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ON THE MECHANISMS BY WHICH A LARVAL PARASITE (*POLYMORPHUS PARADOXUS*, ACANTHOCEPHALA) ALTERS THE BEHAVIOR OF ITS INTERMEDIATE HOST (*GAMMARUS LACUSTRIS*, CRUSTACEA)

by

SIMONE MARIE HELLUY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

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Date...FEBRUARY 1st, 1988.....

DEDICATION

A Marguerite, ma mère

ABSTRACT

Some larval helminths alter the behavior of their invertebrate intermediate hosts by changing the hosts' responses to environmental stimuli. The mode of action of these parasites was investigated using the acanthocephalan *Polymorphus paradoxus* in its intermediate host *Gammarus lacustris* (Crustacea). The worm induces, in this fresh water shrimp, an altered escape response and a shift in habitat. Infected gammarids escape towards a light source, skim the surface, cling to any material and remain immobile in a flexed posture. Uninfected gammarids escape away from a light source, do not skim and do not cling. The shift in habitat is associated with a shift in light preferendum towards zones of higher illumination (Holmes and Bethel, 1972).

The flexed posture of clinging infected gammarids was reminiscent of the flexed posture of lobsters injected with the biogenic amine serotonin (Livingstone et al., 1980); therefore, the effects of injected amines on the behavior of gammarids were tested. Serotonin (5-hydroxytryptamine, 5-HT), 1 to 20 µg, injected into uninfected gammarids, transiently induced three components of the "altered behavior": higher photopositivity, skimming and clinging responses. Octopamine (5, 10 µg) antagonized the clinging response but not the photopositivity of infected gammarids. Dopamine (10 µg) and noradrenalin (10 µg) had lesser antagonistic effects on clinging. Histological sections did

not reveal any qualitative difference between the eyes of infected and uninfected gammarids. However, the accessory screening pigment was, on average, more light adapted in infected gammarids than in uninfected ones. The increased photopositivity induced by serotonin was not accompanied by a migration of the accessory screening pigment.

The results are interpreted as follows. Serotonin may modulate the different components of the altered escape behavior in the CNS of infected gammarids; that is, the transient directional response to light, and the skimming and clinging behaviors. The position of the accessory screening pigment (serotonin independent) may mediate the persistent graded response to light (i.e., the light preferendum), and hence participate in the habitat shift of infected gammarids.

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I. INTRODUCTION

In the last two decades a number of studies have explored an intriguing phenomenon: the alterations of behavior induced by larval helminths in their invertebrate intermediate hosts. In many cases, the altered behavior can be reduced to changed responses to environmental stimuli (Holmes and Bethel, 1972). However, in none of these cases has the mode of action of the parasites been determined. Therefore, the aim of this study is to investigate that aspect of the alterations of behavior.

There are by now more than a dozen cases known where larval helminths, mainly Acanthocephala and Trematoda, change the behavior of their invertebrate intermediate hosts, mainly Crustacea, Mollusca, and Insecta (Table I-1). In the cases listed in Table I-1, the altered behavior occurs in an intermediate host which must be ingested by the definitive host of the parasite for the cycle to continue (Fig. I-1; see reviews in Holmes, 1976; Combes, 1980, 1983; Helluy, 1983a; Moore, 1984). These definitive hosts are diverse comprising fishes, birds and mammals. However, where laboratory predation tests have been performed, the definitive host has eaten significantly more infected than uninfected intermediate hosts (Table I-2). Therefore, the altered behavior of the invertebrate intermediate host makes it more vulnerable to predation by the definitive host of the parasite, thus probably enhancing the parasite's transmission.

Table I-1. Larval helminths that alter the behavior of their invertebrate intermediate hosts.

Intermediate host (ingested by definitive host)	Parasite	Reference
	Acanthocephala:	
Amphipoda	*Polymorphus paradoxus*	Bethel and Holmes, 1973
	Polymorphus marilis	
	Corynosoma constrictum	
	Polymorphus minutus	Hindsbo, 1972
	Pomphorhynchus laevis	Kennedy et al., 1978
Isopoda	*Acanthocephalus jacksoni*	Muzzall and Rabalais, 1975
	Acanthocephalus dirus	Camp and Huizinga, 1979
	Plagiorhynchus cylindraceus	Moore, 1983a
Ostracoda	*Octospiniferoides chandleri*	Demont and Corkum, 1982
Insecta	*Moniliformis moniliformis*	Moore, 1983b; Wilson and Edwards, 1986
	Nematoda:	
Isopoda	*Dispharynx nasuta*	Moore and Lasswell, 1986
	Cestoda:	
Amphipoda	*Diplocotyle sp.*	Stark, 1965
	Trematoda:	
Insecta	*Dicrocoelium dendriticum*	Hohorst and Graefe, 1961; Anokhin, 1966; Spindler et al., 1986
	Dicrocoelium hospes	Romig et al., 1980
	Brachylecithum mosquensis	Carney, 1969
Amphipoda	*Microphallus papillorobustus*	Helluy, 1983b, 1984
Chaetognatha	Hemiuridae	Pearre, 1979
Gastropoda	*Leucochloridium spp.*	Ulmer, 1971
	Neoleucochloridium sp.	
Bivalvia	*Parvatrema affinis*	Swennen, 1969

Table I-2. Experimental predation tests involving invertebrates exhibiting altered behavior induced by parasites.

Intermediate host	Infected by	Eaten x times more than uninfected host	By definitive host	Reference
Gammarus lacustris (Amphipoda)	Polymorphus paradoxus (Acanthocephala)	4 *	Mallard	Bethel and Holmes, 1977
Gammarus pulex (Amphipoda)	Pomphorhynchus laevis (Acanthocephala)	4 *	Minnow	Kennedy et al., 1978
Asellus intermedius (Isopoda)	Acanthocephalus dirus (Acanthocephala)	7.5	Minnow	Camp and Huizinga, 1979
Armadillidium vulgare (Isopoda)	Plagiorhynchus cylindraceus (Acanthocephala)	1.6 *	Starling	Moore, 1983
Gammarus insensibilis (Amphipoda)	Microphallus papillorobustus (Trematoda)	2.4	Gull	Helluy, 1984

* calculated from the authors' data.

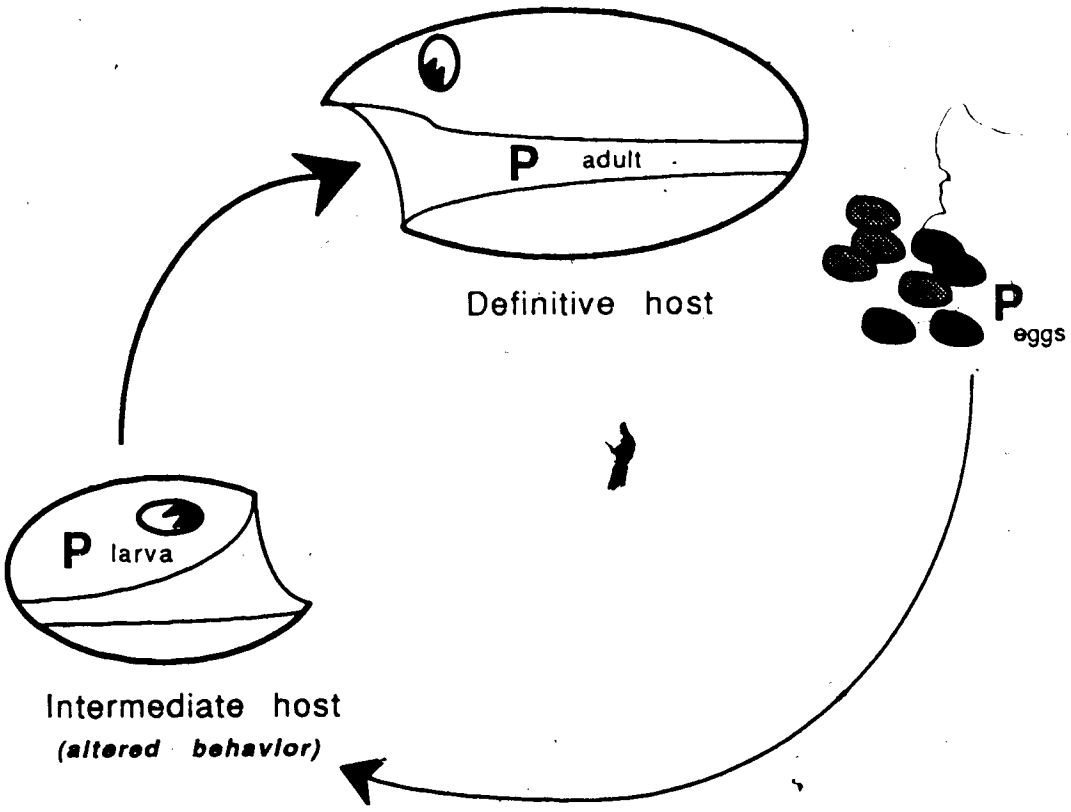


Fig. I-1. Life cycle of an acanthocephalan parasite (P).
The adult lives in the intestine of the definitive host, a vertebrate. The female lays eggs which are passed out with the feces of the host. An egg eaten by an arthropod develops parenterally into an infective larva, called a cystacanth. The cycle is completed when the intermediate host is ingested by the definitive host.

In other groups of helminths, additional stages and additional intermediate hosts may be added between the egg and the larval stage that is infective to the definitive host (thin arrow). However, in all cases listed in Table I-1, the altered behavior occurs in the intermediate host that is ingested by the definitive host.

Larval helminths have been shown to change responses in the invertebrate intermediate host to virtually all types of environmental stimuli: light, temperature, humidity, mechanical stimuli, gravity and chemical stimuli (Table I-3). Proprioceptive stimuli have not been mentioned. Different host-parasite systems involve altered responses to different stimuli that seem to fall into two main categories (terminology that of Camhi, 1984).

1) Altered responses to persistent stimuli, leading to habitat shifts. For a stimulus with a gradient, infected animals choose a different intensity of the stimulus than uninfected animals. This shift in preferendum brings the infected hosts into a different habitat. For example, isopods infected with *Plagiorhynchus cylindraceus* prefer less humid areas than uninfected isopods, and gammarids infected with *Polymorphus marilis*, *Polymorphus paradoxus* or *Microphallus papillorobustus* prefer zones of higher illumination than uninfected gammarids.

2) Altered responses to transient stimuli, modulating subsequent responses. Altered responses to a sequence of stimuli require a triggering stimulus to be expressed; a new behavioral sequence is created. For example, mechanical disturbance triggers altered escape responses in gammarids infected with *P. paradoxus* or *M. papillorobustus*, including a change in the orientation of the escape (towards the light source, rather than away from it).

Table I-3. Environmental stimuli involved in the altered behavior of invertebrate intermediate hosts infected by various larval helminths.
Only cases where the authors have studied the responses of infected hosts to a definite stimulus, eliminating variation in other parameters, are listed; references as in Table I-1.

Stimulus involved in altered behavior of intermediate host	Parasite	Host
Light	*Polymorphus paradoxus*	Gammarid
	Polymorphus marilis	Gammarid
	Polymorphus minutus	Gammarid
	Corynosoma constrictum	Hyalellid
	Pomphorhynchus laevis	Gammarid
	Octospiniferoides chandleri	Ostracod
	Moniliformis moniliformis	Cockroach
	Microphallus papillorobustus	Gammarid
	Brachylecithum mosquensis	Ant
	Dispharynx nasuta	Terrestrial isopod
Temperature	*Dicrocoelium dendriticum*	Ant
	B. mosquensis	Ant
Chemical stimuli	*M. moniliformis*	Cockroach
Humidity	*Plagiorhynchus cylindraceus*	Terrestrial isopod
Mechanical stimuli	*P. paradoxus*	Gammarid
	M. papillorobustus	Gammarid
	P. cylindraceus	Terrestrial isopod
Gravity	*M. papillorobustus*	Gammarid

In addition, responses to different physical characteristics of the same stimulus can be altered. In the *P. paradoxus* system, responses to both direction and intensity of light are altered in gammarids. Also, mechanical stimuli can play diverse roles in the altered behavior. As a vibration or as a transient tactile stimulus, it can trigger an altered behavioral sequence (*P. paradoxus* system); as a persistent stimulus, it can lead to a thigmotactic response (*P. cylindraceus* infected isopods, clinging of *P. paradoxus* infected gammarids).

Besides being of interest to a parasitologist, the host-parasite systems listed in table I-1 represent a unique opportunity for a neuroethologist. In a single species, in a single population, in animals of the same age, a stereotyped behavior like an escape reaction consists of two totally different behavioral sequences, depending on whether the individual is parasitized or not. Behavioral changes are common in invertebrates, but they occur normally as compulsory modifications in the ontogeny of a species, and are often associated with changes in hormone levels. Truman and Riddiford (1977, 1978) distinguish two general categories in ontogenic behavioral changes induced by hormones: releaser effects and modifier effects.

> "A releaser action of a hormone is a direct triggering of a particular piece of behavior... In silk moths, for example, the eclosion hormone acts on the moth CNS to trigger a stereotyped program of

movements that begins 10-15 minutes after hormone application and results in the escape of the moth from the pupal cuticle and cocoon (Truman, 1976). Modifier actions are more subtle and serve to alter the responsive state of the CNS such that a given stimulus provokes a new behavioral response. This action often requires hours or days in which to become manifest. An example is the action of juvenile hormone in promoting sexual behavior in female grasshoppers [Loher and Huber, 1966]" (Truman and Riddiford, 1977 :285).

The host-parasite systems listed in Table I-1 seem to provide interesting models in which to study modifier effects.

A. *Polymorphus paradoxus* / *Gammarus lacustris* and related systems

The host-parasite system chosen for a study of the mode of action of parasites in their intermediate host comprises the acanthocephalan *Polymorphus paradoxus* Connell and Corner, 1957 (Palaeacanthocephala: Polymorphidae), and its intermediate host, the crustacean *Gammarus lacustris* Sars, 1864 (Amphipoda: Gammaridae). The definitive hosts in the system are mainly mallard ducks, muskrats and beavers (see life cycle in Denny, 1969; Bethel and Holmes, 1977). This host-parasite system is quite common in the ponds and lakes around Edmonton (Alberta, Canada). The altered behavior of

P. paradoxus infected *G. lacustris* has been investigated by Holmes and Bethel (1972), and Bethel and Holmes (1973, 1974, 1977). It consists of both a habitat shift and an altered escape behavior. The habitat shift is related to a shift in light preferendum towards higher illuminations (a graded component of the photic response).

The altered escape behavior is more complex. When a *P. paradoxus* infected *G. lacustris* is disturbed, it escapes toward the source of light (toward the surface in natural conditions, but toward the bottom in aquaria lighted from below), and then skims the surface until it finds some solid material to which it clings firmly with the dactylopodites of its gnathopods (the claws of the first two of the seven pairs of thoracic legs). It then remains immobile in a flexed posture (Fig. III-1). An uninfected *G. lacustris* seems less sensitive to disturbance (Bethel and Holmes, 1973). When disturbed, it dives toward the bottom and hides in the mud.

During the full escape pattern of *P. paradoxus* infected *G. lacustris*, three different stimuli are involved, leading to two distinct motor patterns. The transient triggering stimulus is a mechanical one, such as a disturbance of the water around the gammarid (shadow responses do not seem to play a part in this host-parasite system). This disturbance prompts the *P. paradoxus* infected *G. lacustris* to escape toward the light source (a directional component of the photic response). When the surface is encountered (i.e.,

when the phototactic response cannot go further), a thigmotactic stimulus leads the *P. paradoxus* infected *G. lacustris* to cling. The whole sequence is not compulsory. An infected gammarid may cling to a support in a Petri dish after tactile stimulation without the directional response to light being expressed (Chapter III).

Two other polymorphids develop in the haemocoel of *G. lacustris* in Alberta (Denny, 1969), *Polymorphus marilis* and *Polymorphus contortus*. Both cystacanths are smaller than *P. paradoxus*. Little is known of the effects of *P. contortus*. *P. marilis* provokes in *G. lacustris* only an altered preferendum along a light gradient; infected gammarids seek zones of higher illumination. However, their escape reaction is normal; after disturbance, they swim away from the source of light and they do not cling (Bethel and Holmes, 1973). The different altered behaviors elicited by *P. paradoxus* and *P. marilis* appear to be adaptations to the feeding niches of their respective definitive hosts. *P. marilis* infects mainly diving ducks, and *P. paradoxus* mainly dabbling ducks or mammals feeding on the surface.

Presently, there are seven different combinations of *Gammarus* species and helminth species in which infected hosts have been documented to exhibit an altered behavior: *G. lacustris / P. paradoxus*, *G. lacustris / P. marilis* (Canada; Bethel and Holmes, 1973), *G. lacustris / P. minutus* (Denmark; Hindsbo, 1972), *G. pulex / Pomphorhynchus laevis* (England; Kennedy et al., 1978), *G. zaddachi / Diplocotyle*

sp. (England; Stark, 1965), *G. insensibilis / Microphallus papillorobustus* and *G. aequicauda / M. papillorobustus* (France; Helluy, 1983). The variety is not only in the geographic distribution of the pairs or in the taxa of helminth involved (acanthocephalan, cestode and trematode), but also in the microhabitats of the parasite in the gammarids. The acanthocephalan and cestode larvae float freely in the haemocoele of the gammarids when mature. The only larvae of the trematode *M.papillorobustus* that induce an altered behavior are those located in the cerebral ganglia.

The stimuli involved in the altered behavior have been investigated in all studies except the earliest one. In the six pairs studied, light has been shown to be of paramount importance as a stimulus in the altered responses. However, there are distinct variations in the type of alteration noted from one pair to the next. Although it is difficult to assess experimentally the difference between graded and directional responses to light, it has been shown that a directional response is not involved in *G. lacustris* infected by *P. marilis*, while it is involved in the same species of gammarid infected by *P. paradoxus*. *G. lacustris / P. paradoxus* is the only pair in which a clinging behavior is exhibited. Altered responses to gravity have been implicated only in *G.insensibilis / M. papillorobustus* (*G. aequicauda / M. papillorobustus* was not studied in that respect).

Other effects of the parasites on their hosts also differ. Altered haemolymph color has been reported only in *G. lacustris* / *P. minutus*. No trace of ovaries could be found in female *G. zaddachi* infected by *Diplocotyle* sp., and very few female *G. lacustris* infected by *P. paradoxus* present mature ovaries or carry a brood, but progeny were very abundant in female *G. insensibilis* infected by *M. papillorobustus*. Thus, each species of parasite mentioned above has a different action on its gammarid host, even in the same host species. Other larval helminths have been shown to have no perceptible action on the behavior of their gammarid host [such as the larval cestodes *Lateriporus spp.* in gammarids (Bethel and Holmes, 1973)]. Thus, the "altered behaviors" probably represent convergent adaptations in helminths to enhance their transmission (Helluy, 1983b).

