeches, feeding to engorgement with a blood meal results in an abrupt depression of activity (Whitman 1898). The modulatory effect of feeding on activity has been examined quantitatively in Haementeria ghilianii de Filippi by Sawyer et al. (1981) who showed that all host detection behaviour stopped following a blood meal. Following feeding, the tendency to become active increases until a plateau is reached, about the time the individual is

ready to feed again (Davies 1984). Thus, readiness to become active may be regarded as an operational definition of starvation in sanguivorous leeches (Davies 1984; Sawyer 1986).

This study investigated the effects of starvation on the short-term habituation and expression of activity by T. rude to repetitive presentation of surface waves, examining the numbers of individuals responding to stimulation (n), total activity time (Ttime), and the times spent body waving (Twave) and inchworm crawling (Tinch).

5.2. Methods

Three groups of T. rude (30 individuals per group) hatchlings (wet weight 0.19 ± 0.80 (S.D.) mg) were allowed to feed on domestic ducks (Anas platyrhynchos) using techniques described in Davies (1984). The leeches were maintained in the laboratory at 20°C with a 12:12 hr light-dark cycle in aerated pond water. Four, 6 and 8 months post-feeding (regimes 1, 2 and 3, respectively), T. rude were exposed to surface wave stimulation. Individuals which were < 4 months post-feeding, were partially gut filled and non-responsive to surface wave stimulation, while a > 8 months post-feeding period, resulted in moribund T. rude. Thus, the behaviour of individuals which were < 4 months and > 8 months post-feeding was not investigated.

Each group of T. rude was exposed to surface waves

generated from a drip height of 20.0 cm. The mean ($\pm$ S.E.) surface wave amplitudes (mm) and frequencies (Hz) used are listed in Table 2.1. Surface waves were administered systematically as five consecutive blocks for each group. A block consisted of seven continuous stimulation periods of 0.5 min, each separated by a 1.0 min (no stimulus) interval, followed by a 10.0 min no stimulus (recovery) period. The time interval between successive blocks in each regime was 19.5 min.

5.3. Results

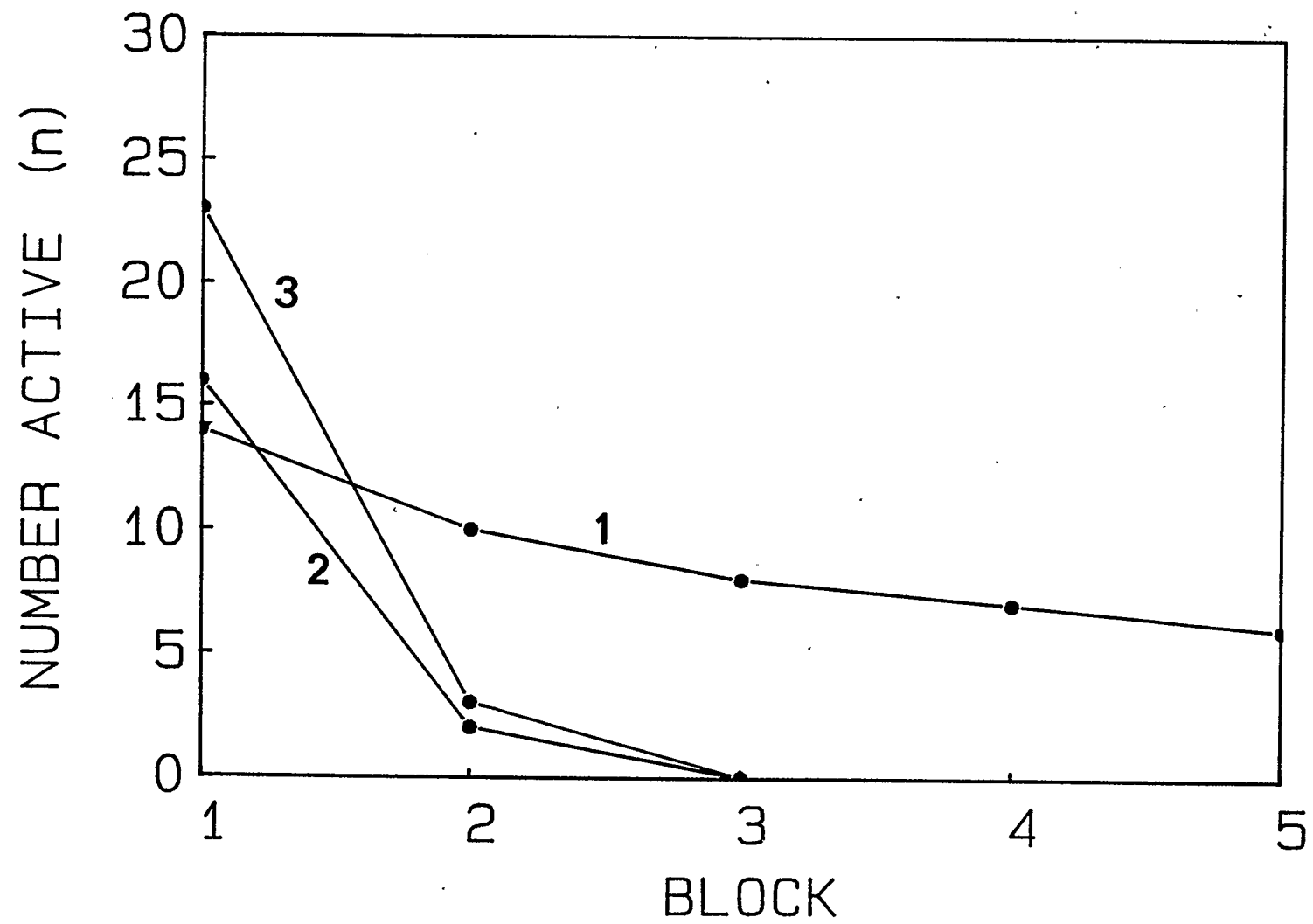
5.3.1. Repeated measures ANOVA of individuals active

Post-feeding time did not significantly effect the mean numbers of individuals responding to surface wave stimulation between regimes ($F_{0.05,2,8} = 2.52$; Table 5.1). Short-term habituation of numbers of T. rude responding to surface wave stimulation (n) at 4, 6 and 8 months post-feeding, was indicated by the decline in n values across blocks (Table 5.1, Figure 5.1). At 6 and 8 months post-feeding, the response n, asymptoted more quickly than at 4 months post-feeding. Increasing post-feeding time, increased the initial responsiveness of T. rude within block 1, which as percentages of the number of individuals stimulated were; 47% at 4 months, 57% at 6 months and 77% at 8 months post-feeding. Tukey's test showed that the mean number of individuals responding to stimulation in block 1 was

Table 5.1: Repeated measures ANOVA of the number of Theromyzon rude active (n) in response to surface waves presented 4, 6 and 8 months post-feeding. R, regime; B, block.

Source of variation	DF	SS	MS	F	Pr>F
R	2	66.53	33.27	2.52	0.1414
B	4	564.93	141.23	10.71	0.0027
B(R)	8	105.47	13.18		

Figure 5.1: Frequency polygon showing the number of Theromyzon rude active to presentation of surface waves after 4, 6 and 8 months post-feeding. 1, regime 1; 2, regime 2; 3, regime 3.



significantly different from that in blocks 2, 3, 4 and 5, which were not significantly different from each other (Table 5.2).

5.3.2. Repeated measures ANOVA of activity times

Body waving was the first activity exhibited by all T. rude responding to surface wave stimulation. Following initiation of body waving activity, T. rude alternated between inchworm crawling and body waving. Total mean activity time by T. rude was significantly greater at 4 months post-feeding (regime 1), than at 6 (regime 2) or 8 (regime 3) months post-feeding, which were not significantly different from each other. The total mean ($\pm$ S.E.) activity times (Ttime) for each regime were: regime 1 = 372.03 ± 40.81 sec; regime 2 = 195.84 ± 50.47 sec; regime 3 = 195.53 ± 29.41 sec. Total mean body waving time (Twave) by T. rude was significantly greater at 4 months post-feeding (160.51 ± 14.98 sec), than at 6 (88.26 ± 14.05 sec) or 8 (119.92 ± 16.18 sec) months post-feeding, which were not significantly different from each other. Total mean inchworming time (Tinch) by T. rude was significantly greater at 4 (211.51 ± 35.75 sec) months post-feeding, than at 6 (107.58 ± 46.92 sec) or 8 (75.61 ± 18.70 sec) months post feeding, which were not significantly different from each other.

Post-feeding time did significantly affect individual Ttime, Twave and Tinch between regimes (Table 5.3) and

Table 5.2: Tukey's analyses of number active (n) and activity time (Ttime, Twave and Tinch) by Theromyzon rude in response to surface waves presented 4, 6 and 8 months post-feeding. Ranked sample means in parentheses.

Dependent variable	Regime	Block
n		1 2 3 4 5 (18.0)(5.0)(2.7)(2.3)(1.7)
Ttime	1 2 3 (372.0)(195.8)(195.5)	
Twave	1 2 3 (160.5) (88.3)(119.9)	
Tinch	1 2 3 (211.5)(107.6) (75.6)	

Table 5.3: Repeated measures ANOVA of activity (Ttime, Twave and Tinch) times by Theromyzon rude in response to surface waves presented 4, 6 and months post-feeding. R, regime; B, block; I, individual.

Activity	Source of variation	DF	SS	MS	F	Pr>F
Ttime	R	2	422843.21	211421.61	4.25	0.0288
	I(R)	51	2537059.55	49746.27		
	B	3	76419.00	25473.00	0.89	0.4617
	R*B	2	2720.33	1360.17	0.05	0.9536
	B*I(R)	21	599885.19	28565.96		
Twave	R	2	141913.33	70956.67	4.19	0.0030
	I(R)	51	363400.29	7125.49		
	B	3	25206.00	8402.00	1.52	0.2378
	R*B	2	5155.47	2577.73	0.47	0.6331
	B*I(R)	21	115857.07	5517.00		
Tinch	R	2	301812.90	150906.45	3.98	0.0251
	I(R)	51	1858509.18	36441.36		
	B	3	67851.33	22617.22	0.81	0.5026
	R*B	2	8828.33	4414.17	0.16	0.8548
	B*I(R)	21	586426.61	27925.08		

Tukey's test showed that mean Ttime, Twave and Tinch by T. rude was significantly higher in regime 1 than in regimes 2 and 3, which were not significantly different from each other (Table 5.2). No short-term habituation of Ttime, Twave and Tinch by T. rude across blocks occurred for the three levels of post-feeding (Table 5.3).

5.4. Discussion

Attempts to initiate activity of H. ghilianii, Theromyzon tessulatum and T. rude with partially filled guts are unsuccessful (Sawyer 1986; van der Lande 1983; Davies 1984). Sawyer (1986) proposed that a readiness to become active be regarded as an operational definition of starvation in sanguivorous leeches. Using this definition, starvation of T. rude kept in the laboratory at 20°C begins 4 months post-feeding (when individuals first become responsive to surface wave stimulation). Between 4 and 8 months post-feeding, starvation did not result in high mortality of T. rude. However, beyond 8 months post-feeding starvation resulted in high mortality of individuals.

Davies (1984) showed that T. rude require a minimum of three blood meals before sexual maturity is attained with the majority of the population take all three meals within 6 months of hatching. This study showed a slower digestion of a blood meal by T. rude fed and maintained in the laboratory. The reasons for the slower digestion by T. rude

are not clear.

As the starvation period increased, the block 1 numbers of T. rude responding to surface wave stimulation increased, indicating that starvation increases the level of sensitization affecting the initiation of activity by T. rude. The rate of habituation in numbers of T. rude active after 6 and 8 months post-feeding is faster than after 4 months post-feeding, suggesting that sensitization does not dissipate as quickly at 4 months post-feeding. It is not known whether the increased sensitization seen after 4 months post-feeding was due to resistance to decay, or increased recovery which may have occurred between continuous stimulation periods (10.0 min). These results suggest that starvation affects the sensitization process which influences the sensory motor pathways (where habituation is hypothesized to occur) responsible for producing activity by T. rude.

Three characteristics have been ascribed to a stimulus which initiates a behaviour (Petrinovich 1984). This study demonstrated all three when examining the numbers of T. rude responding to surface wave stimulation after 4, 6 and 8 months post-feeding. It elicited a response through a stimulus-response sensory motor pathway, it influenced the physiological state of the leech, and habituation and sensitization occurred independently but interacted to yield the final behaviour. Starvation produced an increase in the

level of sensitization responsible for initiation of activity, while repetitive presentation of surface waves produced a decrease in sensitization.

No evidence of habituation of total activity time of individuals or the times spent body waving and inchworm crawling was observed, thus none of the assumptions of habituation (Petrinovich 1984) were demonstrable. However, at the onset of starvation, total, body waving and inchworming times were significantly higher than for those individuals who were starved for longer periods of time.

Anholt and Davies (1985) showed that activity of the predaceous leech Nepheleopsis obscura was higher before than after feeding. For T. rude, this study showed that the total, body waving and inchworming activity times are greatest at the onset of starvation. However, as starvation progresses, activity by T. rude decreases, but the number of individuals responding to stimulation increases. In the field, after complete digestion of a blood meal, T. rude move from sheltered locations (Davies and Wilkialis 1980) and are more active following stimulation by surface waves, perhaps positioning individuals in locations with higher probability of host encounter. As the starvation period is increased, surface waves initiate activity by more individuals but the tendency to move to new locations and attach to a potential host is reduced perhaps as a means to conserve energy.

CHAPTER 6

HABITUATION AND EXPRESSION OF BODY WAVING AND INCHWORM CRAWLING ACTIVITIES TO CONCURRENT STIMULATION BY SURFACE WAVES AND ILLUMINATION

6.1. Introduction

Several studies have shown that for sanguivorous leeches, stimuli of different modality initiate the same activity, e.g. surface wave, tactile and illumination stimuli all initiate swimming by Hirudo medicinalis (Kretz et al. 1976; Friesen 1981; Young et al. 1981; Debski and Friesen 1985; Lockery et al. 1985), while surface wave (Chapter 3) and illumination (Chapter 4) stimuli initiate body waving and inchworming by Theromyzon rude. However, under natural conditions leeches are rarely subjected to isolated stimuli. The importance of concurrent stimulation by two different stimuli to sanguivorous leeches has not been examined. For T. rude, whether surface waves and illumination changes are hierarchally ordered, i.e. by modality, previous experience and the context within which the stimuli are received (Petrinovich 1973), is unknown.

This study investigates the effect of repetitive presentation of concurrent surface wave and illumination stimuli on short-term habituation and expression of the total activity time (Ttime), the activity times spent body waving (Twave) and inchworm crawling (Tinch) and the numbers

(n) of T. rude initiating host detection activity.

6.2. Methods

Four groups of T. rude (30 individuals per group) were exposed to four combinations of concurrent surface wave (high or low) and illumination (high or low) stimuli. The characteristics of the surface wave amplitudes (mm) and illumination changes (lux) used for each regime were: regime 1 - 0.29-0.58 mm, 1000 lux; regime 2 - 1.80-3.93 mm, 1000 lux; regime 3 - 0.29-0.58 mm, 15634 lux; regime 4 - 1.80-3.93 mm, 15634 lux.

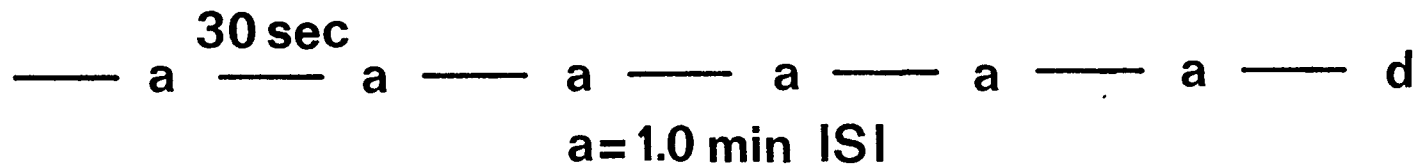
Stimuli were presented systematically in five consecutive blocks for each regime. A block consisted of: seven continuous surface wave stimulation periods of 0.5 min, each separated by 1.0 min (no stimulus) intervals; and concurrently, illumination stimulation consisted of three sets of two 10 sec increased illumination periods, with a 10 sec decreased illumination period interposed. Each illumination set was separated by a 4.0 min decreased illumination interval. A 10.0 min recovery period (no stimuli) followed both the seventh 0.5 min surface wave period and the third illumination set.