B. Speculations on the mode of action

If responses to environmental stimuli are altered, it indicates that reflex pathways are modified in an infected intermediate host, most probably through a chemical or a mechanical action of the parasite. Bethel and Holmes (1973) suggest that a chemical action, an allomone, is more likely than a mechanical action, by pressure of the larvae on surrounding tissues, for at least three reasons:

1) The same altered behavior is elicited by parasites in somewhat different locations. For example, *P. paradoxus* is mostly found laterally in the haemocoele, at the junction of

thorax and abdomen (Fig. III-1), but is also active when present at the posterior end of the abdomen. The metacercaria of *D. dendriticum* is generally located in the suboesophageal ganglion of the ant and induces from dusk to dawn a contraction of the mandibles anchoring the ant to a plant. However, a metacercaria located in the supraoesophageal ganglion still induces a typical contraction of the mandibles (Romig et al., 1980).

2) Different parasites in approximately the same location, such as *P. paradoxus* and *P. marilis*, induce different types of altered behaviors in the same host species.

3) There are no changes in the behavior of the intermediate host for up to two months after infection for *P. paradoxus* in *G. lacustris* (Bethel and Holmes, 1974), one month for *Dicrocoelium hospes* in *Camponotus compressicapus* (Lucius et al., 1980), or approximately one month for *M. papillorobustus* in *G. insensibilis* (Helluy, 1981). Only when the larvae are mature and infective to the definitive host (several days after acquiring their maximum size) does the altered behavior appear (Bethel and Holmes, 1974).

If the action of the parasite is chemical, the active factor(s) has to be blood born before reaching its target. The circulatory system is open in crustaceans. Blood circulation is completed in less than 60 seconds in large decapods (Barnes, 1980). Little is known of the physiology of gammarids; however, the active factor(s) is likely to reach within seconds (see Chapter II-B) any target including

the central nervous system. The CNS of gammarids comprises a supraoesophageal ganglion, a suboesophageal ganglion, and a ventral nerve cord with seven thoracic and four abdominal ganglia (Macpherson and Steele, 1980a, 1980b).

Once it is postulated that the action of the parasite is chemical, several obvious questions are raised.

1) Are there one or several factors released by the parasite and responsible for the different aspects of the altered behavior?

2) What is the nature of the factor(s) released by the parasite?

3) Does this factor(s) act directly on its target (e.g., a nerve cell), or indirectly by influencing some biochemical pathway at an earlier stage?

4) Are the reflex pathways of the host affected at the peripheral sensory receptor level, at the central level, or at the peripheral effector level?

5) Is a reflex pathway created de novo in infected intermediate hosts, or is a preexisting reflex pathway elicited in the "wrong circumstances"? In other words, is the parasite rerouting an afferent input to a different efferent limb or is it inducing in its host the physiological characteristics of a special behavioral state?

6) As Truman and Riddiford (1977) put it: "Do the new behaviors occur by the facilitation of specific pathways or by the removal of inhibition from the appropriate circuit?"

7) Is the parasitic factor(s) released once, irreversibly modifying some host neural circuits, or is it produced continuously or at least intermittently, inducing a variable response?

The partial answers I will be able to provide emerged initially from the analogy of the flexed posture of infected gammarids with the flexed posture induced by the biogenic amine serotonin in lobsters and crayfishes (Livingstone et al., 1980). In these crustaceans, another biogenic amine, octopamine, produces an opposite extended posture. Serotonin and octopamine have also opposite actions on the escape behavior of the crayfish (Glanzman and Krasne, 1983). In addition, serotonin is involved as a neuroregulator in various sensory-motor processes (see Chapter VI-B, and Fig. VI-2). Thus, the effects of injected serotonin and octopamine on the clinging behavior of gammarids were tested. Nothing is known about amines in amphipods. However, several potential neuroregulators of invertebrates, dopamine, noradrenalin, and GABA (Leake and Walker, 1980), were arbitrarily chosen, and their effects on the clinging behavior also tested. High doses of serotonin (1 to 20 µg) injected into uninfected gammarids elicited, as a response to tactile stimulation, the clinging behavior induced by *P. paradoxus* in infected hosts. The other amines did not elicit the clinging behavior. Octopamine (5, 10 µg) and, to a lesser extent dopamine (10 µg) and noradrenalin (10 µg), injected in infected gammarids suppressed the clinging

behavior for hours. These results are reported in Chapter III.

If serotonin and octopamine have opposite actions on the clinging behavior, what are the effects of the two biogenic amines on the other components of the altered behavior, more specifically on the photobehavior of gammarids? Serotonin was found to induce a strong transient photopositivity when injected into uninfected gammarids, while octopamine did not affect the photobehavior of infected animals. Dopamine, morphine, and leu-enkephalin were also tested because they were suspected of controlling accessory screening pigment movements in the eyes. (These three substances are known to act as releasing factors of chromatophorotropins (Fingerman, 1985).) Neither the amine, nor the two peptides had any significant influence on the photobehavior of infected gammarids. It was also shown that serotonin reproduces in uninfected gammarids the skimming behavior (interpreted as being derived from the directional photic response). The data regarding the photobehavior are analysed in Chapter IV.

If light is so widely implicated in the altered behavior induced by helminths, could their hosts' photoreceptors be modified in a perceptible way? In crustaceans, photomechanical adaptations play an important part in adjusting the light intensity reaching the sensory structures of the compound eyes (Autrum, 1981). In gammarids, two sets of screening pigments, the retinular

screening pigment and the white accessory screening pigment, undergo migrations (Debaisieux, 1944). Their positions control the number of photons admitted at the level of the rhabdom. To answer the last question, the eyes of infected and uninfected gammarids were examined histologically in a light and dark adapted state. It appeared that there were no qualitative differences in the anatomy of the eye of infected and uninfected gammarids at the light microscope level. However, external quantitative examination of the eyes showed that on average, the white accessory screening pigment was slightly but significantly more superficial (i.e., more light adapted), in infected than in uninfected gammarids. The time-course of light and dark adaptation was studied and it was found that individual gammarids, whether infected or uninfected, have approximately the same range of amplitude of migration of their accessory pigment but at a different set point. The increased photopositivity induced by serotonin was not accompanied by a migration of the accessory screening pigment. The results concerning the study of the eyes are presented in Chapter V.

In the discussion (Chapter VI), I attempt to demonstrate that, although the quantities of serotonin necessary to obtain behavioral effects in gammarids were high, the effects were likely to be specific, rather than responses of a nervous system overwhelmed by a powerful biogenic amine. The possible level of action of serotonin in the reflex pathways leading to clinging is examined in the

light of recent literature on the behavioral action of serotonin in different invertebrate systems. The actual involvement of serotonin in the host-parasite system is also debated. The dual nature of the photic responses of gammarids is discussed. An explanation is proposed integrating the results obtained on the photic behavior, screening pigments, and action of amines. Part of this explanation assumes that serotonin modulates the different components of the altered escape behavior (the directional response to light, skimming, and clinging behavior), whereas the graded response to light (serotonin independent) is mediated, at least partly, through the position of the accessory screening pigment.

II. MATERIALS AND METHODS

A. Nature and origin of experimental hosts and parasites

Gammarus lacustris Sars, 1864 (Amphipoda: Gammaridae), uninfected or infected by the larval acanthocephalan *Polymorphus paradoxus* Connell and Corner, 1957 (Palaeacanthocephala: Polymorphidae), were collected at a pond near Polar Park, 27 km southeast of Edmonton (Alberta, Canada), from October 1983 until May 1984, and at Lakeview, South Cooking Lake, 34 km southeast of Edmonton, from June 1984 until August 1987.

For 22 months, ice depth or water temperature were measured at Lakeview during monthly visits to the station (Table II-1). Ice depth was measured about 100 m from the shore line after digging a hole with a 6 inch (15 cm) auger. In both years, the lake froze over in November. The thickness of the layer of ice increased gradually to about 80 cm in February - March; free water appeared in April. Water temperature was measured about 1 m from the shoreline, near the bottom at about 20 cm depth. It reached a peak of 22 C in August. In the correlation between water temperature and photic behavior (Chapter V), water temperature under the ice is arbitrarily considered to be 0 C. Monthly duration of day light at the approximate latitude of Edmonton (54 N) was taken from the Smithsonian Meteorological Tables (List, 1949).

Table II-1. Water temperature and ice depth at Lakeview, South Cooking Lake.

Date	Water Temp (°C)	Ice Depth (cm)	Date	Water Temp. (°C)	Ice Depth (cm)
Sept 11, 1985	10.5	-	Sept 14, 1986	11.0	-
Oct 12	4.0	-	Oct 11	6.0	-
Nov 14	.5	forming	Nov 15	-	25
Dec 12	-	60	Dec 13	-	50
Jan 18, 1986	-	65	Jan 17, 1987	-	65
Feb 15		85	Feb 14		70
Mar 15	-	80	Mar 14	-	85
Apr 12	-	5, near shore	Apr 18	1.0	thin layer
Apr 26	7.0	-	-	-	-
May 17	12.0	-	May 16	17.5	-
Jun 14	18.0	-	Jun 13	19.0	
Jul 19	20.0	-			
Aug 11	22.0	-			

Gammarids were present all year round and were captured with a dip net. Most of the infected gammarids were picked up by hand from vegetation, roots and logs near the shore line. In winter, the gammarids surged up with the water in the hole bored in the ice. Moving the auger up and down like a piston brought more gammarids to the surface.

The life cycle of *G. lacustris* is annual (Menon, 1966). When using wild gammarids as experimental animals, it is important to be aware that their physiological state changes over the course of a year. Populations of *G. lacustris* and of *P. paradoxus* were observed carefully for 22 months; a summary of these observations is presented in Table II-2. Gammarids in precopula, the male anchored on the back of the female (Fig. III-3), appeared as early as January. Gravid females bearing eggs or juveniles in their brood pouch were noticeable from April to June. Juveniles were released from the brood pouch in June and July and by August many young of the year were nearly the size of the parent generation. From September on, it was difficult to differentiate the two generations. Gammarids infected with *P. paradoxus* were available as soon as free water appeared near the shore in April. Gammarids with mature cystacanths were difficult to find in June, but harbored at that time many acanthellae (developing acanthocephalan larvae); this new generation of *P. paradoxus* matured in the parent gammarid generation by July. The first mature *P. paradoxus* in the young of the year were noticed in August.

Table II-2. Summary of field observations on Gammarus lacustris and Polymorphus paradoxus populations at Lakeview, South Cooking Lake.

Month	Observations on Host population	Observations on Parasite population
Jan	Some precopulating gammarids	P. paradoxus not found when ice present
Feb	"	"
Mar	Many precopulating gammarids	"
Apr	Many precopulating gammarids Few gravid females	Numerous mature P. paradoxus in overwintering gammarids
May	Few precopulating gammarids Many gravid females	"
Jun	Many gravid females Many juveniles	Few mature P. paradoxus in overwintering gammarids but numerous developing acanthocephalan larvae
Jul	Many adults, many juveniles	Numerous mature P. paradoxus in overwintering gammarids, presumably mostly the new generation
Aug	Young of the year nearly parent size	Mature P. paradoxus in spring gammarids
Sep	Difficult to differentiate the generations	Numerous mature P. paradoxus
Oct	"	"
Nov	"	P. paradoxus not found when ice present
Dec	"	"

In the laboratory, the amphipods were kept in lake water or in tap water left several days to dechlorinate. The water was aerated constantly using air stones. The tanks were situated in an environmental chamber at 17 -19 C with 12 hr dark, 12 hr light.

Adult gammarids, both male and female, weighing from 30 mg to 100 mg depending on the season, were used as experimental animals. However, in any one experiment, gammarids were as similar as possible. When injecting drugs, no correction was made for the differences in weight. The weight of the parasite represented approximately 0.5 % of the weight of the host. The average individual wet weight of 100 cystacanths of *P. paradoxus* (measured in groups of 20) was 340 µg. The average individual dry weight (after freeze drying) of 97 cystacanths (measured in groups of 5) was 51 µg.

B. Injecting gammarids - Chemicals used

Before an experiment, gammarids were housed in numbered individual plastic Petri dishes (diameter: 5.5 cm) filled with lake water. For the injection, a gammarid was placed head first in a well hollowed in plasticine, and immobilized with a strip of the same material. A 10 µl Hamilton syringe fitted with a special needle (0.26 mm in diameter, 17 mm long) was inserted laterally between two abdominal segments. Generally groups of 5 (clinging behavior tests) or 6 (photic behavior tests) were injected at a time. The most

satisfactory results were obtained with the following procedure: 1) the syringe was loaded with 2 µl solution, 2) the gammarid was dropped on paper towel to dry the cuticle, 3) it was fixed in the plasticine well, 4) the injection was performed under the dissecting microscope, 5) the time of injection was noted, 6) the syringe was loaded for the next gammarid, and 7) the injected animal was returned to its Petri dish. The whole process took approximately 2 minutes. For experiments lasting several days, the cuticle of the lateral abdomen was rubbed with 70 % alcohol prior to injection. It was once tested whether or not injected substances were distributed throughout the animal; 2 µl of diluted China ink were injected into gammarids; within seconds, the entire organism became darker. The quality of the injection improved with practice. Towards the end of the study, death following treatment was rare; the gammarids survived for several days (as long as observed), with only a black scar (due to deposition of melanin) at the point of insertion of the needle. The performance of a gammarid dying at any time during a test or immediately after was not considered in data analysis. All experiments were performed at room temperature (21 - 23 C), during the day. Nearly all experiments were started in the morning.

The composition of the crustacean saline was taken from Van Harreveld (1936; in Lockwood, 1961); per liter: Na Cl (12 g), KCl (0.4 g), $CaCl_2$ (1.5 g), $Mg\ Cl_2$ (0.25 g), $Na\ HCO_3$ (0.2 g), to which 0.3 g of glucose was added (Butterworth,

1968). The saline was stored at -20 C as a stock concentrated solution according to the technique of Coulombe (1970). The sodium bicarbonate was added on the day of use and the saline was never kept for more than two days after dilution.

The following chemicals were injected into the gammarids: ϑ-amino-n-butyric acid (GABA), (±) arterenol (norepinephrine, noradrenalin), 5,7-dihydroxytryptamine creatinine sulfate, [D-Ala^2, D-Leu^5]-enkephalin, 5-hydroxytryptamine creatinine sulfate and 5-hydroxytryptamine hydrochloride (5-HT, serotonin), 3-hydroxytyramine hydrochloride (dopamine), DL-p-hydroxyphenylethanolamine hydrochloride (octopamine), morphine sulfate, SQ 10,643 hydrochloride (cinanserin). These substances were purchased from Sigma Chemical Company, except for the two peptides from Peninsula Laboratories, and for cinanserin donated by E.R. Squibb and Sons. The molecular weights of these substances are listed in Appendix II-1. For most substances, 10 µg per gammarid was used and the corresponding number of moles is also listed in Appendix II-1. The substances were dissolved immediately prior to injection. However, serotonin was sometimes stored frozen at -20 C in samples of 1 ml for up to a week (but no longer, to avoid using an oxidized solution). When 10 µg was to be injected in a 2 µl volume of saline, 50 mg of the substance was dissolved in 10 ml of saline.

The volume of haemolymph in *Gammarus pulex* is equivalent to 26% of its wet weight (Butterworth, 1968). *Gammarus lacustris* ranging from 30 to 100 mg, depending on the season, were used; by analogy, their haemolymph volume ranged from 7.8 to 26 µl. Ten µg 5-HT HCl represent 2.58×10^{-8} moles, whereas 10 µg 5-HT Creatinine Sulfate represent 4.70×10^{-8} moles (Appendix II-1). The highest concentration of serotonin was obtained after injection of 4.70×10^{-8} moles into 7.8 µl of haemolymph, which gives a concentration of 6.0×10^{-3} M [$4.7 \times 10^{-8} \times (10^6 / 7.8)$]; the lowest concentration was obtained after injection of 2.58×10^{-8} moles into 26 ul of haemolymph, which gives a concentration of 9.9×10^{-4} M [$2.58 \times 10^{-8} \times (10^6 / 26)$]. Therefore, the level of serotonin in the haemolymph of gammarids, immediately after injection, was in the low 10^{-3} M range.

Cystacanth extracts were prepared by crushing in a tissue grinder 20 fresh cystacanths of *P. paradoxus* in 70 µl saline (Appendix III-3, experiment Jun, 17) and 40 fresh cystacanths in 40 µl saline (exp. Aug, 31). The solution was then transferred to a microtube, centrifuged, and the supernatant was immediately injected into uninfected gammarids (Jun, 17). For the second experiment, the supernatant was frozen at -70 C and two weeks later thawed and then injected (Aug, 31).

C. Clinging behavior

To assess the clinging response, gammarids were placed in individual plastic Petri dishes (diameter: 5.5 cm) lined with cheese-cloth (thread diameter: .16 mm, mesh: .26 mm) (Fig. III-1, below). The gammarid was stroked with a paint-brush for a few seconds. Its clinging response was recorded as positive if it grasped the cheese-cloth with its gnathopods, curled its abdomen and became immobile. Two types of ambiguous clinging responses were observed and they were both recorded as negative: 1) The gammarids tried to cling to the cheese-cloth but kept moving; this type of response seemed characteristic of a low "drive" for clinging. 2) The gammarids curled completely, stopped moving but clung to their antennae; this appeared to be an "over-clinging" response.

Generally 5, ~~sometimes~~ 6, gammarids were injected at a time. Injecting 5 *Gammarus* took approximately 10 min. The clinging behavior was assayed every 15 min: at the hour, and 15, 30, and 45 min after the hour. The first assay was usually done at the next checking time after the injection of the last gammarid in the group. Each group of 5 was considered to have been injected at the same time. When many gammarids were assayed together, the lag time between the injection of groups of 5 (15 min) was taken into account.

In some experiments, the duration of the clinging response was recorded using a stopwatch or, if the response lasted hours, the time of initial stimulation was noted as

well as the time when the gammarid stopped clinging.

D. Photic behavior

To test the photic behavior of *Gammarus*, a long plexiglass box (140 x 35.5 x 11 cm) was constructed (Fig. II-1). Eleven removable partitions (6.5 cm high) formed 12 tracks, each 2.5 cm wide. The bottom and sides were black, the ends were transparent. The tray was filled with lake water to a depth of 4 or 5 cm, the night prior to an experiment. The water was aerated vigorously overnight with an air stone, which was removed at the beginning of the test.

Two types of experiments were performed. For the first one, with fixed light source (Fig. II-1, above), a removable black lid covered half the box forming a dark zone. A small additional black vertical partition (which extended down to the water surface, but not below) was added in the center, parallel to the smaller faces of the tray, to create a darker zone under the lid. A 60 W light in a 16 cm white reflector was placed at a distance of 25 cm away from the end without the lid, and 25 cm above it. Even after several hours of experiment, the difference in temperature at the two ends of the tray was never more than one degree Celsius. The illuminance, measured in foot-candles with a photometer, ranged over 3 log units from approximately 40 foot-candles (approximately 400 lux) at the lighted end, to 2 ft-c (approximately 20 lux) in the middle of the tray, and 0.03

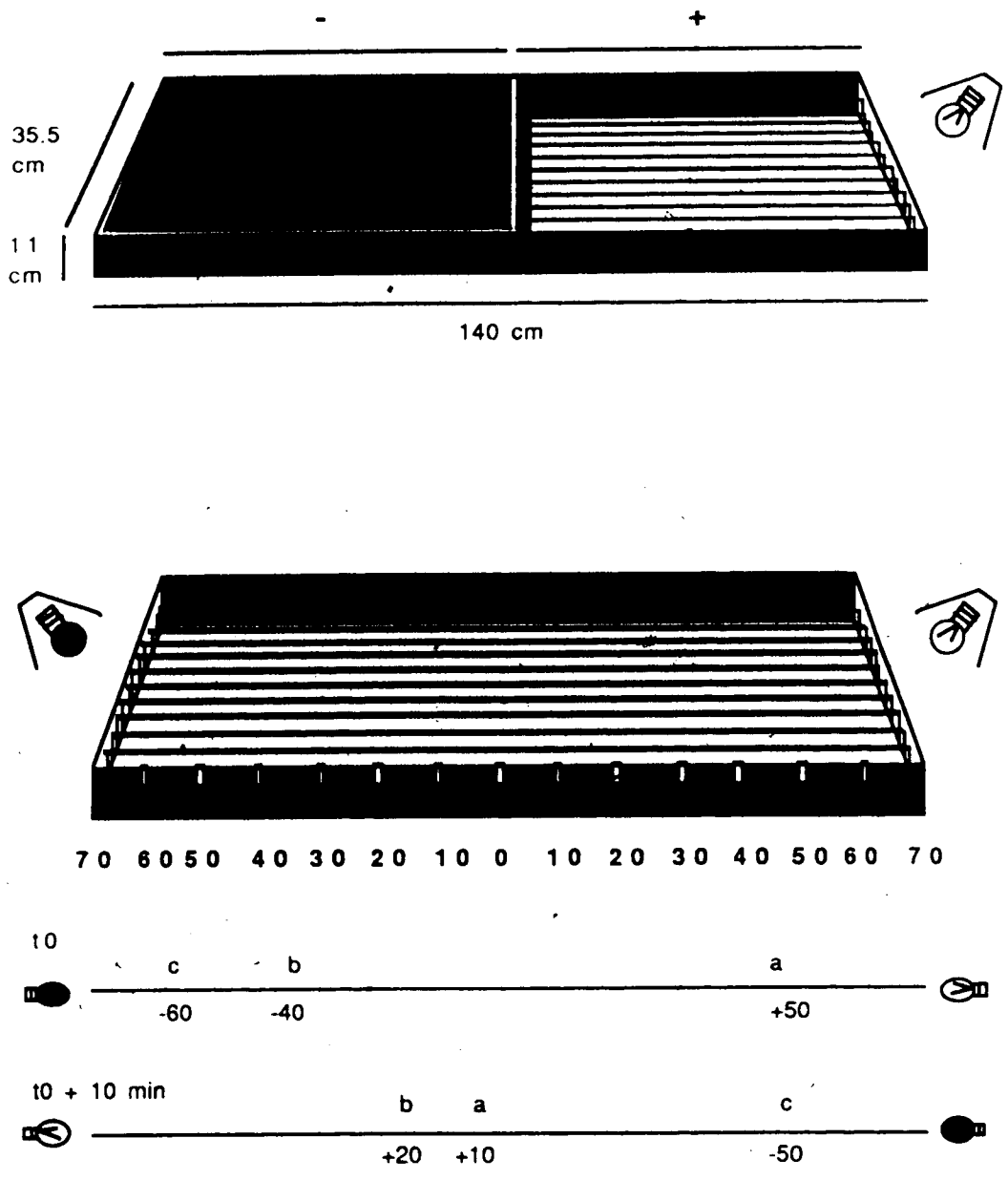


Fig. II-1. Testing apparatus for photic behavior of gammarids.
Above: experiments with fixed light source.
Below: experiments with alternating light source; "a", "b", and "c" indicate positions of individual gammarids; in ten minutes, the minimum distance covered by gammarid (a) was + 60 cm, (b) - 20 cm, and (c) - 110 cm .

ft-c (approximately 0.32 lux) at the darkest end. The absolute values were liable to change depending on the turbidity of the water. There was one gammarid per track, and the tracks (and the gammarid in each track) were identified by numbers. A gate with indentations fitting the partitions was brought down for a short time every 5 minutes and the numbers of the gammarids present in the lighted zone of their tracks were recorded.

In the experiment with alternating light sources, the black lid was removed, a second 60 W lamp was installed at the other end of the tray and the light was switched on alternately at each end every 10 minutes (Fig. II-1, below). Before each light switch, the position of each gammarid in the tray was recorded to the nearest 10 centimeters.

Six gammarids were injected in a row; all six were considered to have been injected at the time of the medianth injection (9:10 in the example in Fig. II-2). In experiments with a fixed light source, the 6 gammarids were placed in the track with the number corresponding to that of their Petri dish, uninfected gammarids in the half away from the lamp, infected ones in the half nearest to the lamp, for 5 minutes with the gate down. The gate was lifted and 5 minutes later the first record of several hours of observation was taken. Another batch of 6 was treated in the same way, and placed in the remaining tracks. When analysing the data for the 12 gammarids, the lag time between the injection of the two groups was taken into consideration. In

		Individuals						
		1	2	3	4	5	6	
Injection	t0	9:05	9:07	9:09	9:11	9:13	9:15	
9:30	t0+20	+	+	+	+	+	+	6
9:35	t0+25	+	+	+	-	+	+	5
9:40	t0+30	+	+	+	+	+	+	6
9:45	t0+35	-	-	+	+	+	+	4
9:50	t0+40	+	+	+	-	-	-	3
9:55	t0+45	-	-	+	-	+	-	2
10:00	t0+50	+	-	+	-	+	-	3
10:05	t0+55	-	-	+	+	-	-	2
Total		5	4	8	4	6	4	31

1) Average individual performance: $n = 6$ $\bar{x} = 5.17 \pm 1.60$ (65%)

2) Average performance per checking time: $n = 8$ $\bar{x} = 3.88 \pm 1.64$ (65%)

3) Percentage positive: $\frac{31 \times 100}{48} = 65$

4) Time-course:

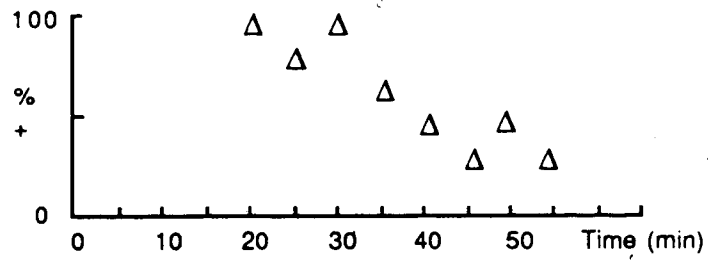


Fig. II-2. Sample of data recording for behavioral tests involving gammarids, and primary steps of data analysis.
"+" may refer to clinging or to presence in the lighted portion of the photic testing apparatus (see text).

experiments with alternate opposite light source, the procedure was similar, except that the observations were made every 10 minutes, and both infected and uninfected gammarids were placed initially at the end away from the light. Most experiments (see Appendix IV-1 and IV-2) involved 12 control gammarids and 12 gammarids injected with the substance tested. In that case, either 6 infected controls and 6 drug injected animals were treated on one day and the procedure repeated on the next day, or, the 12 drug injected were treated on day 1, and the 12 controls on day 2. For an experiment running on two consecutive days, special care was taken that gammarids of the same origin would be used and handled in exactly the same way. Experiments were started in the morning.

E. Distribution of accessory screening pigment

The position of the white accessory screening pigment in the compound eye of *G. lacustris* was assessed on living or freshly killed gammarids. The amphipods were dropped for a few seconds in sub-boiling water to prevent further migration of the pigments. The eyes were observed immediately after death because the reflecting pigment is photolabile; the white color in reflecting light disappeared after a few hours (Debaisieux, 1944).

The superficiality of the white pigment was rated under the dissecting scope in arbitrary units from 1 to 5 (with 0.5 unit steps), 1 representing the darkest eye (the most

dark adapted observable externally) and 5 the whitest eye (the most light adapted) (Fig. V-2). This method, as far as I know, has not been applied to the reflecting pigment of crustaceans. However, it has been used previously to estimate the dispersion of the retinular screening pigment in gammarids in histological sections (Ali and Steele, 1961), and extensively to study pigment migration in tegumental chromatophores of crustaceans, after the original method of Hogben and Slome (1931). Each experiment was made in a blind fashion with 12 gammarids in individual small Petri dishes bearing a number on a tag stuck on the bottom and invisible during handling. The dispersion of the white pigment was generally similar in both eyes, and although both eyes were observed, only one value was recorded. The 12 gammarids were observed twice each, the second rating being performed unaware of the first result. The average of the two ratings, called eye score, was used. When 6 uninfected gammarids and 6 infected gammarids were studied, the thorax and abdomen were cut off and removed before observation.

This method gave coherent results (Chapter V), but had two limitations. 1) The whole potential range of migration was not covered at the lowest end. When the eye was scored 1, further proximal migration could have taken place, inapparent to the observer. 2) Within experiments, scoring was consistent; however, scores may not have been consistent between experiments, the whole scale from 1 to 5 may have varied slightly.

Several time-course studies were performed on the migration of the reflecting pigment upon dark and light adaptation in living gammarids. In the first experiment (S19,20), performed over two days, 12 infected and 12 uninfected gammarids were taken from their common storage tank, subjected to 2 hours light adaptation 30 cm away from a 60 W lamp, tested for eye score, subjected to 2 hr dark-adaptation and tested again. In experiment S23,24, 12 infected and 12 uninfected gammarids were taken from the tank, immediately tested, subjected to 2 hours light-adaptation and tested again. In experiment N20,21, 11 infected gammarids and 13 uninfected gammarids were taken from the tank, tested, subjected to 2 hours light adaptation and tested one more time. Experiment N27,28 was a longitudinal study of dark-adaptation in 12 uninfected gammarids; taken from their tank, they were tested, placed in a dark cabinet and tested every hour for 6 hours, left in the dark overnight and tested again 24 and 25 hours after the beginning of the experiment. The fifth experiment (S6,7) involved 6 infected and 5 uninfected gammarids. The starting protocol was exactly as for N27,28, but after the test at t_{0+24} the animals were placed under the 60 W lamp and tested every hour for 6 hours.

F. Histological sections

To study the anatomy of the compound eye of *G. lacustris*, histological sections were prepared for examination in light microscopy. The heads were processed using a LKB 2218-500 Embedding Kit (LKB, Bromma). On day 1, gammarids were killed in sub-boiling water, antennae cut off, and the heads dropped in Bouin's solution. On day 2, the heads were rinsed in several baths of 90% and then 100% ethanol, infiltrated overnight with a mixture of resin and 100% ethanol and embedded on day 3 in the synthetic resin. Three-micrometer sections were obtained with glass knives and the slides were stained with haematoxylin and eosin following standard procedure.

The heads of eight gammarids were sectioned transversly for histological examination of the eyes. Four infected and four uninfected gammarids were processed, two of each after dark-adaptation, the other two light adapted. In order to make sure that infected animals were photopositive and uninfected ones photonegative, the gammarids were first tested in the testing apparatus for photic behavior. For the light adapted group, 6 infected and 6 uninfected animals were placed in the apparatus for three hours. The two most photopositive infected individuals and the two most photonegative uninfected ones were then killed with sub-boiling water and processed. For the dark adapted group, the same general procedure was repeated, this time leaving the gammarids one hour in the testing apparatus and then one

hour in the dark cabinet.

G. Data recording and analysis

The data for the clinging behavior and for the photic behavior tested with a fixed light source were always recorded as matrices of "+" and "-", "+" corresponding to clinging or to present in the lighted compartment, and "-" to non clinging or to absent from the lighted compartment. On the recording sheets, gammarid individuals were spread horizontally and time vertically (Fig. II-2). The matrices were analysed in different ways, as demonstrated in Figure II-2. 1) The average number of "+" per column in the first hour of observation following treatment yielded the average performance per gammarid. 2) The number of "+" per row represented the average performance per checking time. 3) In some tests, the total number of "+" out of the total number of observations was considered. 4) The time-course of the clinging or photic behavior was often plotted with the number of "+" per row, expressed as a percentage of maximum, against time.

Several statistical tests were performed. The significance level was 0.05. Calculations were done by hand, with a scientific calculator (Casio fx-4000p), or with the programs Statworks or Statview 512 on a Macintosh microcomputer. Graphs and best fit curves were plotted using the Cricket Graph program on the Macintosh microcomputer.

III. CLINGING BEHAVIOR

A. Characterization of clinging behavior

Infected gammarids

Clinging is the last and most distinctive component of the altered escape behavior induced by the acanthocephalan *Polymorphus paradoxus* in its intermediate host *Gammarus lacustris* (cf. Chapter I). However, an infected gammarid subjected to a tactile stimulus in a confined container will cling to a suitable object (tissue fiber, wood fiber, hair, feather), even though the first steps of the altered escape behavioral sequence have not been performed. This allows the clinging response to be studied independently of other aspects of the altered escape reaction. The clinging behavior itself consisted of the following sequence. After a single tactile stimulus, the gammarid grasped the first available object with the claws of two or more of its four gnathopods, curled its abdomen tightly (Fig. III-1, above) and remained immobile. At some later time, the gammarid, still holding firmly to the supporting object with its gnathopods, relaxed its abdomen and resumed the beating of its pleopods (the first three abdominal appendages), which circulate water past the ventral thoracic gills (Fig. III-1, below). Still later, the gammarid relaxed its gnathopods and moved.

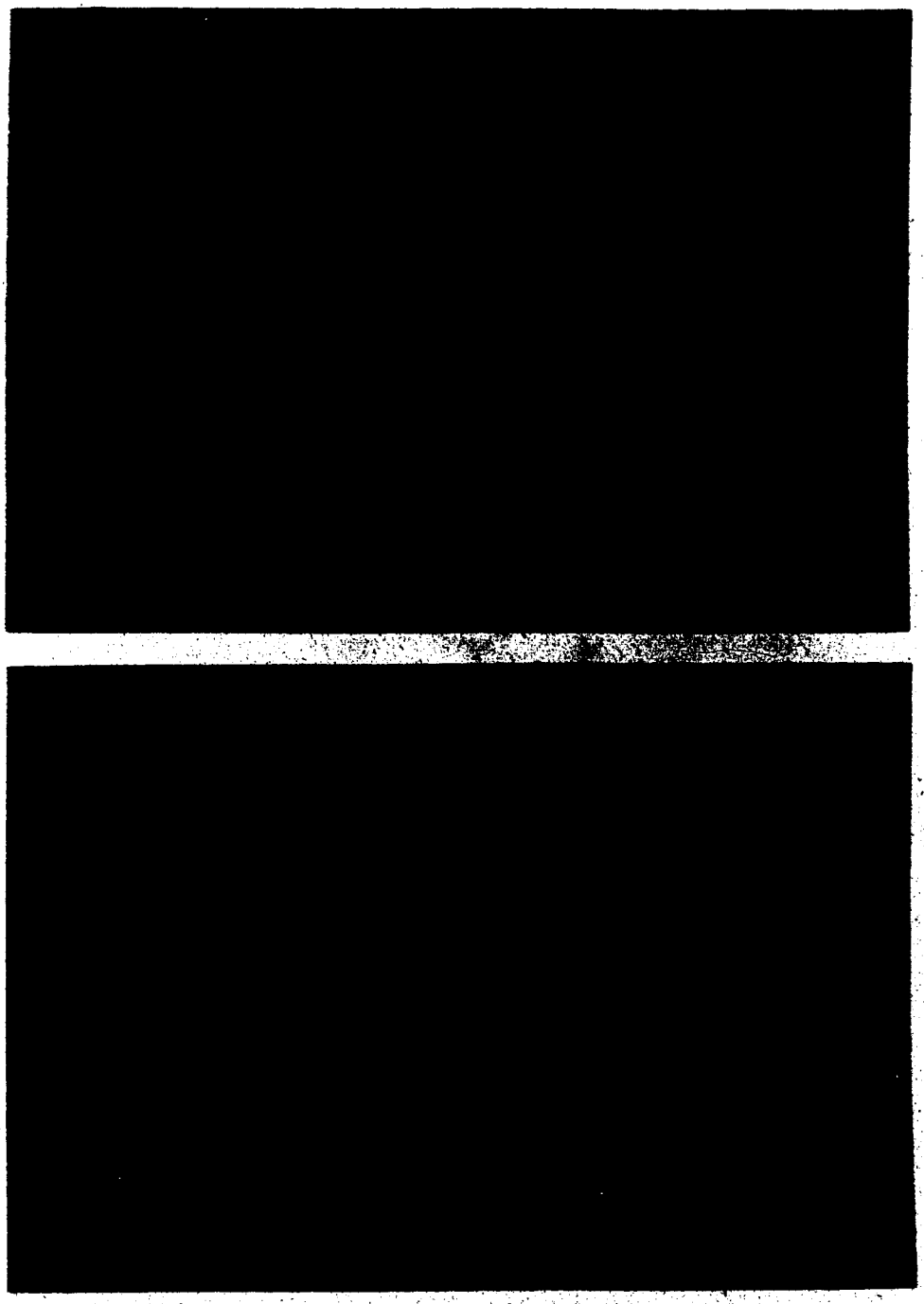


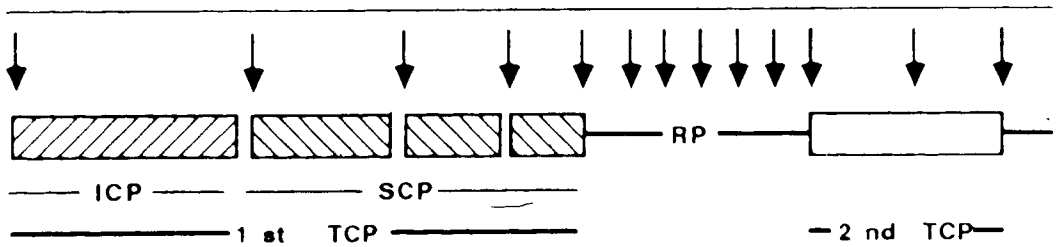
Fig. III-1. Clinging behavior in *Gammarus lacustris* infected by the larval acanthocephalan *Polymorphus paradoxus* (orange dot). Above: extreme flexion following tactile stimulation; below: later, abdomen slightly relaxed, pleopods beating (x10).

Two experiments assessed the duration and pattern of the clinging behavior in *P. paradoxus* infected *G. lacustris*: L1 (April, 1985, n=18, 12 females, 6 males), and L2 (April, 1985, n=20, 14 females, 6 males). Infected gammarids were collected the day before the experiment from a stock tank by vigorously moving a net through the water. Any clinging gammarid was then swiftly transferred to a finger bowl until the next day. The next morning each gammarid was induced to cling to a circular piece of cheese-cloth, placed in a small numbered Petri dish filled with pond water, stroked with a paint brush and the time recorded. The gammarids were observed continuously. When a gammarid stopped clinging (i.e., when it opened its claws and moved), the time was noted, and the gammarid stroked with the paint brush. Observations on each gammarid continued until it no longer responded to stimulation by clinging. Experiment L1 was terminated at that point. During L2, this refractory period (RP) was monitored and gammarids were stimulated every 15 minutes until they resumed clinging. During this second clinging period, gammarids were stimulated as soon as they stopped clinging (the time was not noted), until they reached the second refractory period, which was not monitored.