The three illumination sets coincided with the first, fourth and seventh 0.5 min surface wave stimulations (Figure 6.1). The 10.0 min (no stimulus) recovery periods for each regime were concurrent. The interval between successive

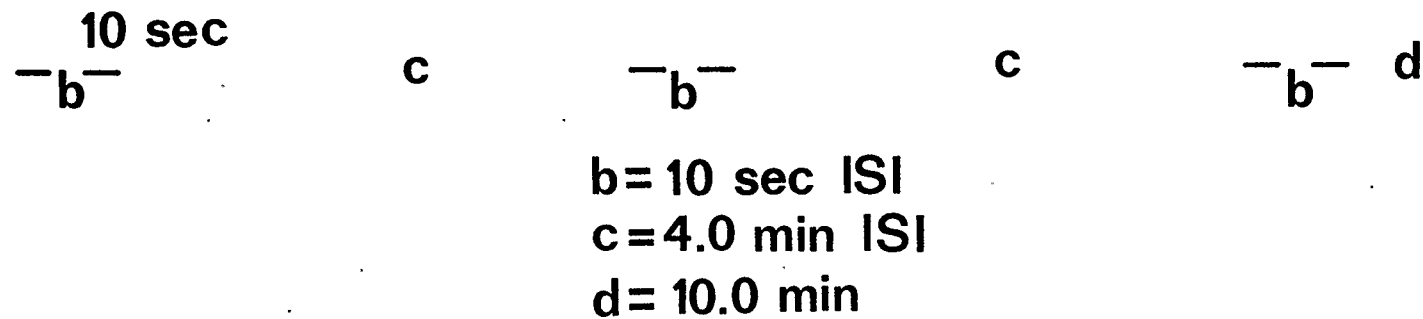
Figure 6.1: Schematic of concurrent surface wave and illumination changes presented to Theromyzon rude. (-) denotes stimulation. Not drawn to scale.

BLOCK = 19.5 min

1



2



blocks for each regime was 19.5 min.

6.3. Results

6.3.1. Repeated measures ANOVA of individuals active

Concurrent stimulation of T. rude did not significantly affect the total numbers of individuals active between regimes ($F_{0.05,3,12} = 1.64$; Table 6.1). However, more individuals in regime 4 became active within block 1 (53%), than in regimes 1 (7%), 2 (13%) or 3 (27%) (Figure 6.2).

Short-term habituation of T. rude was indicated by the significant block effect ($F_{0.05,4,12} = 5.72^{***}$; Table 6.1) and Tukey's test showed that n in block 1, was significantly different from n in blocks 2, 3, 4 and 5, which were not significantly different from each other (Table 6.2).

6.3.2. Repeated measures ANOVA of activity times

Body waving was the first activity exhibited by all T. rude responding to concurrent stimulation. Following initiation of body waving activity, T. rude alternated between inchworm crawling and body waving.

The total activity (T_{time}), body waving (T_{wave}) and inchworming (T_{inch}) times were not significantly different between regimes (Table 6.3). The mean ($\pm$ S.E.) T_{time} , T_{wave} and T_{inch} times by T. rude were: regime 1 ($T_{time} = 183.65 \pm 48.81$ sec; $T_{wave} = 138.25 \pm 30.41$ sec; $T_{inch} = 45.40 \pm 21.60$ sec); regime 2 ($T_{time} = 182.37$

Table 6.1: Repeated measures ANOVA of the number of Theromyzon rude active (n) in response to concurrent surface waves and illumination changes. R, regime; B, block.

Source of variation	DF	SS	MS	F	Pr>F
R	3	36.55	12.18	1.64	0.2326
B	4	170.00	42.50	5.72	0.0082
B(R)	12	89.20	7.43		

Figure 6.2: Frequency polygon showing the number of Theromyzon rude active to presentation of concurrent surface waves (high, low) and illumination changes (high, low). 1, regime 1; 2, regime 2; 3, regime 3; 4, regime 4.

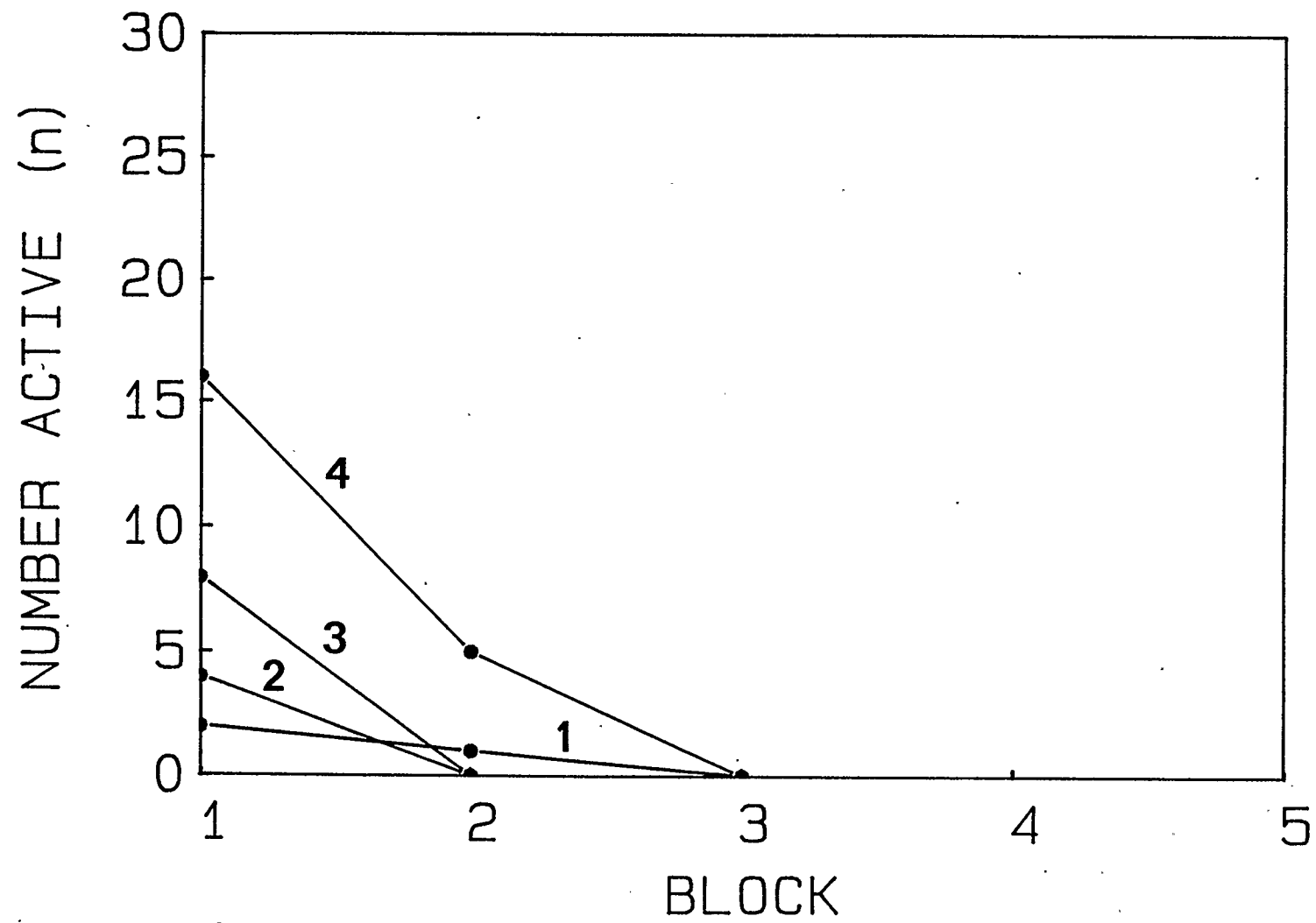


Table 6.2: Tukey's analyses of number of Theromyzon rude active (n) in response to concurrent surface waves and illumination changes, across blocks. Ranked sample means given in parentheses.

Dependent variable	Block				
n	<u>1</u> (7.5)	<u>2</u> (1.25)	3 (0.0)	4 (0.0)	<u>5</u> (0.0)

Table 6.3: Repeated measures ANOVA of activity (Ttime, Twave and Tinch) times by Theromyzon rude in response to concurrent surface waves and illumination changes. R, regime; B, block; I, individual.