The clinging period from the initial tactile stimulation until the gammarid first released its grasp and began to move, the initial clinging period (ICP), was highly variable, from 2 minutes to 4 hours, with median values of

approximately 40 and 45 minutes for L1 and L2, respectively (Fig. III-2). The clinging period elicited by subsequent stimulations after the gammarid had stopped clinging for the first time (i.e., after the ICP) was referred to as the subsequent clinging period (SCP). This period was also highly variable, from none at all to more than 13 hours. The SCP lasted on average longer than the ICP in the two experiments (although that relationship was not shown by the median values in experiment L2). The sum of the initial clinging period and the subsequent clinging period was called the first total clinging period. It ranged from 21 min to more than 15 hours; half the gammarids clung more than 1:30 hr in both L1 and L2. The refractory period (RP) lasted from 5 minutes to 8 hours, with a median value half that of the TCP. The second total clinging period ranged from 3 minutes to 110 minutes and was shorter than either the first total clinging period or the first refractory period.

The high variability among individuals was accompanied by a clear pattern within individuals. There was a significant positive correlation between the length of the initial clinging period and the length of the subsequent clinging period (Fig. III-2), and between the length of the first total clinging period and the length of the refractory period. There was no significant correlation between the length of the first TCP and the length of the second TCP.



	ICP	SCP	1 st TCP	RP	2 nd TCP
L1					
n	18	18	18		
$\bar{x}$ ± s.d (min)	53 ± 42	231 ± 239	283 ± 267		
median (min)	38	77.5	97.5		
range (min)	2 - 108	0 - 796	31 - 855		
L2					
n	20	20	20	17 *	17 *
$\bar{x}$ ± s.d. (min)	79 ± 77	116 ± 192	195 ± 252	85 ± 115	38 ± 35
median (min)	46.5	40	100	49	25
range (min)	10 - 247	0 - 683	21 - 922	5 - 482	3 - 110

Correlations	ICP and SCP	1 st TCP and RP	1 st TCP and 2nd TCP
L1			
n	18		
r	0.61	–	–
p	$p < 0.01$		
L2			
n	20	17 *	17 *
r	0.71	0.64	0.33
p	$p < 0.01$	$p < 0.01$	$0.1 < p < 0.2$

* 3 gammarids did not resume clinging during the period of observation

Fig. III-2. Duration of clinging behavior in gammarids infected with the acanthocephalan Polymorphus paradoxus.
Arrows represent tactile stimulations; boxes indicate periods of clinging;
ICP: Initial Clinging Period; SCP: Subsequent Clinging Period; TCP: Total Clinging Period;
RP: Refractory Period; n = number of gammarids; $\bar{x}$ ± s.d. = mean ± standard deviation.

Uninfected gammarids and precopulation

There is at least one clear cut example of clinging in uninfected *G. lacustris*. It is part of the reproductive behavior, in which the male grasps the female with the claws of two contralateral gnathopods, one under the first thoracic segment of the female, the second under the fifth thoracic segment, in a position typical for the genus *Gammarus* (Le Roux 1933, in Charniaux-Cotton, 1957) (Fig. III-3). The male rides the female for several days until her eggs descend into the ventral brood pouch where he fertilizes them. The "riding" behavior is referred to as precopulation or mate guarding (Dunham, 1986).

To study the relationships between clinging and precopulation, gammarids were collected at Polar Pond, on April 7, 1985. Thirty pairs in precopula were isolated on April 14, the male and the female were separated by gently pulling them apart, and each was then tested for the clinging response. Thirty more pairs in precopula were assessed on April 15. The same process was repeated on May 14 and May 21 with animals collected from Polar Pond on May 10. In April, 17 to 43% of the animals did cling, for 1 to 131 minutes (Table III-1); further stimulation did not elicit a subsequent clinging response. In May, clinging responses could be elicited in only 2 out of 60 males (for 6 and 10 minutes), and 1 out of 60 females (for 33 min).

To check whether clinging could be elicited in postcopulating gammarids, 70 pairs in precopula (never

Table III-1. Clinging response in uninfected gammarids,
a) precopulating gammarids separated and tested immediately,
b) postcopulating gammarids (see text for details).
F = females, M = males.

Date	Sex	Number of Gammarids		Duration of response		
		Responders / Tested	(%)	$\bar{x}$ ± s.d. (min)	median (min)	range (min)
a) Precopulating						
April 14	F	5/30	(17)	7 ± 5	7	1 - 14
	M	13/30	(43)	43 ± 35	38	5 - 131
April 15	F	11/30	(37)	16 ± 15	15	1 - 45
	M	11/30	(37)	18 ± 15	13	3 - 48
b) Postcopulating						
1 day	F	6/30	(20)	11 ± 17	2	1 - 45
	M	13/30	(43)	16 ± 20	9	1 - 75
1week	F	0/25	(0)	0	0	0
	M	1/16	(6)	1	1	1
2 weeks	F	2/19	(11)	4 ± 3	4	2 - 6
	M	0/5	(0)	0	0	0
3 weeks	F	0/10	(0)	0	0	0
	M	0/3	(0)	0	0	0

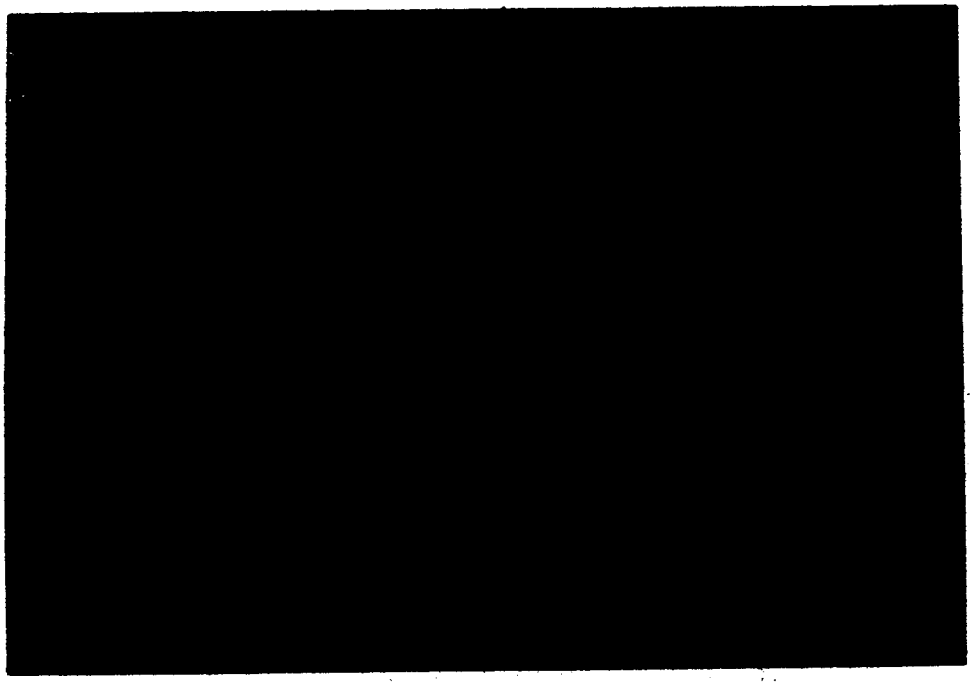


Fig. III-3. Gammarids in precopula (male: left, female: right, x 10).

tested before) were isolated in finger bowls on April 15. The next day, animals which had voluntarily parted were assayed. All postcopulating females had eggs in their brood pouches. Clinging was elicited in 43% of the males, for 1 to 75 min, and in 20% of the females, for 1 to 45 min (Table III-1). The same animals were tested 1, 2, and 3 weeks after separation; clinging was rarely elicited then (Table III-1).

To determine whether or not a seasonal pattern existed, gammarids were collected monthly in Cooking Lake. Each month 30 gammarids were assayed the day of collection and 30 more, 5 days later. Precopulating gammarids were found from January until May. During these months both precopulating and non precopulating gammarids were tested. Clinging was elicited in only 1 of 720 non-precopulating gammarids (one collected in May) and in 3 of 252 precopulating individuals (one each in January, February, and May). Details are shown in Appendix III-1. (Note that these results are inconsistent with those from the April experiment on precopulating gammarids. Whether or not this difference is due to the difference in protocol is not clear.)

Clinging in uninfected gammarids was also observed in the field during the spring. In May 1986, at Cooking Lake, the dip net was swept vigorously in a cluster of gammarids at the bottom very near the shore. A total of 128 of those collected were clinging to the net. Each was dissected and sexed; there were 110 males and 18 females, none infected with the cystacanth of *P. paradoxus*.

B. Biogenic amines and clinging behavior

The studies outlined above indicated that the responses of infected gammarids varied over time. In order to examine these responses systematically, gammarids injected with different chemical substances were assayed at regular intervals. Thus, after injection, each gammarid was stroked with a paint brush every 15 minutes, whether still clinging or not; its immediate response was recorded. The quantities of chemical substances injected are expressed in micrograms. Corresponding numbers of moles for a 10 µg dose are tabulated in Appendix II-1.

Variation in response among gammarids was analyzed using the number of gammarids clinging in the sample, and the average number of clinging responses per gammarid for the hour following treatment (maximum = 4). The mean numbers of clinging responses per gammarid per hour are listed for all experiments in Appendix III-2 (infected gammarids) and Appendix III-3 (uninfected gammarids). This mean number of clinging responses per gammarid is referred to as the average individual clinging performance.

The time-course of the response was analysed using the percentages of gammarids clinging at each assay for 3 to 10 hours post treatment. In these time-course studies, only experiments with more than 6 animals were considered.

Infected gammarids

All untreated infected gammarids (43) tested for the first time responded by clinging at least twice (Table III - 2), while all saline injected gammarids (58) responded by clinging at least once, during the first hour of observation.

The number of clinging responses per gammarid per hour in untreated animals averaged 3.7 (Table III-2; range 3.2 to 4.0 in 5 experiments), and that in saline - injected animals averaged 3.5 (range 3.1 to 4.0 in 6 experiments). A two level nested anova with unequal sample sizes indicated that there was no significant difference in clinging performance between untreated and saline injected animals, and that treatment accounted for only 6 % of the variance (Table III-3a). Although variation among individuals accounted for most of the variance (85 %), there was a significant variation among experiments, which accounted for 9 % of the variance. A seasonal factor of variability was isolated; there was a significant positive regression of the mean number of clinging responses on the date of the experiment ($n = 11$, $r = 0.75$, $p < 0.01$; Fig. III-4a). From May to November, the average number of clinging responses increased.

Time-course studies indicated that in untreated (Fig. III-5) or saline injected individuals (Fig. III-6), the number of clinging responses in the sample decreased with time. The slopes of the linear regressions, all negative,

Table III - 2. Clinging behavior in uninfected gammarids and those infected with the acanthocephalan Polymorphus paradoxus during the hour following treatment with different substances.
The means and standard deviations (s.d.) represent the number of clinging responses per gammarid per hour (4 observations per hour). Individual data are pooled across experiments. All experiments are listed in Appendix III-2 and III-3.
PCPA = p-chlorophenylalanine.

	Number of Experiments	Responders / Tested	Mean number of clinging responses ± s.d. per responder	per tested	(%)
INFECTED					
Untreated	5	43/43	3.7 ± 0.6	3.7 ± 0.6	(94)
Saline	6	58/58	3.5 ± 0.9	3.5 ± 0.9	(86)
Octopamine					
5 µg	1	3/8	1.0 ± 0.0	0.4 ± 0.5	(9)
10 µg	1	0/7	0	0	(0)
Dopamine	1	8/9	2.4 ± 0.9	2.1 ± 1.2	(53)
Noradrenalin	1	3/10	2.3 ± 1.2	0.7 ± 1.3	(18)
PCPA	1	7/7	3.6 ± 0.8	3.6 ± 0.8	(89)
Cinanserin					
5 µg	1	10/10	3.8 ± 0.4	3.8 ± 0.4	(95)
10 µg	2	16/19	2.8 ± 1.1	2.3 ± 1.4	(58)
20 µg	1	4/6	2.0 ± 1.4	1.3 ± 1.5	(33)
UNINFECTED					
Untreated	2	0/10	0	0	(0)
Saline	4	0/35	0	0	(0)
Serotonin					
1 µg	1	2/5	1.0 ± 0.0	0.4 ± 0.6	(10)
10 µg	5	27/40	2.5 ± 1.0	1.7 ± 1.4	(43)
20 µg	1	3/4	1.3 ± 0.6	1.0 ± 0.8	(25)

Table III-3. Analysis of variance of the clinging response in gammarids.

a) Gammarids infected with the acanthocephalan Polymorphus paradoxus, untreated or injected with saline.
Two level nested anova with unequal sample sizes.

	df	SS	MS	Fs	Variance %
Untreated vs Saline	1	3.30	3.30	2.60 ns	6
Experiments within groups	9	11.50	1.28	2.01 *	9
Individuals within experiments	90	56.91	0.63		85
Total	100	71.71			100

* significant at 0.05 , F0.05 [9,80] = 1.99

b) Uninfected gammarids injected with 10 µg serotonin.
One level nested anova with unequal sample sizes

	df	SS	MS	Fs	Variance %
Among experiments	4	11.97	2.99	1.53 ns	6
Individuals within experiments	35	68.49	1.96		94
Total	39	80.46			

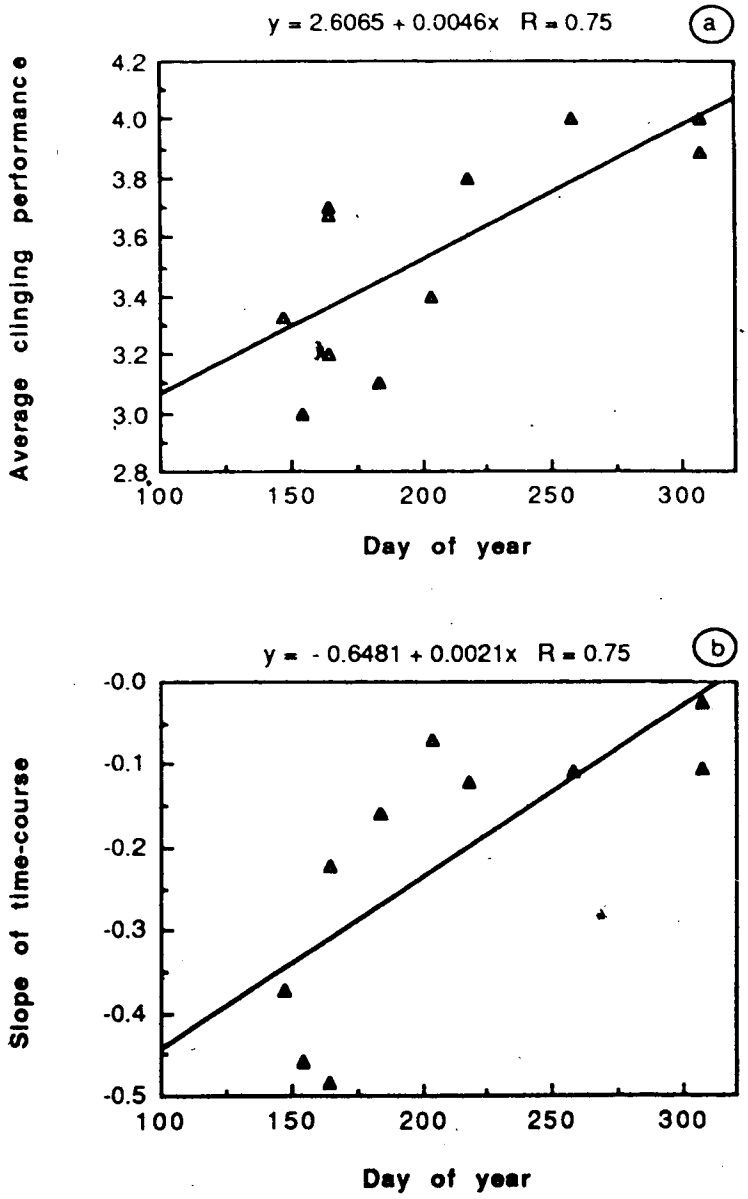


Fig. III-4. Seasonal variation in the strength of the clinging behavior in control gammarids infected with the acanthocephalan Polymorphus paradoxus (untreated or injected with saline).
a) average clinging performance vs day of year;
b) slope of time-course of the clinging response vs day of year.
Linear regression equations are followed by correlation coefficient (R).

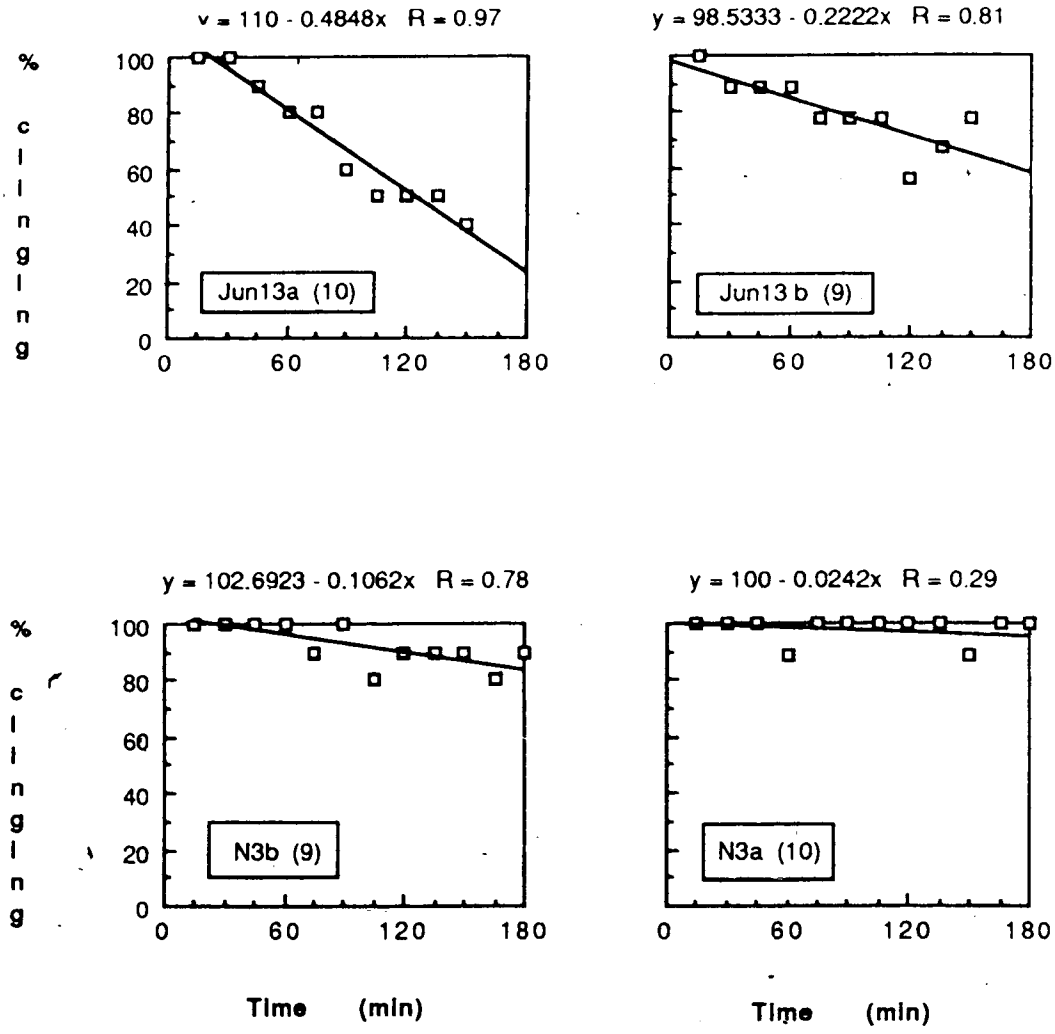


Fig. III-5. Time-course of clinging behavior in untreated gammarids infected with the acanthocephalan Polymorphus paradoxus.
Legends in boxes are date of experiment and, in parenthesis, sample size; linear regression equations are followed by correlation coefficient (R).

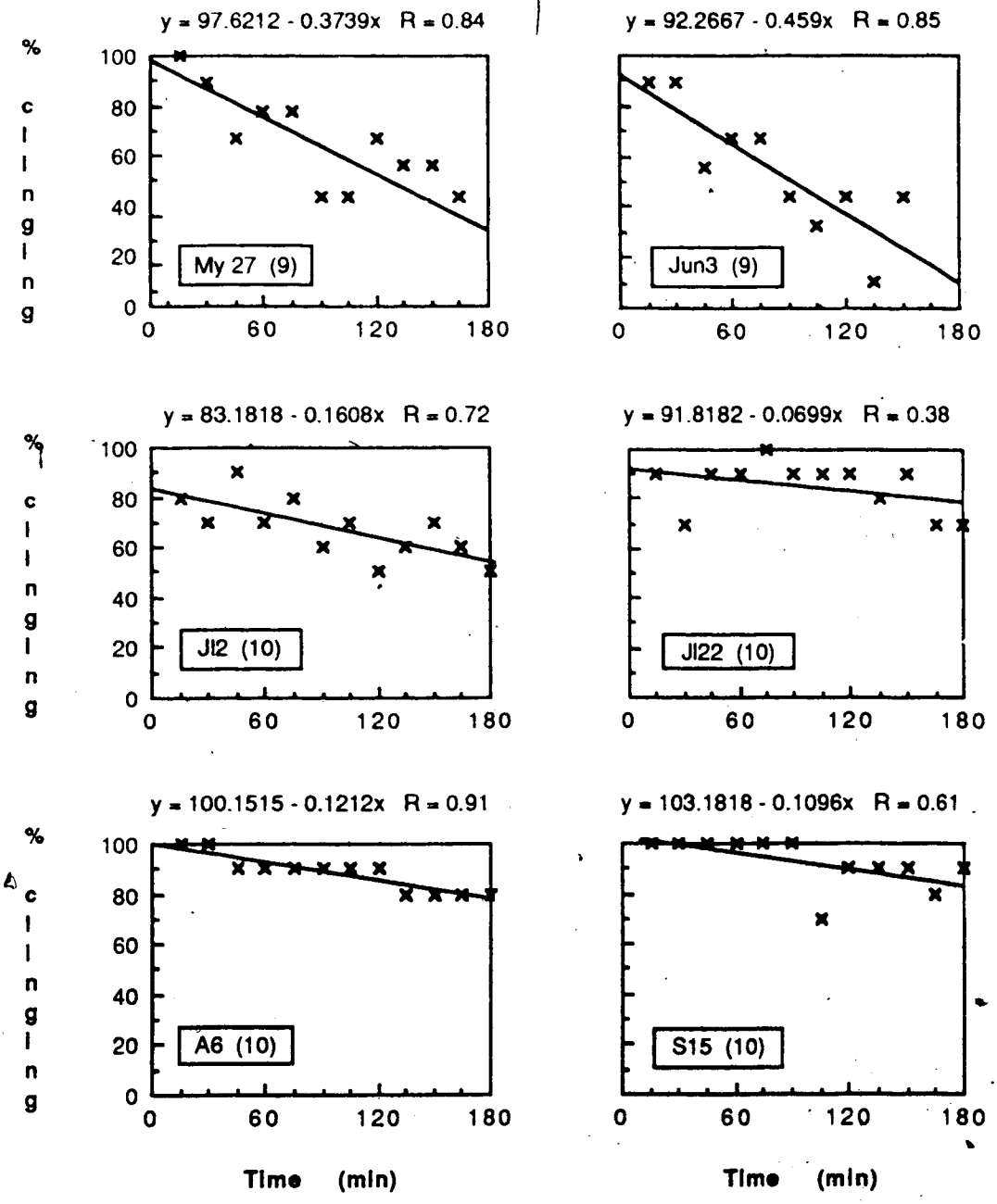


Fig. III-6. Time-course of clinging behavior in untreated gammarids infected with the acanthocephalan Polymorphus paradoxus, injected with saline at time 0. Legends in boxes are date of experiment and, in parenthesis, sample size; linear regression equations are followed by correlation coefficient (R).

and the proportion of the variance explained by the regressions, varied considerably among experiments, but not between untreated and saline injected animals (Table III-4). The seasonal variability among experiments was again clearly shown by the regression of slope on the date of the experiment ($n = 10$, $r = 0.75$, $p < 0.05$; Fig. III-3b). From May to November, the slopes were less and less negative; i.e., the clinging responses persisted longer.

The effects of octopamine on the clinging behavior of infected gammarids were dramatic as well as dose-dependent. Only 3 out of 8 infected animals injected with 5 µg octopamine responded by clinging in the first hour (and only once each), while 10 µg octopamine totally suppressed the clinging behavior (Table III-2). The time-course of the clinging response (Fig. III-7) also differed drastically from that in controls; the slope of the linear regression of the time course was positive with both dosages. The slope for 5 µg (0.28) was twice as steep as the slope for 10 µg (0.14). With 5 µg octopamine, the return to a normal clinging behavior began almost immediately while with 10 µg, all gammarids stopped clinging for 3:30 hr. With 5 µg octopamine the return to a normal clinging behavior took about 5 hours, while with 10 µg it took about 10 hours.

Both dopamine (10 µg) and noradrenalin (10 µg) caused a significant reduction in the number of clinging responses after injection; 2.11 and 0.70 respectively compared to 3.45 for the saline controls (Table III-2, t-test, $p < 0.05$). In

Table III.4. Time-course of clinging behavior in control gammarids infected with the acanthocephalan Polymorphus paradoxus: average slope of linear regression s.d. = standard deviation.

	Number of Experiments	Slope (b) Mean ± s.d. (Range)	% Variance Explained (R^2) Mean ± s.d. (Range)
Untreated	4	-0.209 ± 0.201 (-0.024 to -0.485)	57 ± 36 (8 to 94)
Saline injected	6	-0.216 ± 0.160 (-0.070 to -0.459)	55 ± 26 (38 to 91)

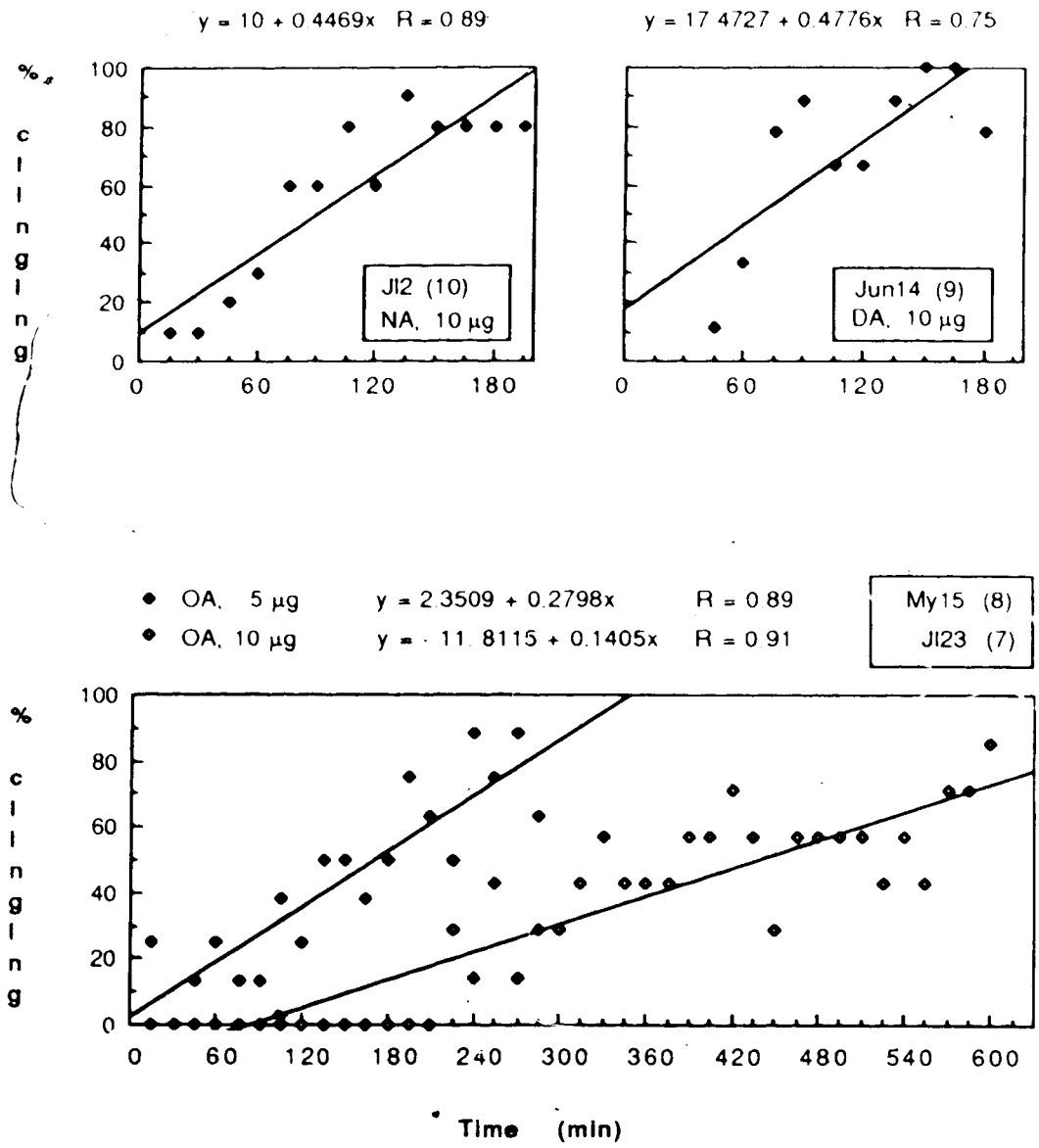


Fig. III-7. Time-course of clinging behavior in gammarids infected with the acanthocephalan Polymorphus paradoxus, injected with 10 µg dopamine (DA), 10 µg noradrenalin (NA), 5 and 10 µg octopamine (OA), at time 0.
Additional legends in boxes are date of experiment and, in parenthesis, sample size; linear regression equations are followed by correlation coefficient (R).

both cases, the slope of the regression line of the time-course was positive (Fig. III-7) and about twice as steep as the slope of the time-course for 5 µg octopamine. In both cases, the clinging behavior was back to that normal for infected gammarids within about two hours, which was less time than for gammarids injected with 5 µg octopamine.

Two attempts were made to inhibit the clinging behavior of infected gammarids by reducing their serotonin levels, one with PCPA (p-chlorophenylalanine), the other with cinanserin (SQ 10,643). PCPA is an inhibitor of the synthesis of serotonin and has its maximum depleting effect in mammalian brain 2-3 days after a single high dose (Vogt, 1982). Just after treatment, the individual clinging performance of animals injected with 10 µg PCPA was not significantly different from the performance of saline controls (3.6 vs 3.5) (Table III-2, t-test, $p > 0.05$). Over the first 180 minutes, the clinging response persisted better than in controls run at the same time (Fig. III-8a) but the slope was similar to those of other controls (Fig. III-4). A follow up of the performance 6:30, 24, 34, 48 and 72 hr post injection indicated that either the treatment itself (the injection of 2 µl fluid), or the time spent clinging during the first test, had a strong negative influence on subsequent performances, principally at 6:30 and 24:00 hr later (Fig. III-8b). This was true for both PCPA and saline injected gammarids. From these results, no clear difference appeared between the long term clinging

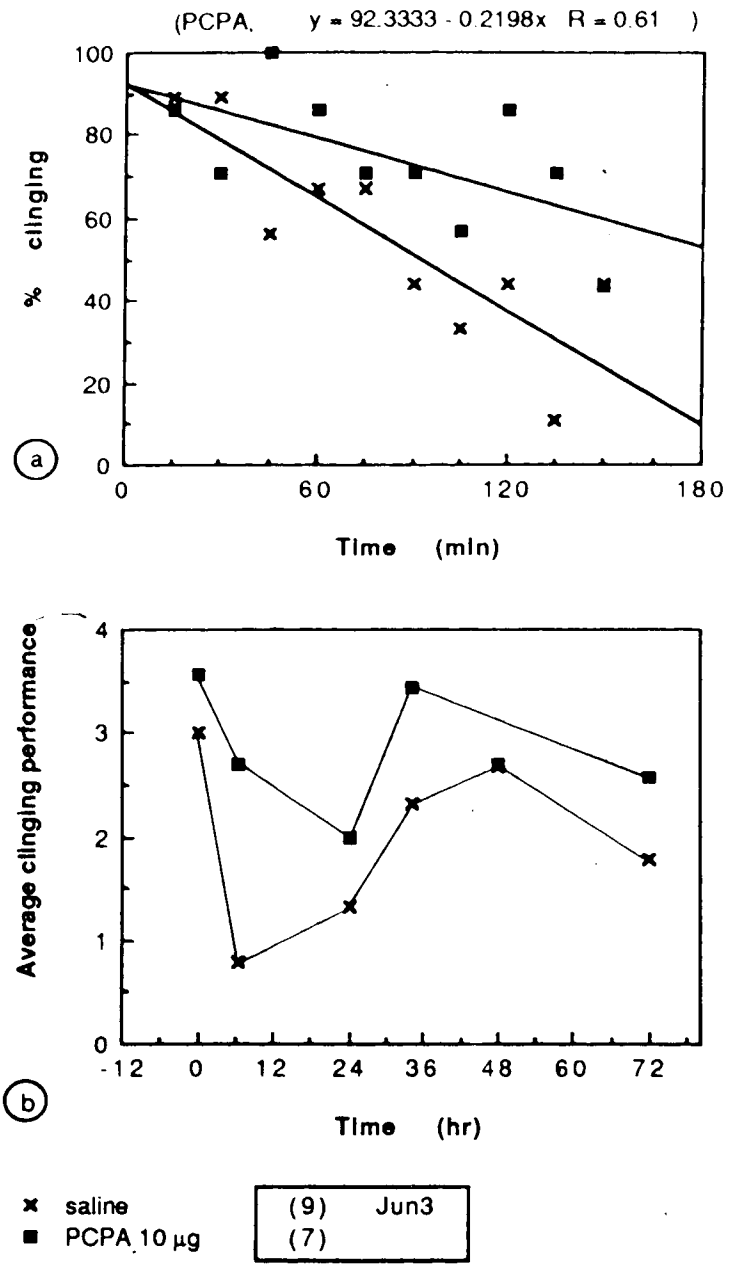


Fig III-8. Clinging behavior in gammarids infected with the acanthocephalan Polymorphus paradoxus, injected with 10 µg PCPA (p-chlorophenylalanine) or saline at time 0.
a) time-course of clinging response immediately following treatment;
b): long-term average individual performance post treatment.
Legends in boxes are date of experiment and, in parenthesis, sample size; the linear regression equation is followed by correlation coefficient (R).

behavior of PCPA and saline injected infected gammarids.

Cinanserin (SQ 10,643) is an antagonist of serotonin that appears to bind selectively to 5-HT_2 sites (Glennon, 1986). It has been used to block serotonin receptors on presynaptic terminals of sensory neurons in the abdominal ganglion of *Aplysia* (Brunelli et al., 1976). Its effects on infected gammarids were dose dependent. Those injected with 5 µg cinanserin did not differ significantly in average individual performance; 3.8 vs 3.5 for the controls (t-test, $p > 0.05$), and only slightly in time course (Fig. III-9a). Ten micrograms cinanserin reduced significantly the average individual performance in the two experiments performed; 2.3 vs 3.5 for the saline controls (Table III-2, $p < 0.05$). However, the time-course of the clinging response in gammarids injected with 10 ug cinanserin differed markedly between experiments (see Fig. III-9b and c); 20 µg cinanserin significantly reduced the average individual performance (1.3 vs 3.5, t-test, $p < 0.05$), and the gammarids failed to resume a normal clinging behavior even after 4 hours (fig III-9d). Cinanserin injected animals exhibited a significant, dose-dependent, reduction in the number of their clinging responses compared to controls. However, the absence of a clear pattern in the time-course suggests a general pathological effect of the drug, rather than of a specific inhibition of the clinging response.