Activity	Source of variation	DF	SS	MS	F	Pr>F
Ttime	R	3	204786.66	293451.57	1.82	0.1645
	I(R)	26	975174.55	37506.71		
	B	1	592.90	592.90	0.01	0.9327
	R*B	1	49491.23	49491.23	0.70	0.4638
	B*I(R)	3	211681.38	70560.46		
Twave	R	3	186321.29	62107.10	2.95	0.0548
	I(R)	26	547384.59	21053.25		
	B	1	1988.10	1988.00	0.11	0.7665
	R*B	1	4389.03	4389.03	0.23	0.6622
	B*I(R)	3	56476.38	18825.46		
Tinch	R	3	26476.30	8825.43	1.92	0.1750
	I(R)	26	119511.08	4596.58		
	B	1	409.60	409.60	0.02	0.8912
	R*B	1	83356.90	83356.90	4.50	0.1241
	B*I(R)	3	55588.50	18529.50		

± 75.68 sec; $T_{\text{wave}} = 158.50 \pm 65.32$ sec; $T_{\text{inch}} = 23.87 \pm 16.13$ sec); regime 3 ($T_{\text{time}} = 55.25 \pm 16.98$ sec; $T_{\text{wave}} = 54.25 \pm 16.98$ sec; $T_{\text{inch}} = 1.0$ sec); regime 4 ($T_{\text{time}} = 168.66 \pm 68.36$ sec; $T_{\text{wave}} = 145.00 \pm 81.67$ sec; $T_{\text{inch}} = 23.66 \pm 11.86$ sec).

No short-term habituation of T_{time} ($F_{0.05,1,3} = 0.01$), T_{wave} ($F_{0.05,1,3} = 0.11$) or T_{inch} ($F_{0.05,1,3} = 0.02$) across blocks was observed for any of the stimulus regimes (Table 6.3).

6.4. Discussion

A dearth of information exists for the effects of concurrent stimulation on the expression of activity by sanguivorous leeches. This study shows that for T. rude, repetitive presentation of four combinations of concurrent surface waves and illumination changes produced short-term habituation of the numbers of T. rude that were active. In addition, concurrent stimulation produced different initial response levels in T. rude. Specifically, when high surface wave amplitude and high illumination change were combined (high-high), the result was a higher initial number of individuals responding to stimulation. For the other combinations of stimuli presented to T. rude, a much lower response (n) was observed, even though individuals may have been receiving high surface wave amplitude (concurrent with low illumination change) or high illumination change

(concurrent with low surface wave amplitude) stimuli.

The increase in numbers of active T. rude to concurrent high-high regimes compared to the other regimes, suggests that sensitization of initiation of activity is influenced by the characteristics of concurrent stimuli, i.e. surface wave amplitude and level of illumination change. No short-term habituation of total activity time, or the times spent body waving and inchworm crawling.

In the field, as a waterbird moves towards T. rude surface waves of increasing amplitude will be produced as the attenuation distance is reduced. Waterbirds in close proximity to T. rude will probably also produce illumination changes during the day. However, the level of change will vary on a daily and seasonal basis. Concurrent stimulation of T. rude by high-high (regime 4) surface waves and illumination changes, increases the block 1 numbers of T. rude that are active compared to stimulation by low-low (regime 1), low-high (regime 2) or high-low (regime 3) regimes. Thus, T. rude respond to concurrent high-high stimuli as better indicating the presence of a potential host.

Both isolated surface waves (Section 3.2) and isolated illumination changes (Chapter 4) are used by T. rude to detect the presence of a potential host; increase of surface wave amplitude and level of illumination change increases the number of individuals that are active. Comparing the

total numbers of T. rude active in response to concurrent surface wave and illumination stimuli, isolate surface wave and isolate illumination change stimulation (Table 6.4), shows that isolate high surface waves initiate higher activity by T. rude than the other regimes. The four concurrent stimuli regimes decreased total numbers active compared to isolate surface wave (high and low) regimes, but increased total numbers active when compared to isolate illumination change (high and low) regimes. This suggests that concurrent surface waves and illumination changes have an antagonistic effect on initiation of activity in T. rude. In the field, isolate high amplitude surface waves are better indicators of the presence of a potential host than are any combination of concurrent (surface waves and illumination changes) or isolate illumination change regimes. The reasons for these differences are not clear.

There were significant differences between the activity times by T. rude in response to concurrent, isolate surface wave and isolate illumination change regimes (Table 6.5). Concurrent high-high and high-low regimes resulted in a significant reduction of inchworming time (and hence total activity time) compared to isolated high surface wave regime and a significant increase of body waving activity (and thud total activity time) when compared to isolated illumination change (high and low) regimes (Table 6.5). This suggests, that T. rude integrate concurrent surface wave and

Table 6.4: Comparison of total numbers (n) of Theromyzon rude responding to stimulation by concurrent (CS) and isolate (IS) surface wave (SW) or illumination (I11) stimuli (H high or L low).

Concurrent Stimulation			Single Stimuli				Comparison CS vs. IS
			SW		I11		
SW	I11		H	L	H	L	
H	H	20	34	-	15	-	SW>CS>ILL
H	L	8	34	-	-	5	SW>CS>I11
L	H	4	-	6	15	-	I11>SW>CS
L	L	3	-	6	-	5	SW>I11>CS

Table 6.5: Repeated measures ANOVA of activity times (Ttime, Twave and Tinch) by Theromyzon rude comparing stimulation by concurrent (CS) and isolate (IS) surface wave (SW) (H high) or illumination (Ill) changes (H high or L low) with Tukey's test for significant differences in parentheses. N.S. non significant.

Concurrent Stimulation			Isolate Stimuli		
SW	ILL		SW	Ill	
		Time	H	H	L
H	H	Ttime	** (<u>IS</u> <u>CS</u>)	*** (<u>CS</u> <u>IS</u>)	
		Twave	N.S.	*** (<u>CS</u> <u>IS</u>)	
		Tinch	** (<u>IS</u> <u>CS</u>)	N.S.	
H	L	Ttime	*** (<u>IS</u> <u>CS</u>)		*** (<u>CS</u> <u>IS</u>)
		Twave	N.S.		*** (<u>CS</u> <u>IS</u>)
		Tinch	*** (<u>IS</u> <u>CS</u>)		N.S.

illumination change stimuli to modify the expression of activity, i.e. illumination changes (regardless of intensity) reduce inchworming time while surface waves increase body waving activity. The two activities, body waving and inchworm crawling serve different functions, with respect to successful host contact. Body waving frees the anterior sucker from the substrate and allows for attachment to a host, while inchworm crawling serves to move the leech to new locations, perhaps with a higher probability of host contact. Decreasing inchworm activity and increasing body waving activity to concurrent high-high surface wave and illumination change, increases the probability of host encounter for two reasons: (i) it proportionally increases body waving activity necessary for successful host contact, (ii) it keeps T. rude at the location where probability of host contact is high.

There was no significant difference in activity times (total, body waving and inchworming) between; (i) the concurrent low-high regime and isolate high illumination change regime, and (ii) the concurrent low-low regime and isolate low amplitude surface wave regime (Table 6.6). However, the low-high concurrent regime significantly decreased activity time by T. rude compared to the isolate low surface wave amplitude regime, while the concurrent low-low regime significantly increased activity time compared to the low illumination change regime. The reasons why the

Table 6.6: Repeated measures ANOVA of activity times (Ttime, Twave and Tinch) by Theromyzon rude comparing stimulation by concurrent (CS) and isolate (IS) surface wave (SW) (L low) or illumination (Ill) changes (H high or L low) with Tukey's test for significant differences in parentheses. N.S. non significant.

Concurrent Stimulation			Isolate Stimuli		
			SW	Ill	
SW	ILL	Time	L	H	L
L	H	Ttime	*** (<u>IS</u> <u>CS</u>)	N.S.	
		Twave	*** (<u>IS</u> <u>CS</u>)	N.S.	
		Tinch	*** (<u>IS</u> <u>CS</u>)	N.S.	
L	L	Ttime	N.S.		** (<u>CS</u> <u>IS</u>)
		Twave	N.S.		** (<u>CS</u> <u>IS</u>)
		Tinch	N.S.		* (<u>CS</u> <u>IS</u>)

concurrent low-high regime reduced activity by T. rude are not clear, but may be related to the reduction of inchworming time by concurrent (low or high) illumination stimulation. Increasing activity to low-low concurrent stimulation T. rude may move (by inchworming) to a location where the probability of host encounter is high.