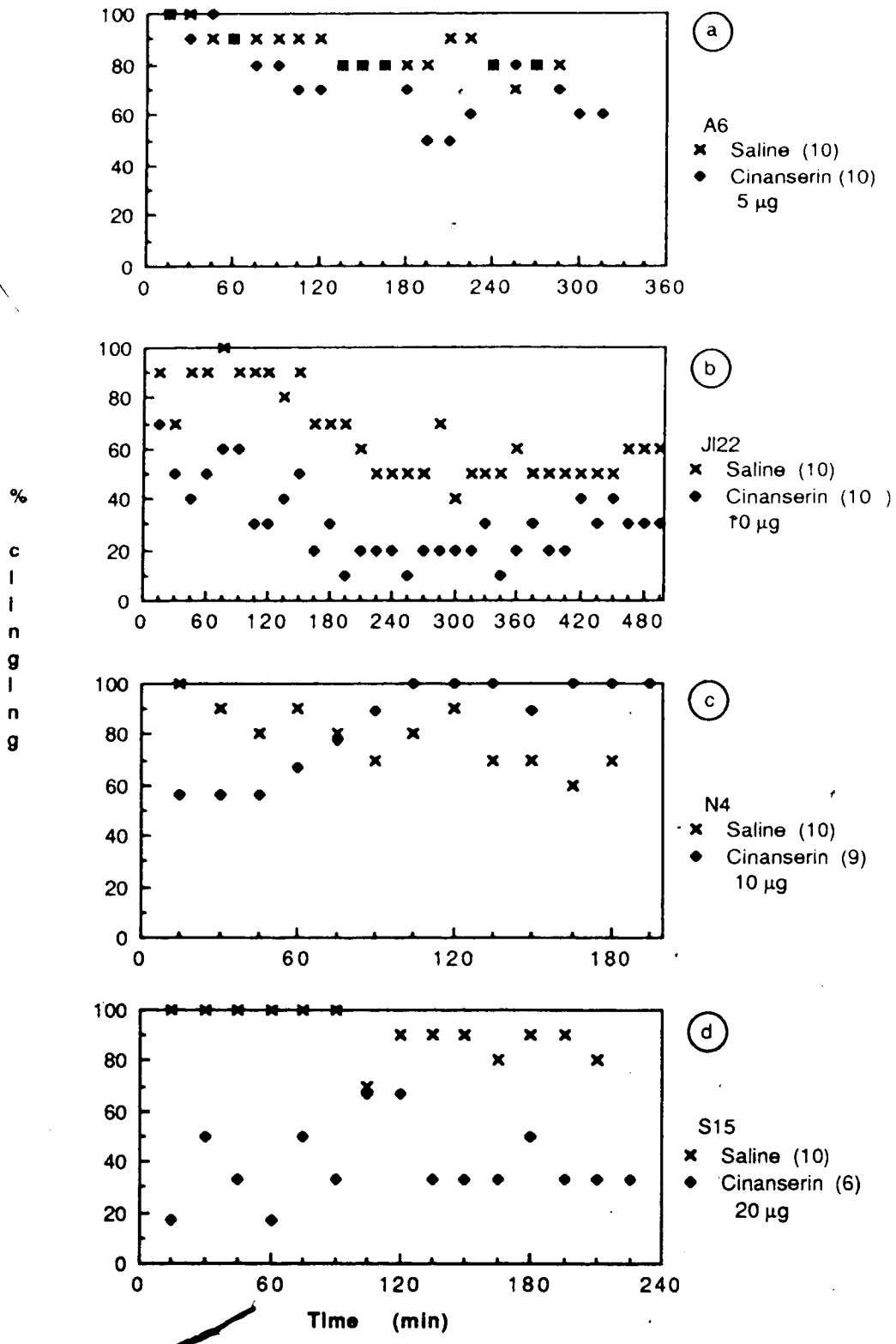


Fig. III-9. Time-course of clinging behavior in gammarids infected with the larval acanthocephalan Polymorphus paradoxus, injected with 5, 10, and 20 µg of cinanserin (SQ 10, 643) at time 0. Additional legends are date of experiment and, in parenthesis, sample size.

Uninfected gammarids

In contrast to control infected gammarids, which were all clinging nearly all the time in the first hour of the test, uninfected gammarids, either untreated or injected with saline, never responded by clinging (Table III-2). The lack of response persisted over time except on one occasion, with one gammarid clinging once.

Serotonin injected into uninfected gammarids qualitatively elicited the clinging behavior induced by the larval acanthocephalan *Polymorphus paradoxus* in infected hosts. It did so in a dose-dependent manner. Only two out of the 5 gammarids injected with 1 µg 5-HT responded by clinging, and only on a single occasion each; that dose was obviously inadequate. Three out of 4 gammarids injected with 20 µg 5-HT exhibited a few typical clinging responses but also many exaggerated responses, in which they curled and grasped their antennae with their gnathopods; such responses were not counted as clinging. Most experiments therefore were performed with 10 µg serotonin, which elicited at least one clinging response in 27 out of 40 gammarids. The number of clinging responses per gammarid tested averaged 1.7 (Table III-2; range 0.9 to 2.3 in 5 experiments, Appendix III-3). The number of clinging responses in responding gammarids was somewhat higher, 2.5, but still considerably lower than in the infected controls, 3.5 (Table III-2, $p < 0.05$). There were two likely sources of variability among experiments: the slight difference in molecular weight

between serotonin HCl and serotonin creatinine sulfate and hence in the number of moles injected (Appendix II-1), and seasonality. However, a single level nested anova showed that there was no significant variation among experiments in the number of clinging responses per gammarid tested (Table III-3b).

The duration of single clinging responses induced by 10 ug serotonin was measured in two experiments (Table III-5); it lasted from a few seconds to 16 minutes, far less than in infected gammarids which clung from a few minutes to a few hours (Fig. III-2). Because of the brevity of the response, it was impractical to study the details of the different clinging periods (initial, subsequent and refractory).

The time-course of the response induced by 5-HT in uninfected gammarids was quite variable (Fig. III-10). However, the peak of clinging responses occurred in the hour following treatment and the effects of the biogenic amine did not extend beyond three hours.

None of the other neurotransmitters tested elicited clinging responses. Single injections of 1 µg GABA (number of gammarids = 5), 10 µg GABA (n=5), 5 µg octopamine (n=5), 5 µg dopamine (n=3), 10 µg dopamine (n=4), or 10 µg noradrenalin (n=10) failed to induce any clinging behavior in uninfected gammarids (Appendix III-3). With 10 µg GABA, the gammarids were apparently paralyzed for up to one hour. The failure of the other three neurotransmitters to elicit the clinging response is not surprising, because all three

Table III-5. Duration of clinging response in uninfected gammarids injected with 10 µg serotonin and tested every 15 minutes post-treatment.

	Exp June 10	Exp Sept 22
Number of gammarids tested	9	10
Number of gammarids responding	7	8
Number of clinging responses	29	20
Mean duration ± s.d. (sec)	101 ± 209	62 ± 80
Median duration (sec)	27	29
Duration range (sec)	5 - 960	5 - 300

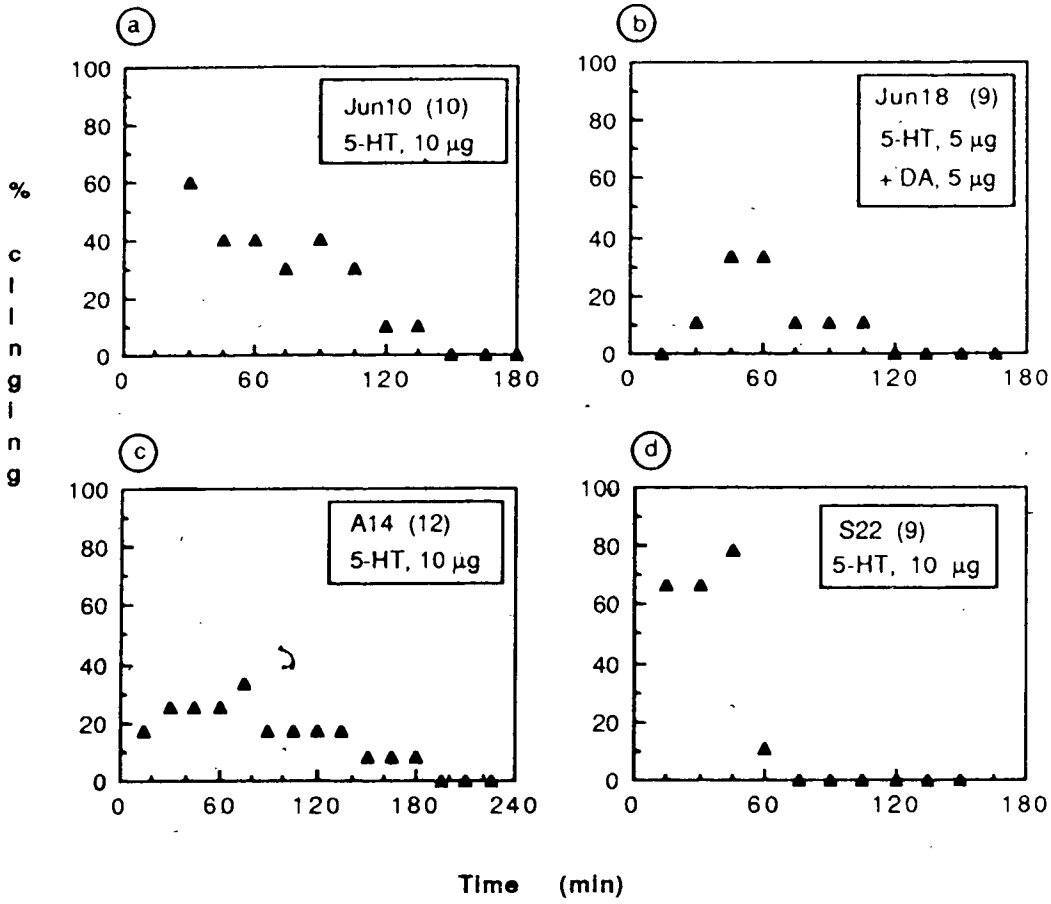


Fig. III-10. Time-course of clinging behavior in uninfected gammarids injected with 10 µg serotonin (5-HT), or with 5 µg serotonin + 5 µg dopamine (DA) at time 0. Legends in boxes are date of experiment and, in parenthesis, sample size.

inhibited clinging in infected gammarids. However, injecting concurrently 5 µg dopamine and 5 µg 5-HT elicited the same qualitative response as 10 µg dosages of 5-HT alone (Fig. III-10b).

Because a chemical action of the parasite on the nervous system of its host is suspected, it was logical to inject haemolymph of infected gammarids, as well as cystacanth extract, into uninfected gammarids to try to reproduce the clinging behavior. However, injection of haemolymph was impossible for technical reasons. Only a very small volume of haemolymph could be sampled from one individual without squashing it, and it was thus necessary to collect blood from several animals; even though heparinized capillary tubes were used, the haemolymph clotted immediately when transferred into the Hamilton syringe. Extracts of cystacanths of 20 fresh cystacanths of *P. paradoxus* in 70 µl saline, or 40 fresh cystacanths in 40 µl saline (see details in Chapter II), produced clinging in 1/10 and 2/10 gammarids, in each case only once in the hour following treatment and for a very short period of time. These results, although not impressive, do suggest that cystacanth extracts contain a substance involved in producing the clinging behavior.

Serotonin injected into uninfected gammarids elicited qualitatively the clinging behavior typical of infected gammarids. However, serotonin (10 µg) did not reproduce the quantitative effects of the parasite. Not all serotonin

injected gammarids responded by clinging, those which did responded significantly fewer times than did infected gammarids, and the clinging response lasted from a few seconds to a few minutes, while in infected gammarids it lasted from a few minutes to a few hours. Also, the time-course indicated that the effects of serotonin were transitory, and the clinging response could only be elicited for two to three hours after treatment.

IV. PHOTIC BEHAVIOR

A. Seasonal variation of photic behavior in uninfected untreated gammarids

Early on, the photic behavior of uninfected gammarids appeared very variable. It was therefore tested at least once a month (from March 1986 to June 1987) to serve as a control when studying the effects of amines on the photic behavior. Two uninfected gammarids, collected in the field the preceding day, were placed in each of the twelve tracks of the 140 cm plexiglass tray, which was filled with the water in which the gammarids were collected. The fixed light source design (see details in Chapter II) was used, and the number of gammarids present in the lighted zone was determined at five minute intervals for one hour (12 times). The gammarids were left undisturbed for one hour, then the observations were repeated for one more hour. The data, calculated independently for the two runs, are shown in Figure IV-1. A two way analysis of variance (Table IV-1) indicated that there was a highly significant variance in photic behavior among dates, no consistent difference between runs, and that the interaction between date and run was significant; i.e., the relationship between the photic behavior in the first run and that in the second run varied depending on the date (Table IV-1). One factor of variability among dates of experiments was a marked seasonal pattern with a maximum in June-July, about 60% presence in

Table IV-1 Two way analysis of variance of seasonal photic behavior in uninfected gammarids (Data shown in Figure IV-1.)

	df	SS	MS	Fs
Runs	1	0.334	0.334	0.081 ns
Dates	17	7421.000	436.529	106.367 *
Runs x Dates	17	417.000	24.529	5.977 *
Within subgroups	396	1625.055	4.104	
Total	431	9463.389		

$Fs_{.01\ [15,\ \infty]} = 2.04$

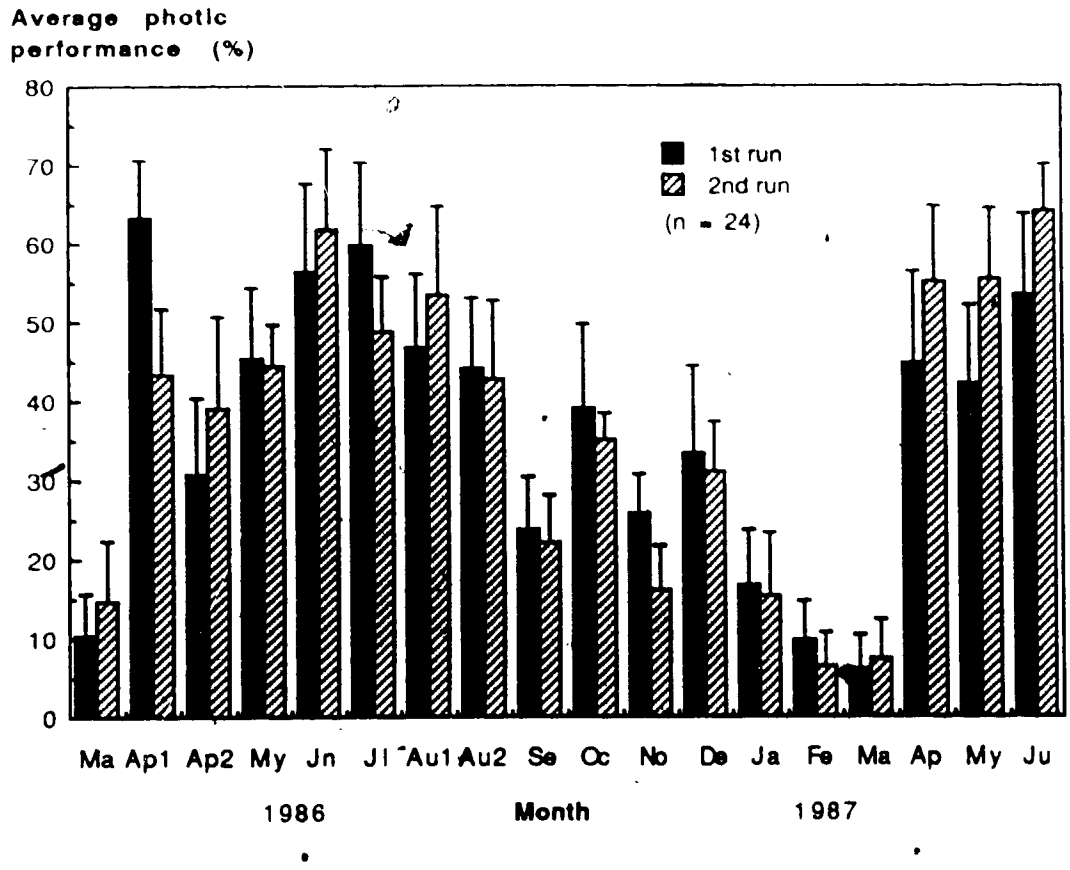


Fig. IV-1. Seasonal variation in photic behavior in uninfected gammarids, one day after collection.
The average photic performance represents the average number of gammarids present in the lighted half of the testing apparatus per checking time; means and standard deviations were converted to percentages for display. The two runs of one hour each were separated by one hour.

the light, and a minimum in February-March, about 10% in the light (Fig. IV-1). There was a significant regression of the average photic performance on lake water temperature ($n = 18$, $r = 0.68$, $p < 0.05$) (Fig. IV-2a), and a still more significant regression on daylength ($n = 18$, $r = 0.78$, $p < 0.01$) (Fig. IV-2b), but no significant regression on water temperature in the tray at the end of the experiment ($n = 11$, $r = 0.58$, $p > 0.05$). Daylength and lake water temperature were obviously correlated ($n = 18$, $r = 0.68$, $p < 0.01$). However, the regression of photic performance on daylength was stronger (greater slope over comparable ranges of the independent variable, see Fig. IV-2) and more important (higher r^2 values) (terminology that of Welden and Slauson, 1986), suggesting that the relationship with daylength is the more relevant one.

B. Biogenic amines and photic behavior with fixed light source

The photic performance of control gammarids and those injected with biogenic amines were tested in two types of experiments, the first with a fixed light source, the second with alternating opposite light source (next section). For the fixed light source experiment, the same apparatus as above was used but with only one gammarid per track.

As for the clinging behavior, the raw data were handled in two ways. 1) The number of times each gammarid was present in the half of the tray nearest to the light in one

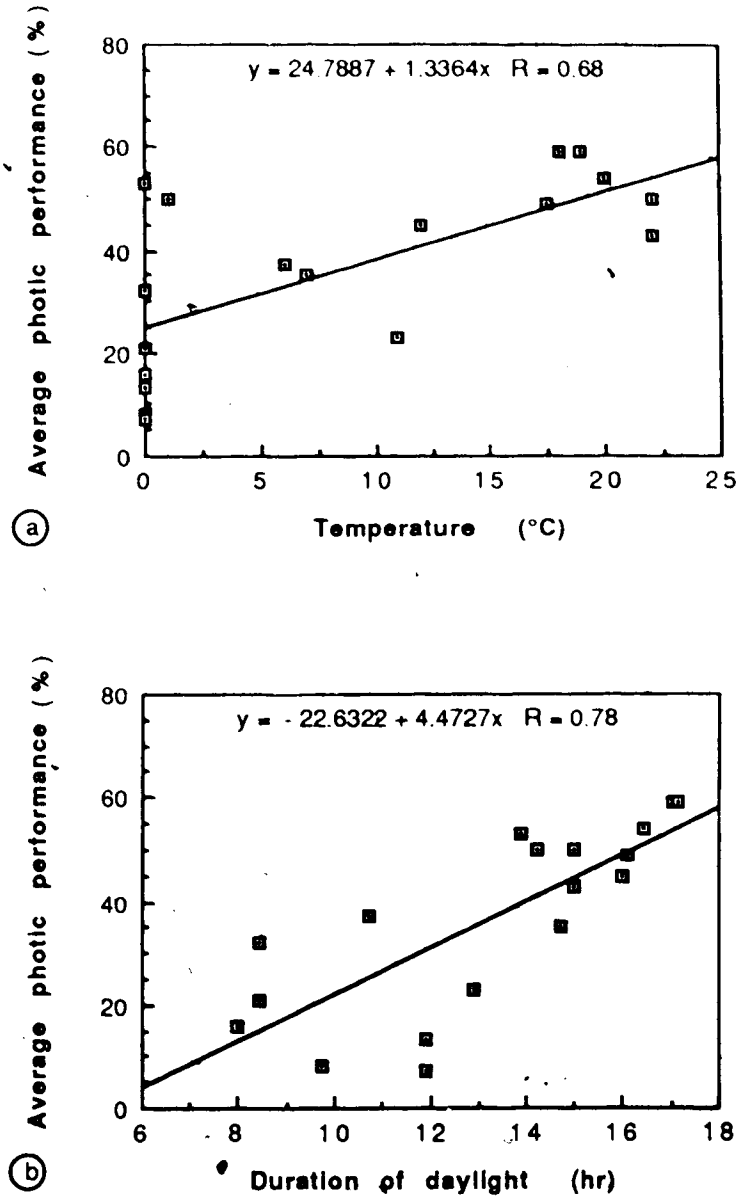


Fig. IV-2. Relationship between a) water temperature and photic behavior of uninfected gammarids, and b) duration of daylight and photic behavior. Water temperatures at Cooking Lake are listed in Table II-1; hours daylight at latitude 54 °N are taken from the Smithsonian Meteorological Tables (List, 1949). Linear regression equations are followed by correlation coefficient (R).

hour (12 observations) was calculated. The mean of this value for all gammarids in each experiment, referred to as average individual photic performance, is listed in Appendix IV-1 (uninfected gammarids) and Appendix IV-2 (infected gammarids). 2) The time-course of the behavior was studied by examining changes over time in the number of gammarids present in the lighted half of the tray per checking time.