CHAPTER 7

SENSE ORGANS USED FOR THE DETECTION OF SURFACE WAVES AND ILLUMINATION CHANGES

7.1. Introduction

Leeches possess a variety of epidermal sense organs (e.g. eyes, sensilla, sensory buds and labial sense organs) structurally specialized to respond to stimulus modalities (e.g. light, water movement and chemicals). These sense organs consist of photoreceptive cells and (or) bipolar cells with apical cilia. Light microscopy has identified ciliated sense organs on numerous species which have been described using scanning electron microscopy in four families: Glossiphoniidae - Haementeria ghilianii by Sawyer (1986), Batracobdella picta (Verrill) by Dessler and Weller (1977), Piscicolidae - Oceanobdella sexoculata (Malm), Johanssonia arctica (Johansson), Malmiana brunnea (Johansson), M. scorpii (Malm) by Khan and Emerson (1981), Hirudinidae - Hirudo medicinalis by DeRosa and Friesen (1981), Phillips and Friesen (1982), Elliott (1987), and Erpobdellidae - Erpobdella montezuma Davies et al. (1985) by Blinn et al. (1986).

The ciliated sense organs of sanguivorous leeches are fixed in number and restricted in position to the neural annuli within body segments (Dessler and Weller 1977; Khan and Emerson 1981; DeRosa and Friesen 1981; Sawyer 1986)

while in predaceous species, the ciliated sense organs are neither fixed in number nor restricted to the neural annuli (Blinn et al. 1986). Sawyer (1986) suggested that ciliated sense organs are functionally more important in sanguivorous leech species inhabiting lentic ecosystems where they are used for host detection, than in lotic ecosystems and in predaceous species which capture prey by random encounter. Thus, Sawyer would predict that all sanguivorous leeches should possess fixed numbers of ciliated sense organs positioned on the neural annuli.

Eye pairs occur in stereotyped positions on the dorsal head region of leeches and are, in sanguivorous species, serially homologous with the ciliated sense organs (Sawyer 1986). However, within and between families, variation occurs in the number of eye pairs present (Sawyer 1986). For example, the minimum number of eye pairs (Moore 1946), occurs in the piscicolid Myzobdella lugubris Leidy which has one pair of widely separated eyes facing posteriorly, while the maximum number of five eye pairs (DeRosa and Friesen 1981) occurs in H. medicinalis.

This scanning electron microscopy study was undertaken to describe the morphology and distribution of the eyes and ciliated sense organs of T. rude involved in the detection of illumination changes and surface waves, to compare them to previously described species, and to determine whether the number, size and distribution of sensilla in T. rude

supports Sawyer's hypothesis on their function.

7.2. Methods and materials

Theromyzon rude were collected in May-June 1986 from Stephenson's Pond. They were maintained in the laboratory at 15°C in filtered aerated pond water and divided into three size-classes. Size-class I (0.19 ± 0.02 (S.D.) mg), collected from the ventral surface of brooding adults, had not taken a blood meal. Size-classes II (1.38 ± 0.50 mg) and III (14.77 ± 1.93 mg) comprised specimens which according to their size had imbibed their first and second blood meals, respectively (Davies 1984).

Five individuals of each size-class were fixed in buffered glutaraldehyde containing 2.5% glutaraldehyde, 0.2M Millonig's phosphate buffer (pH 7.4), and 0.14M sodium chloride (Cloney and Florey 1968) for 30 min at 20°C, and then washed in phosphate buffer (pH 7.4). After fixation the specimens were dehydrated in graded ethanol and dried in a Bomar SPC-900/EX critical point apparatus using carbon dioxide as the transitional fluid. The dried specimens were mounted (three dorsal and two ventral side up) on aluminum studs, coated with gold-palladium in a Technics Hummer V Sputtering system and examined with an AMR 1000 scanning electron microscope (SEM).

7.3. Results

7.3.1. Eyes and sensilla

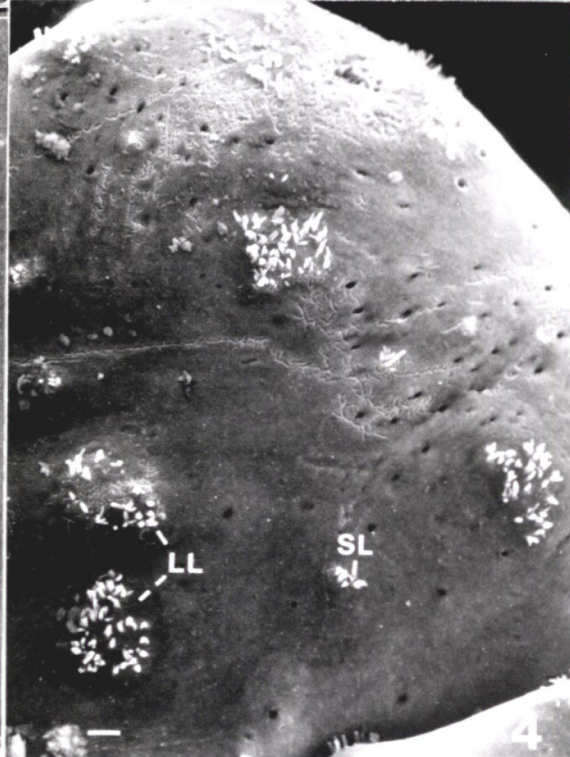
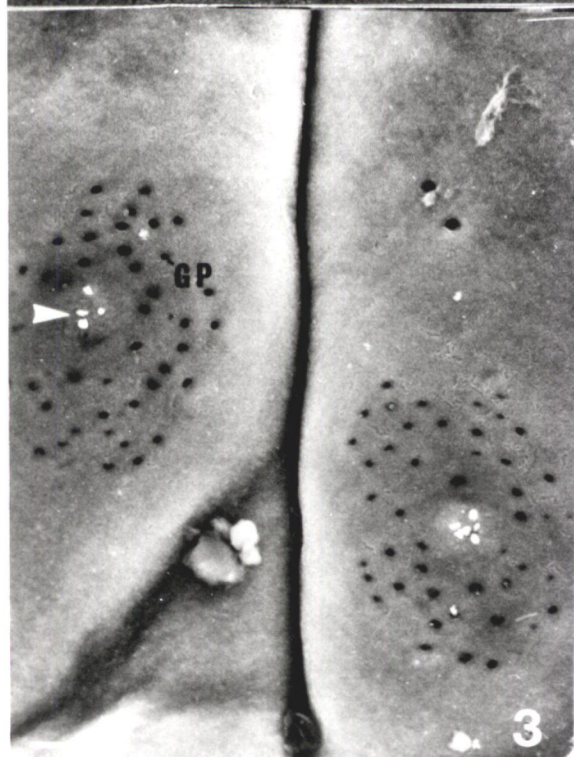
Two distinct paramedial rows of segmental sensilla (one pair per body segment on neural annulus a2) occur on the dorsal surface extending to but not onto the posterior sucker and are serially homologous with the four pairs of eyes present on body segments II, III, IV and V. The distance between the two paramedial rows of segmental sensilla was proportional to the body width. Each segmental sensillum consisted of a circular or elliptical patch of specialized epidermis devoid of gland pores. The shape and diameter of each segmental sensillum varied with the contraction, expansion and location on the body. Sensillum diameter ranged from 20 μm on antero-posterior segments to 40 μm on midbody segments. Using the nomenclature of DeRosa and Friesen (1981) modified by Blinn et al. (1986), two types of cilia occurred in each segmental sensillum: single cilium (S-cilium) and groups of cilia (G-cilia) with epidermal depressions encircling both types of cilia. Both S- and G-cilia (diameter 0.3 μm) projected 3.0 - 4.0 μm above the apex of the specialized epidermal surface (Figures 7.1 and 7.2) with G-cilia more numerous than S-cilia. The distinctions between S- and G-cilia are not as clear in T. rude as recorded by DeRosa and Friesen (1981) and Elliott (1987) for H. medicinalis. Segmental sensilla were absent from the ventral surface.

Figure 7.1: Segmental sensillum on prostomium showing cilia in a patch of epidermis devoid of pores. R = sensillum boundary ring. Scale bar = 5 μ m.

Figure 7.2: Pore-free sensillum bearing S- and G-cilia. S = S-cilium, G = G-cilia, D = epidermal depression. Scale bar = 5 μ m.

Figure 7.3: Ventral midbody segment showing sensory bud (arrowhead) having few cilia surrounded by 40-50 gland pores (GP). Scale bar = 5 μ m.

Figure 7.4: Prostomium showing large and small labial sense organs. LL = large labial sense organ, SL = small labial sense organ. Scale bar = 5 μ m.



7.3.2. Sensory buds

Irregularly dispersed sensory buds occurred between segment V and the ventral surface only. They ranged between 6.0 and 7.0 μm in diameter and were present on slightly raised regions of epidermis and contained fewer S- and G-cilia than segmental sensilla. Each sensory bud was encircled by an irregularly spaced ring (25-45 μm in diameter) of 40-50 gland pores (Figure 7.3).

7.3.3. Labial sense organs

On the dorsal and ventral surfaces of the prostomium and peristomium (Figures 7.4, 7.5 and 7.6) and segments III to V (Figure 7.7) small labial sense organs ranging from 6.0 to 7.0 μm in diameter occurred. In addition, large labial sense organs ranging from 20.0 to 35.0 μm in diameter occurred only on the prostomium (Figures 7.4 and 7.5). Both sizes of labial sense organ bore S- and G-cilia which projected 1.0 to 1.5 μm above the epidermal surface. In comparison, large labial sense organs possessed more S- and G-cilia than small labial sense organs.

7.3.4. Gland pores

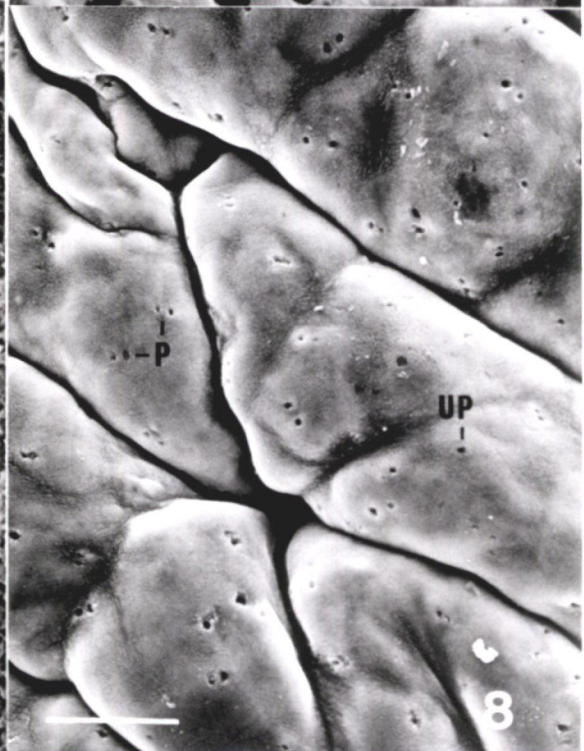
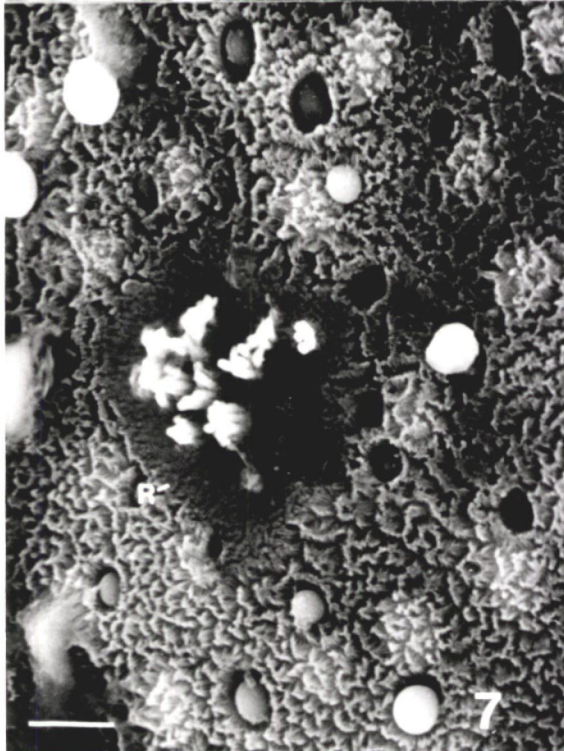
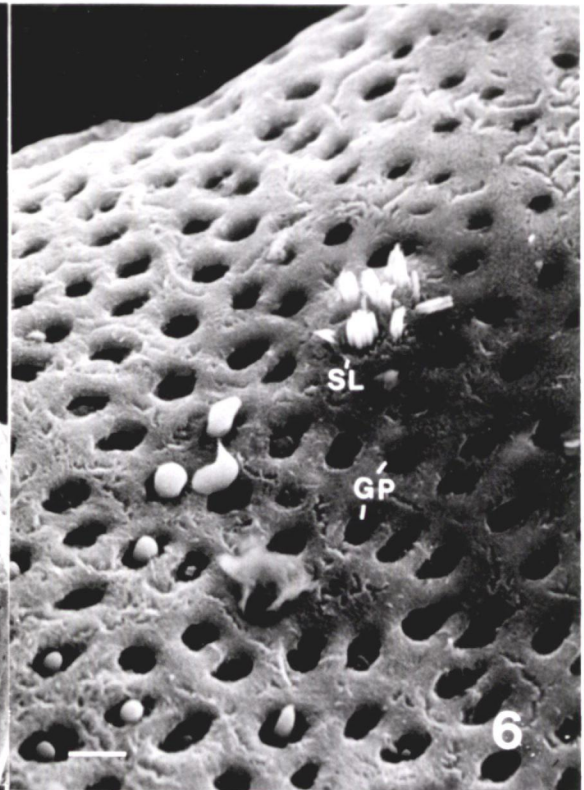
On the dorsal and ventral surfaces, gland pores occurred either singly or in pairs (0.3 to 0.4 μm apart) (Figure 7.8). Gland pores were more abundant on the dorsal surface. On the peristomium a band (100 μm by 260 μm) of

Figure 7.5: Transverse view of dorsal lip of mouth with a band of gland pores (GP) and large labial sense organ (LL) and small labial sense organ (SL). Scale bar = 50 μm .

Figure 7.6: High magnification of band of gland pores on dorsal lip showing small labial sense organ (SL) surrounded by gland pores with secretions. Scale bar = 5 μm .

Figure 7.7: High magnification of labial sense organ within buccal cavity showing ring of pore-free epidermis (R) bearing cilia and surrounding gland pores with secretions. Scale bar = 5 μm .

Figure 7.8: Dorsal midbody segment showing 3 annuli (oriented diagonally) showing numerous paired (P) and unpaired (UP) gland pores. Scale bar = 25 μm .



numerous irregularly dispersed (Figure 7.7) gland pores was present (Figures 7.5 and 7.6). All gland pores were 0.1 to 0.2 μm in diameter.

7.4. Discussion

Differentiation of the ciliated sensory cells into segmentally arranged sensilla, non-segmentally arranged sensory buds, and labial sense organs has been inadequately described in most studies of the epidermal sensory structures of leeches. This has resulted in some confusion and contradictions in comparing the morphology, distribution and function of these organs. Khan and Emerson (1981) refer to "clumps of cilia" which are interpreted by Sawyer (1986) as sensory buds because of their irregular arrangement on M. brunnea, M. scorpii and O. sexoculata despite their arrangement in 12 longitudinal rows around the body in J. arctica. Blinn et al. (1986) refer to sensilla on the dorsal lip of E. montezuma as does Elliott (1986) on H. medicinalis, although from their descriptions the ciliated structures around the mouth are not segmentally arranged and are more likely to be labial sense organs.

It is, therefore, suggested that for comparative purposes, ciliated sense organs should be classified as (i) segmental sensilla, if present on either the dorsal or ventral body surface in a stereotyped segmental pattern, (ii) sensory buds if present on either the dorsal or ventral

surface in a non-segmental and irregularly dispersed pattern, differing in size and shape from sensilla (if present), and (iii) labial sense organs when present on the prostomium, peristomium or within the buccal cavity.

Many leech species have an orderly arrangement of circular to elliptical segmental sensilla in longitudinal rows e.g. H. medicinalis has 14 longitudinal rows around the body (DeRosa and Friesen 1981) with the ventral sensilla (100-200 um) larger than the dorsal sensilla (60-90 um). However, in Hirudinaria manillensis (Lesson) and H. ghilianii some segmental sensilla are elongated so that the longitudinal sensilla axes appear oblique to the body axis. In T. rude there are two dorsal longitudinal paramedial rows of segmentally arranged sensilla extending to, but not onto, the posterior sucker. However, like H. ghilianii and most members of the Glossiphoniidae (Whitman 1892; Livanow 1902,1903), T. rude does not have segmental sensilla on the ventral surface. The segmental sensilla of T. rude are morphologically similar to those described for other species, consisting of a raised, pore free area of epidermis bearing S- and G-cilia.