Average individual photic performance

The number of times uninfected gammarids were present in the lighted half of the tray averaged 4.3 out of twelve in untreated controls (Table IV-2; range 1.8 to 6.9 in 5 experiments), while it averaged 2.7 in saline injected controls (range 0.6 to 5.8 in 6 experiments), and 6.2 in uninfected gammarids injected with 10 micrograms 5-HT (HCl) (range 4.9 to 8.1 in 7 experiments). The number of times infected control gammarids were present in the lighted half of the tray averaged 9.6 out of 12 in untreated controls (Table IV-2; range 7.7 to 11.2 in 12 experiments), 9.7 in saline injected gammarids (range 9.3 to 10.0 in 5 experiments), and 9.1 in infected animals injected with 10 micrograms octopamine (8.3 and 9.5 in two experiments). Three other substances, dopamine, leu-enkephalin and morphine, were assayed (in one experiment each) in an attempt to decrease the photopositivity of infected gammarids. All three were suspected of being able to control photomechanical changes in the compound eye of crustaceans.

Table IV-2. Average individual photic performance in uninfected gammarids and in those infected with the acanthocephalan Polymorphus paradoxus, following treatment with different substances.
The means and standard deviations (s.d.) represent the average number of times gammarids were present in the lighted half of the tray out of 12 observations in one hour. Individual data are pooled across experiments. All experiments are listed in Appendix IV-1 and IV-2.

	UNINFECTED		INFECTED	
	Number of Experiments / Number of Individuals	Mean ± s.d.	Number of Experiments / Number of Individuals	Mean ± s.d.
Pre treatment	5 / 59	4.3 ± 3.3	12 / 129	9.6 ± 3.3
Saline	6 / 65	2.7 ± 3.8	5 / 52	9.7 ± 3.1
5-HT (10 µg)	7 / 77	6.2 ± 4.2	-	-
Octopamine (10 µg)	-	-	2 / 17	9.1 ± 2.6
Dopamine (10 µg)	-	-	1 / 12	7.3 ± 4.2
Morphine (5 µg)	-	-	1 / 12	9.7 ± 3.3
Enkephalin (10 µg)	-	-	1 / 12	9.0 ± 3.4

Following injection of dopamine (10 µg) infected gammarids were present on average 7.3 times in the lighted half, 9.7 with morphine (5 µg), and 9.0 with leu-enkephalin (10 µg)(Table IV-2).

The condition of homoscedasticity was not met for the data regarding the photic behavior of controls and serotonin injected animals, precluding the use of a two level nested anova to compare the performance between groups. However, multiple t-tests (with unequal variances) on pooled data indicated that untreated uninfected controls were significantly more photopositive than saline treated uninfected animals (d.f. = 122, $t = 2.419$, $p = 0.017$), and that serotonin injected animals were more photopositive than both saline injected controls (d.f. = 140, $t = 5.136$, $p < 0.001$) and untreated controls (d.f. = 134, $t = 2.873$, $p = 0.005$), but less photopositive than saline injected infected gammarids (d.f. = 127, $t = 5.296$, $p < 0.001$). The photic performance of saline injected infected controls did not differ significantly from that of untreated infected controls, nor that of octopamine, dopamine, morphine, or leu-enkephalin injected animals. (Note that, had their been a larger sample size, the average photic performance following treatment with dopamine (7.3) might have been significantly lower than that following treatment with saline (9.7).)

One level nested anovas indicated that in uninfected gammarids there was a significant variability among

experiments for both uninjected controls and saline injected animals (Table IV-3). In contrast to the controls, and despite the differences in treatment before the injection (e.g., whether or not the gammarids had been dark adapted, or whether or not they had been tested prior to injection, see Appendix IV-1), there was no significant variance among experiments involving uninfected gammarids injected with 10 ug serotonin (Table IV-3). Also, there was no significant variance among experiments involving infected gammarids, whether in untreated controls, in saline injected animals, or octopamine injected animals (Table IV-4).

The variability among experiments in the photic performance of uninfected gammarids not related to seasonal variation. There was no significant correlation between average photic performance and date of experiment in uninfected gammarids (uninjected: $n = 5$, $r = -0.154$, $p > 0.05$; saline injected: $n = 6$, $r = -0.007$, $p > 0.05$), nor was there any significant correlation between average photic performance and daylength on the date of experiment (uninjected: $n = 5$, $r = -0.142$, $p > 0.05$; or saline injected: $n = 6$, $r = 0.705$, $0.1 < p < 0.2$). However, note that 4 out of 5 experiments involving uninjected gammarids and 4 out of 6 experiments involving saline injected animals were performed in a single month (February). In all experiments, the variability among individuals was high and accounted for more than 70% of the variance within treatments, even in groups in which the variability among

Table IV-3. Variation among experiments in individual photic performance of uninfected gammarids, untreated, injected with saline, or with serotonin (10 μg). Each row is a summary of a one level nested anova.

Treatment	df †	Fs	P	% variance among exps.
Untreated	4 , 54	5.256	0.0012	26.5
Saline	5 , 59	3.786	0.0049	20.5
Serotonin	6 , 70	0.862	0.5272	0.0 ††

† d.f. (among experiments) , d.f. (individuals within experiments).

†† The analysis produces meaningless negative values when MS among is less than MS within; given here as 0.0.

Table IV-4. Variation among experiments in individual photic performance of gammarids infected with the larval acanthocephalan Polymorphus paradoxus, untreated, injected with saline, or with octopamine (10 µg).
Each row is a summary of a one level nested anova.

Treatment	df †	Fs	P	% variance among exps.
Untreated	11 , 117	1.518	0.1339	4.6
Saline	4 , 47	0.080	0.9882	0.0 ††
Octopamine	1 , 15	0.690	0.4191	0.0 ††

† d.f. (among experiments) , d.f. (individuals within experiments).
†† The analysis produces meaningless negative values when MS among is less than MS within; given here as 0.0.

experiments was significant (Table IV-3 and IV-4). This variability in photic behavior among individuals was accompanied by an element of consistency within individuals. A significant positive correlation was found in the photic performance between pre and post treatment with saline in both infected ($n = 52$, $r = 0.623$, $p < 0.001$) and uninfected controls ($n = 24$, $r = 0.530$, $p = 0.008$). There was no significant correlation between the performance pre and post treatment in the 23 individuals injected with serotonin ($r = 0.003$, $p = 0.989$). These features agree with those on variation among experiments and indicate an override of the "normal" photic behavior rather than an additive effect by serotonin in uninfected animals. There was a significant correlation between pre and post treatment for all the infected individuals pooled across the four different treatments (i.e., octopamine, dopamine, enkephalin, morphine) ($n = 53$, $r = 0.414$, $p = 0.002$), although the correlation for octopamine alone was not significant ($n = 17$, $r = 0.316$, $p = 0.159$).

The results concerning the average individual photic performance indicate that 1) the photopositivity of serotonin injected uninfected gammarids was significantly higher than that of uninfected controls but not as high as that of infected controls; 2) serotonin injected uninfected gammarids also resembled infected controls in that their photic behavior was more predictable than that of uninfected controls; 3) there was an element of consistency in the

photic behavior pre and post treatment in uninfected and infected controls that was absent in serotonin injected animals; and 4) octopamine, dopamine, morphine, or [redacted]-enkephalin did not affect significantly the photic behavior of infected gammarids.

Time-course

The patterns of changes in photic behavior over time in two representative experiments involving uninfected gammarids (Fig. IV-3) clearly illustrate several points: 1) the unpredictable, sometimes high, photopositivity of untreated uninfected controls when first placed in the tray; 2) the unpredictable, but lower, photopositivity of saline injected gammarids; and 3) the predictable pattern in the photopositivity of serotonin injected animals, with a sharp rise after injection and a linear decrease thereafter. Experiments F18,19 and F20,21 were performed in February 1986, during the same week, and under the same conditions, but with gammarids of different origin. In experiment F18,19, gammarids had been collected in January, whereas in experiment F20,21, they had overwintered in the laboratory. In contrast the time-courses of photic behavior in infected gammarids, whether untreated (Fig. IV-4a), injected with saline (Fig. IV-4b), or injected with octopamine (Fig. IV-4c), appear very similar; all show an initially high photopositivity, decreasing slightly over time.

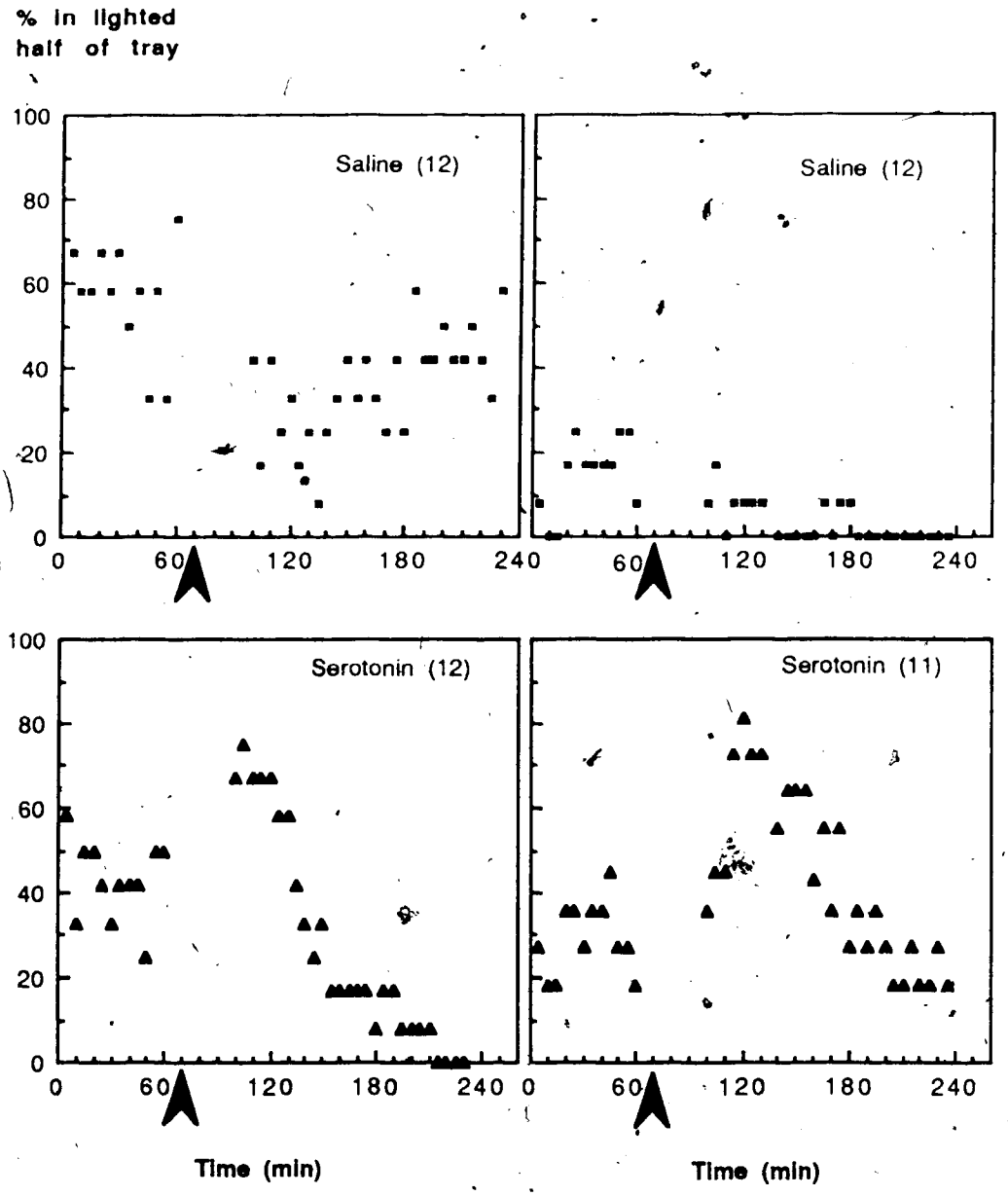


Fig. IV-3. Photic behavior pre and post treatment in uninfected gammarids injected with saline or with 10 µg serotonin in two experiments (F18,19: left; F20,21: right). The arrows indicate the time of injection; sample size in parenthesis.

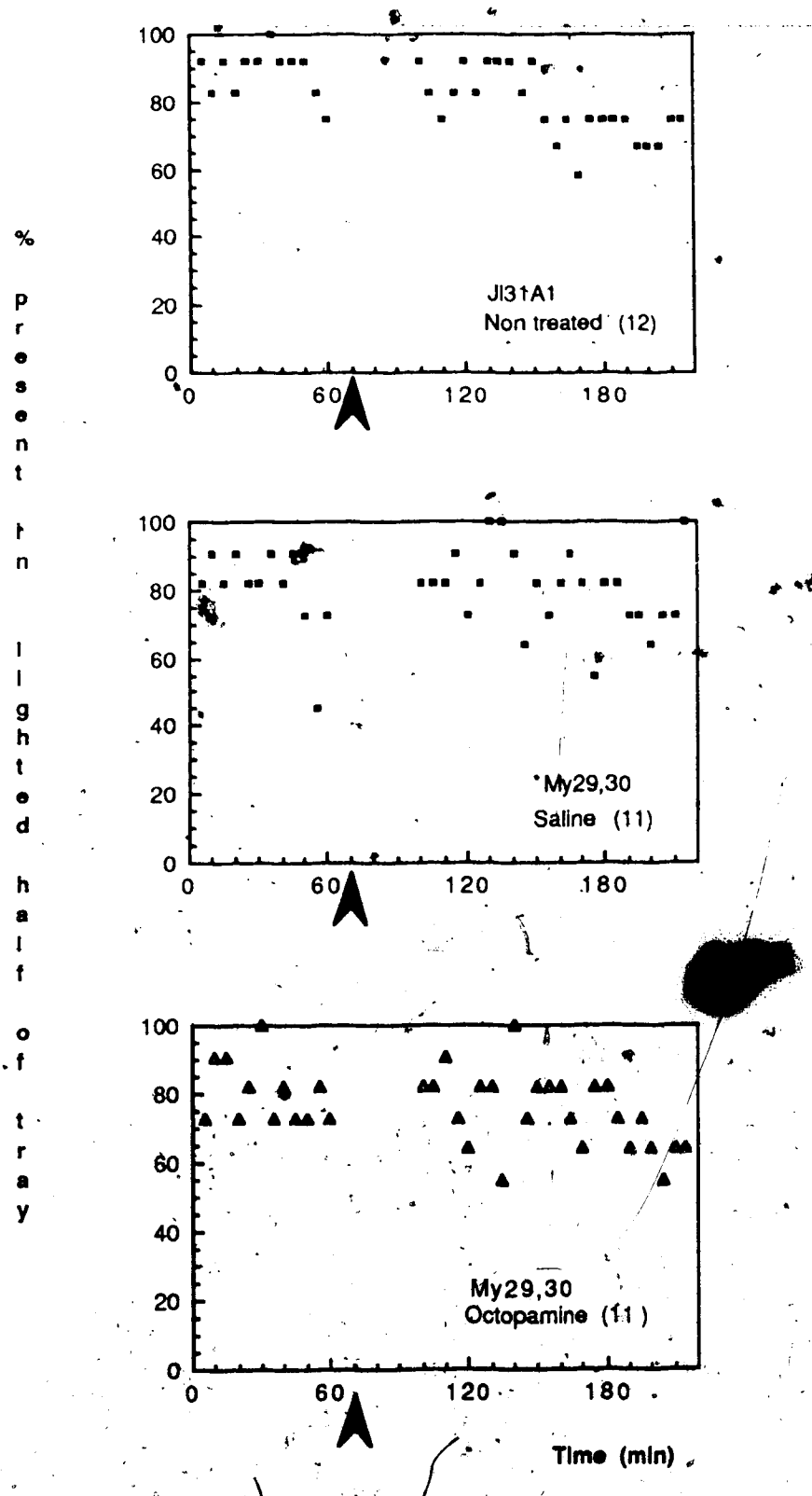


Fig. IV-4. Photic behavior pre and post treatment in gammarids infected with the acanthocephalan Polymorphus paradoxus, untreated, injected with saline, or injected with 10 µg octopamine.
The arrows indicate the time of injection; date of experiment, treatment, and sample size (in parenthesis) in boxes.

To assess whether or not the patterns shown in these examples were consistent throughout all experiments, time-courses of the photic behavior after treatment were fitted with linear regressions (slopes, correlation coefficients and statistical significance are given in Appendix IV-1 and IV-2) In all experiments involving gammarids injected with serotonin (7), there was a consistent pattern. In all, there was a significant linear regression of photic performance on time following injection, and the average slope of the linear regression was significantly different from that involving either uninfected or infected controls injected with saline. (Table IV-5). In addition, the control groups did not show consistent patterns. Linear regressions were significant in only three out of six of the control uninfected groups, and two out of five of the control infected groups. Furthermore, as expected, the average correlation coefficient of the regression line (r) was significantly higher in experiments involving serotonin than in experiments involving uninfected or infected controls (Table IV-5). Thus, both between and within experiments, the pattern over time of the photic behavior in serotonin injected animals was consistent and differed from that in control gammarids.

However, the linear regression of the time-course was not the best description of the photic behavior over time in uninfected gammarids injected with serotonin; for six out of the seven experiments, a third order regression term was

Table IV-5 Linear regression of proportion of gammarids in the lighted zone on time Values for individual experiments are listed in Appendix IV-1 (uninfected gammarids), and in Appendix IV-2 (gammarids infected with the larval acanthocephalan Polymorphus paradoxus)

	Uninfected gammarids		Infected gammarids	Significance 0.05, t-test †
Column #	1	2	3	
Treatment	Saline	5-HT	Saline	
Number of Experiments	6	7	5	
Mean slope of Linear Regression ± s.d.	0.005 ± 0.143	-0.376 ± 0.116	-0.107 ± 0.164	2 ≠ 1 = 3
Mean correlation Coefficient of Linear Regression ± s.d.	0.368 ± 0.279	0.729 ± 0.127	0.454 ± 0.209	2 ≠ 1 = 3

† All possible t-tests per row performed (3).

significant, and explained an additional 10-23% of the variance (Fig. IV-5; correlation coefficients in Appendix IV-1 and IV-2). Third order regressions are characteristic of curves with two inflection points. These six experiments all showed a peak in photopositivity (61 ± 13%) at 50 ± 8 minutes after injection. The photopositivity in the sample then decreased to a minimum of 21 ± 7% (n = 5 experiments, A8 excluded because of its early termination) after a further 68 ± 8 min. In experiment F18,19, it is clear that, had there been some ascending points recorded shortly after injection, the first peak would also be present.