A function of segmental sensilla in H. medicinalis is the detection of water movements (Friesen and Dedwylder 1981; Friesen 1981). Herter (1929) found that Theromyzon tessulatum exhibit positive vibro- and rheotaxis when stimulated by weak water waves and water flow, respectively,

and it has been shown (Chapters 3) that T. rude also respond to water waves. Although S-cilia have been hypothesized to detect water waves (DeRosa and Friesen 1981), the structural basis of rheotaxis has not been demonstrated. Tautz (1979) predicts that the structural differences between S- and G-cilia result in sensory specializations. However, cilia in segmental sensilla responding to water flow, could also respond to water waves. Determination of whether S- and (or) G-cilia respond to water flow in a purely tonic manner or to surface waves in a purely phasic manner, minimizing overlap in response, needs to be examined.

In many predaceous species, sensory buds occur on both the dorsal and ventral surfaces, e.g. Erpobdella octoculata (Linn.) (Havet 1900; Moore 1927) and E. montezuma (Blinn et al. 1986). Sensory buds are especially prominent in some species of Glossiphoniidae and Piscicolidae on the dorsal tubercles, but are absent (DeRosa and Friesen 1981) from H. medicinalis and J. arctica (Khan and Emerson 1981). In T. rude, sensory buds are restricted to the ventral body surface and differ from species so far described in being encircled by a ring of gland pores. The function of sensory buds is not known.

Two sizes of labial sense organs occur on the upper lip of T. rude, H. medicinalis (described by Elliott 1987) and four species of piscicolids examined by Khan and Emerson (1981). In H. medicinalis these receptors are chemosensory

(Elliott 1984, 1985a,b, 1986, 1987) and possibly thermosensory, mediating contact recognition of mammalian hosts (Gee 1913; Herter 1929,1936; Kaiser 1954) and initiation of the sucking response (Galun and Kindler 1966,1968; Galun 1975). However, Blinn et al. (1986) showed that in E. montezuma, the labial sense organs (incorrectly termed sensilla) function as mechanoreceptors, detecting the disturbance created by swimming amphipod prey, Hyalella montezuma Cole and Watkins. The possibility of the labial sense organs of T. rude being similarly used to detect hosts cannot be discounted.

Blinn et al. (1988a) examined the close predator-prey relationship between E. montezuma and H. montezuma, both species endemic to the near thermally constant habitat of Montezuma Well, Arizona, U.S.A. (Cole and Barry 1973; Boucher et al. 1984) a condition which may existed over the last 10000 years (Hevly 1974) or even longer (Batchelder 1974). Using labial sense organs near the mouth, E. montezuma selectively forages on juvenile H. montezuma (despite the presence of a wide variety of prey) based on differences in prey signal (Blinn et al. 1986; Blinn and Davies 1989). The non-specialized feeders E. octoculata and N. obscura, (neither found in Montezuma Well), forage primarily at night on a wide variety of prey types, in a broad range of microhabitats (Davies et al. 1978; Young and Ironmonger 1980; Davies et al. 1981; Davies et al. 1982b) in

a random manner (Greene 1874; Davies et al. 1982b; Sawyer 1986). Both E. octoculata and N. obscura exhibit a significantly lower responsiveness than E. montezuma to the signals of H. montezuma and H. azteca (Blinn and Davies 1989). Thus, in highly predictable conditions and over evolutionary time, close predator-prey (e.g. E. montezuma and H. montezuma) relationships can develop through the use of mechano- reception.

Similar to the ability of E. montezuma to distinguish between different prey type signals, T. rude discriminate between non-informative surface waves from surface waves which are indicators of the approach or presence of a host, based on stimulus characteristics (surface wave ISI and duration) (Chapter 3). The responsiveness of T. rude to surface wave stimuli, is affected by the physiological state of the individual (level of starvation) (Chapter 5) and preceeding experience (Chapter 3). However, unlike E. montezuma, E. punctata and N. obscura (Blinn et al. 1986; Blinn and Davies 1989) which rely on labial sense organs for prey detection, T. rude potentially use sensilla to detect a potential host.

Leeches secrete copius amounts of mucus, which serves a variety of functions including sucker adhesion, skin cleansing, defense against dessication (Sawyer 1986), and maintenance of the cuticle (Desser and Weller 1977). The gland pores present on the dorso-ventral surface of T. rude

are probably the openings of unicellular epidermal glands (Sawyer 1986). The abundance of gland pores within the buccal cavity, on the dorsal lip and surrounding the ventral sensory buds, suggest that these gland pore secretions aid lubrication and (or) enhance the efficiency of stimulus reception. Kennel (1886) described a similar band of gland pores in Cylicobdella joseensis (Grube and Oersted).

Sawyer (1986) suggested that segmental sensilla are functionally less important in predaceous and lotic species, but are well developed in sanguivorous species in lentic waters. Sanguivorous leeches rely heavily on sensilla for distal potential host detection (Friesen 1981; Young et al. 1981), while predaceous species use labial sense organs for prey detection (Blinn et al. 1986; Blinn and Davies 1989). The influence of food source and environment, may account for the differences of ciliated sense organ morphology, and their distribution and arrangement between sanguivorous and predaceous leech species. While the segmental sensilla of the sanguivorous T. rude are relatively large, they are less numerous and more restricted in distribution than on the sanguivorous species previously described and thus do not fully support Sawyer's hypothesis.

CHAPTER 8

GENERAL DISCUSSION AND SUMMARY

Theromyzon rude primarily feeds in the nares of waterbirds. Theromyzon rude is restricted to the nearctic and is characteristically found in lakes and ponds distributed along the Rocky Mountain duck migration routes of the Provinces, Territories and States west of the Great Lakes (Herrmann 1970; Davies 1973) and the American midwest (Klemm 1972, 1977).

Following digestion of a blood meal T. rude move from under rocks and deeper waters to the upper exposed surfaces of rocks, stones and macrophytes of shallow waters (Davies and Wilkialis 1980). Movement from sheltered to exposed regions of lentic ecosystems is related to the reversal of light avoidance behaviour associated with physiological state, i.e. starved T. rude are photopositive while satiated individuals are photonegative (Davies 1984). At or near the surface, T. rude are exposed to surface waves and illumination changes produced by potential hosts (waterbirds) as well as those occurring naturally.

Theromyzon rude use the stimuli produced by waterbird activity to detect a suitable host. The questions addressed in this study were: to which properties of surface waves and illumination does T. rude respond, and how do they respond?

In the field, waterbirds produce surface waves as they swim and (or) dabble (drink and feed) in shallow waters

where T. rude are most abundant (Davies and Wilkialis 1980). Waterbirds approaching T. rude produce continuous surface waves which increase in amplitude as the attenuation distance is reduced. In contrast waterbirds which visit temporarily, skirt and (or) move in and out of this location, produce short and (or) discontinuous surface waves. In addition, during daylight waterbirds passing over T. rude will expose individuals to illumination changes which will vary in intensity on a diel and seasonal basis.

Once activity by T. rude is initiated, differential expression of body waving and (or) inchworming is crucial to successful host contact and subsequent location of the nares where feeding occurs. The two activities (body waving and inchworming) probably serve different functions with respect to successful host contact. Body waving frees the anterior sucker from the substrate and allows for attachment to a host. Inchworming serves more to move the leech to new locations presumably to increase the probability of host contact.

The site of T. rude attachment to a waterbird determines the probability of locating the nares. If T. rude attach to the bill, the probability of locating the nares is much greater than if attachment occurs elsewhere on the body surface. On the bill, T. rude inchworm towards the nares, guided primarily by water streaming from the nares during waterbird feeding and drinking (negative rheotaxis) and

secondarily by thermal gradients on the bill surface (positive thermotaxis) and body waving is rarely displayed (Vandenberg 1987). Temperatures measured on the bill surface, with and without water streaming are $>25^{\circ}\text{C}$, while temperatures recorded on other body surfaces are $\leq 20^{\circ}\text{C}$ due to: (i) the insulatory effect of feathers which reduces the body surface-water temperature differential (ii) water temperatures which in the shallow water zones do not exceed 20°C during summer. This suggests selective thermosensory contact recognition by T. rude of waterbird bills. If T. rude attach (by body waving or inchworming) to other parts of the body (e.g. legs or feathered regions) they usually detach without feeding (Vandenberg 1987).

The main objectives of this study were to examine surface waves and illumination changes as stimuli which elicit host detection behaviour by T. rude, and how expression of activity increases the probability of host encounter. The importance of six variables which affect the ability of T. rude to detect the presence and (or) approach of a waterbird were examined: (i) continuous versus discontinuous surface waves, (ii) surface wave amplitude (iii) intensity of illumination change (iv) concurrent stimulation by surface waves and illumination (v) the context within which a stimulus is presented (vi) preceeding experience, and (vii) physiological state.

Separately, both surface waves (Chapter 3) and change

of illumination (Chapter 4) initiate host detection activity by T. rude. Increases in surface wave amplitude (from 0.29-0.58 mm to 1.80-3.93 mm) or level of illumination change (from 1000 to 15634 lux) increase the number of T. rude initiating activity. In addition, surface waves which are more continuous (reduced ISI) and of longer duration (3.5 min) compared to shorter durations (0.25 and 0.5 min) also increase initiation of activity. Short-term habituation of initiation of activity by T. rude was observed for each level of isolated surface wave amplitude and level of illumination change. However, habituation of initiation of activity at surface wave amplitudes of 1.80-3.93 mm was faster than at surface wave amplitudes of 0.29-0.58 mm and 0.92-1.70 mm. Similarly, habituation of T. rude was faster at illumination changes of 15634 lux than at illumination changes of 1000 lux and 7564 lux. It is suggested that the relative increase of initiation of activity observed after presentation of: (i) surface wave amplitudes of 1.80-3.93 mm, (ii) continuous surface waves of duration 3.5 min and (iii) illumination change of 15634 lux, was due to a transient initial increase of sensitization at the beginning of each regime, which quickly dissipated with repetitive stimulation.

As T. rude are progressively starved from 4 to 8 months post-feeding, the block 1 number of individuals responding to surface wave stimulation (0.92-1.70 mm) increases

(Chapter 5). However, the rate of habituation of initiation of activity after 6 and 8 months post-feeding is faster than after 4 months post-feeding, providing evidence that starvation also affects: (i) the initial level of sensitization, (ii) rate of decay of sensitization.

Leeches are facultative anaerobes which produce adenosine triphosphate (ATP) from glucose (stored as glycogen) in the inner longitudinal muscles by glycolysis (Talesara 1966; Elder and Rogers 1977) and by oxidative metabolism in circular and peripheral muscle (Fischer 1967). Energy expenditure (metabolism) of an organism is most commonly indirectly determined by measuring respiration (Florey 1966; Phillipson 1981). Wrona (1982) showed that oxygen consumption by active Nepheleopsis obscura and Erpobdella punctata was significantly higher than consumption by resting individuals. Prolonged starvation of T. rude likely depletes glycogen stores for synthesis of ATP used for body maintenance and repair. Starvation of T. rude increases the rate of habituation of initiation of activity and may serve as a means to conserve energy.

Concurrent stimulation by high surface waves of amplitudes 1.80-3.93 mm and high illumination changes of 15634 lux, increases the number of individuals active relative to concurrent stimulation by high-low, low-high or low-low surface waves and illumination changes (Chapter 6). High amplitude surface waves presented on their own are

better releasers of activity by T. rude than surface waves with high amplitude concurrent with low or high illumination changes. This suggests that surface waves are better than illumination stimuli as releasers of host detection activity by T. rude, and that surface waves and illumination stimuli have an antagonistic affect on initiation of activity. Short-term habituation of initiation of activity was observed for each concurrent regime. However, the rate of habituation was faster when T. rude were exposed to concurrent high-high surface waves and illuminations. It is suggested that increase of numbers active in block 1 to concurrent high-high stimulation compared to high-low, low-high or low-low concurrent stimulation was due to an transient increase of initial sensitization which dissipated quickly.

Illumination changes of different intensity presented on their own do not affect activity times (total, body waving or inchworming) by T. rude (Chapter 4) and short-term habituation was not observed. This suggests that illumination changes are used only for the detection of a potential host with an all or none response in terms of activity time so that T. rude shows no flexibility in expression of body waving and inchworming.

Surface waves of increasing amplitude presented on their own increase both total activity and body waving time by T. rude, while discontinuous surface waves of reduced

duration increase both total activity and inchworming time (Section 3.2, Chapter 3). No short-term habituation of total activity, body waving or inchworming times was observed for any of the surface wave regimes presented. This suggests that surface wave ISI influences expression of inchworming time by T. rude, while surface wave amplitude influences expression of body waving. Increasing inchworming time in response to a stimulus characteristic of a distant host, perhaps positions T. rude in a new location with higher probability of host encounter. Surface waves of increasing amplitude indicate the close proximity of a potential host and T. rude responds by increasing body waving activity, in readiness for host contact.

Concurrent (high-high) surface waves and illumination changes reduce total activity time by T. rude compared to high surface wave amplitude presented on its own and a significant increase of body waving activity when compared to illumination changes presented on their own (high and low) (Chapter 6). This shows, that T. rude integrate concurrent surface wave and illumination stimuli to modify expression of activity, i.e., illumination changes reduce inchworming time while surface waves increase body waving activity.

The physiological state of T. rude also influences their responses to surface waves. The total activity times of individuals was inversely proportional to numbers active

after 4, 6 and 8 months post-feeding, i.e. total activity time by individuals was reduced as starvation time increased, perhaps as a means of energy conservation. Starvation does not selectively influence the expression of body waving or inchworming.

The use of two-factor dual-process theory (Petrinovich 1984) offers a model within which modification of host detection by T. rude can be explained. The basic premise of the theory is that only stimuli which are different on some dimension (e.g. intensity or modality) act as efficient releasers of a behaviour, while repetitive presentation of a stimulus results in habituation of behaviour. Surface wave amplitude, duration, ISI, and level of illumination change in the field vary in their probability in informing T. rude of the proximity or approach of a potential host. Both stimulus modalities communicate to T. rude varying degrees of information regarding the presence or approach of a potential host. T. rude will initiate activity to both isolate and concurrent surface wave and illumination stimuli which are characteristically different from previous stimuli. The first activity expressed by T. rude after presentation of different surface waves or illuminations is body waving which preceeds attachment to a host. Thus, T. rude initially perceive different surface waves or illuminations as indicating the proximity of a host. However, if T. rude do not contact a host following

initiation of activity, individuals will either increase inchworming time to move to new locations (where perhaps the probability of host encounter is higher) or habituate to the stimulus. Thus, a trade off between the energy investment in moving to new locations versus potential increase of host encounter probability occurs.

Theromyzon rude do not explicitly discriminate between host and non-host generated surface waves and illumination changes. Theromyzon rude responds as if all surface waves and illumination changes different from previous stimuli indicate the potential presence of a host. If during repetitive stimulation, host contact does not occur, habituation of initiation of activity and alteration of the expression of body waving and inchworming occurs. The biological significance of habituation of initiation of activity is apparently: (i) the protection of the initiation of activity mechanism from reactions to non-host generated stimuli (ii) to maintain alertness toward "host generated stimuli", and (iii) as a means of energy conservation. The ecological significance of modifying the expression of body waving and inchworming times is associated with potentially increasing the probability of host encounter.

Blinn and Davies (1989) showed that close predator-prey relationships can develop over evolutionary time in isolated aquatic ecosystems by comparing the mechanosensory abilities of three erpobdellid species, Erpobdella montezuma, N.

obscura and Erpobdella punctata. The mechanosensory receptors (labial sense organs) of Erpobdella montezuma are highly selective for the acoustic vibrations produced by the rapid movement of the pleopods during the swimming action of the endemic Hyalella montezuma prey counterpart (Blinn et al. 1986,1988). In contrast neither E. punctata nor N. obscura who lack labial sense organs (Blinn and Davies 1988) showed highly developed abilities to detect amphipod prey by mechanoreception. This is probably the result of amphipods not being the major prey and the habitats in which these two species usually occur being less suitable for mechanoreception.

Sanguivorous leeches rely on segmental sensilla to detect the surface waves (Friesen and Dedwylder 1978; Friesen 1981) and eye pairs to detect illumination changes (Kretz 1975; Kretz et al. 1976) generated by a potential host. T. rude also rely on sensilla and the four eye pairs to detect surface waves and illumination changes, respectively (Chapter 7). Similar to the ability of E. montezuma to distinguish between different prey type signals, T. rude discriminate between surface waves and illuminations different from previous stimuli experience as indicating the potential presence or approach of a host. Thus, a host-parasite relationship has developed between T. rude and waterbirds through the use of both mechano- and photoreception. It is likely that other sanguivorous leeches

have also developed similar close host-parasite relationships through the use of stimuli used to detect the potential presence or approach of a host.

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