Because of the consistent pattern characteristic of the time-courses of the photic responses of serotonin injected gammarids, the time-courses of control uninfected gammarids were arbitrarily fitted with third order regressions (Fig. IV-6; Appendix IV-1). Two features should be noted. First, there was no consistent shape to the curves. Second, a third order regression was significant for only three of six groups, and a third order term explained a significant amount of variation in only one of those (Fig IV-6; F18,19). Clearly, the photic behavior of uninfected animals was considerably less predictable than that of serotonin injected animals. The time-courses of the photic responses of infected gammarids injected with saline (Fig. IV-7), and those of infected gammarids injected with amines or peptides (Fig. IV-8), were also fitted with third order regressions. As for the uninfected controls, there was no consistent

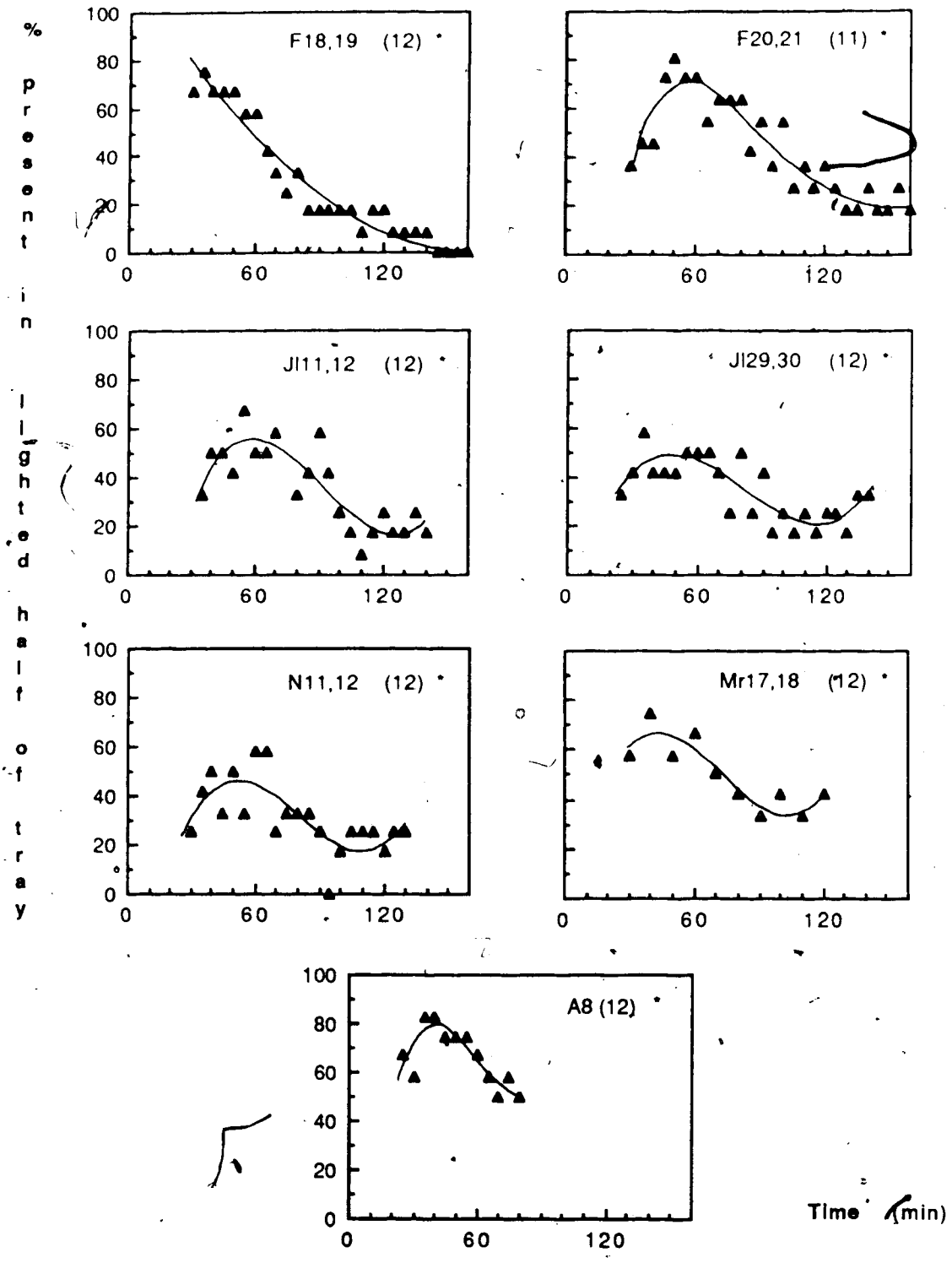


Fig. IV-5. Time-course of photic behavior in uninfected gammarids injected with 10 µg serotonin at time 0. All experiments were performed with a fixed light source, except for Mr17,18 conducted with alternating light source.
The curves are third order curvilinear regressions; correlation coefficients are listed in Appendix IV-1 (* $p < 0.05$). Additional legends in boxes are date of experiment and, in parenthesis, sample size.

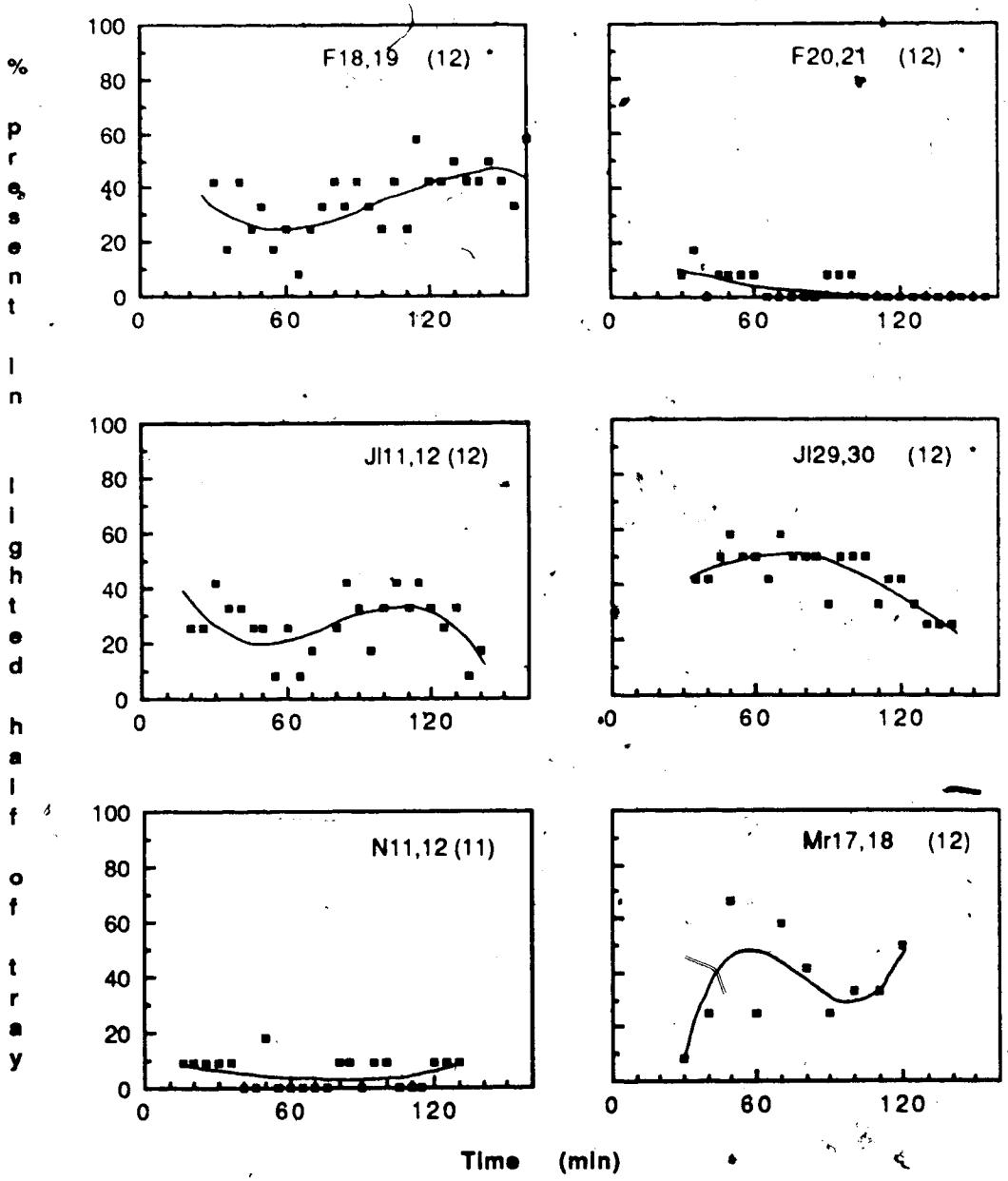


Fig. IV-6. Time-course of photic behavior in uninfected gammarids injected with saline at time 0. All experiments were performed with a fixed light source, except for Mr17,18 conducted with alternating light source.
The curves are third order curvilinear regressions; correlation coefficients are listed in Appendix IV-1 (* $p < 0.05$). Additional legends in boxes are date of experiment and, in parenthesis, sample size.

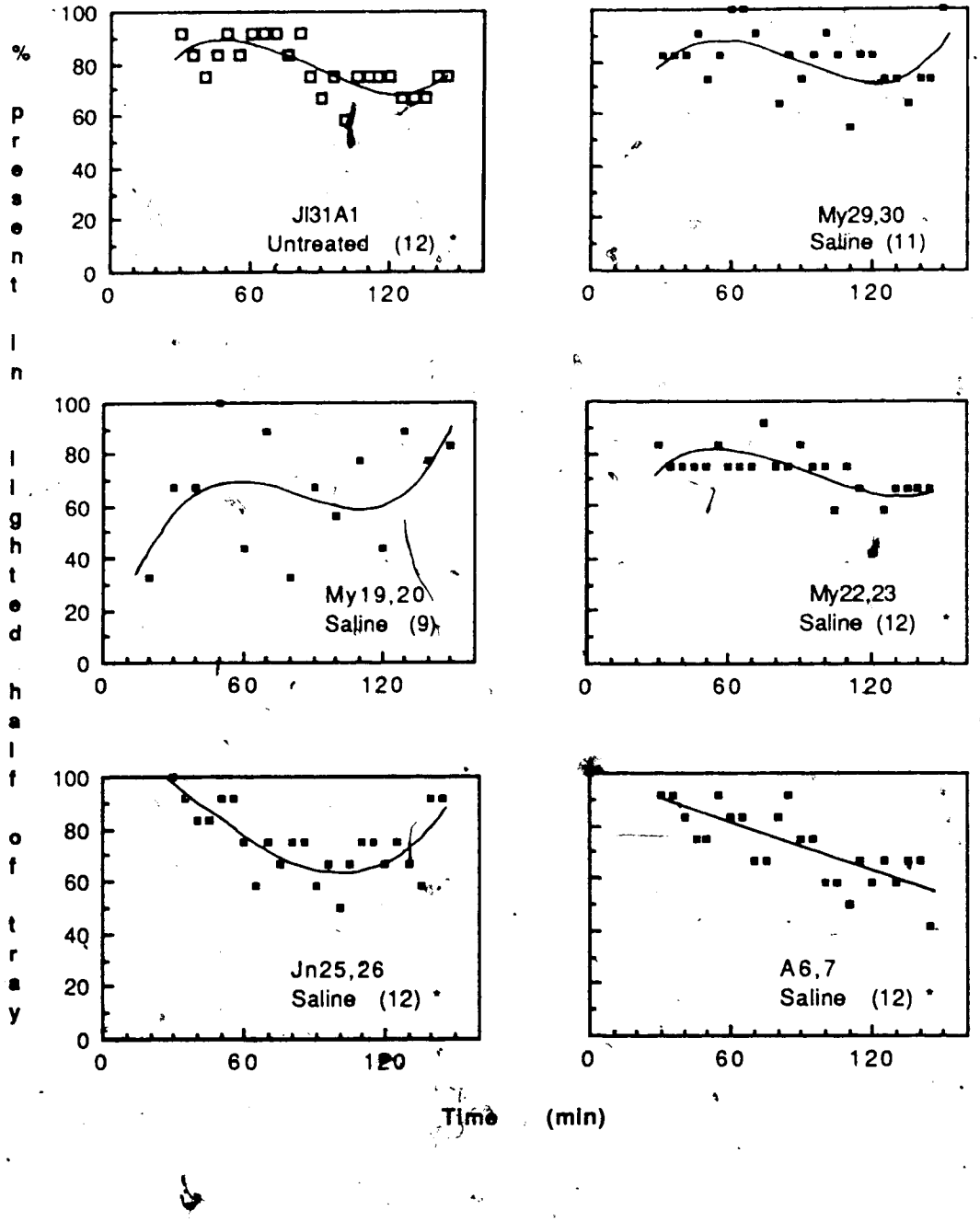


Fig. IV-7. Time-course of photic behavior in gammarids infected with the larval acanthocephalan Polymorphus paradoxus, untreated or injected with saline at time 0. All experiments were performed with a fixed light source, except for My19,20 conducted with alternating light source.
The curves are third order curvilinear regressions; correlation coefficients are listed in Appendix IV-2 (* $p < 0.05$). Additional legends in boxes are date of experiment and, in parenthesis, sample size.

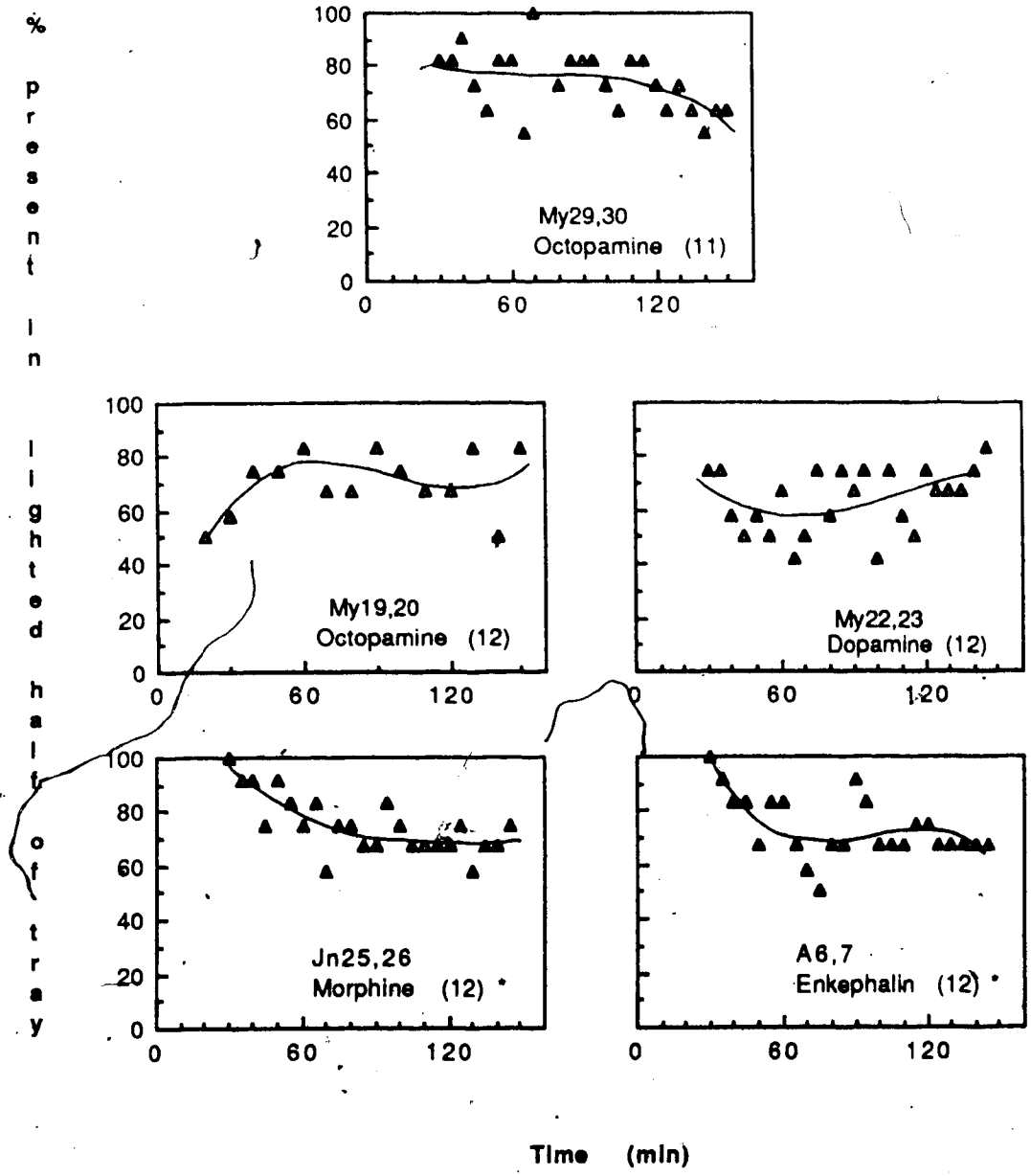


Fig. IV-8. Time-course of photic behavior in gammarids infected with the larval acanthocephalan Polymorphus paradoxus, injected with 10 µg octopamine, dopamine, leu-enkephalin, or 5 µg morphine at time 0. All experiments were performed with a fixed light source, except for My19,20 conducted with alternating light source. The curves are third order curvilinear regressions; correlation coefficients are listed in Appendix IV-2- (* $p < 0.05$). Additional legends in boxes are date of experiment and, in parenthesis, sample size.

shape to the curves. Third order regressions were significant for 4 out of 6 experiments involving saline, for the experiments involving morphine or leu-enkephalin, but not for those involving dopamine or octopamine.

C. Biogenic amines and photic behavior with alternating light source

It was important to test whether or not light was indeed the environmental factor involved when gammarids chose the lighted half of the experimental tray. It was also necessary to determine whether gammarids injected with 10 µg of biogenic amines retained their motor ability. Therefore, the experimental procedures were modified to provide two lamps, one at each end, that were switched on alternately every 10 minutes (Fig. II-1). There was one gammarid per track, and its position in the track was recorded to the nearest 10 cm before each light switch. The net distance traveled from the position occupied 10 minutes earlier, as well as the direction of the move (away from or towards the light source) were determined. All distances are minimum distances; the movements of the gammarids within the 10 minute periods were not monitored.

Two experiments were performed, one involving uninfected gammarids (Mr17,18), with 12 animals injected with saline and 12 with 10 µg serotonin; the other involving infected gammarids, with 12 animals injected with saline and 12 with 10 µg octopamine (My19,20). Three of the 12 infected

gammarids injected with saline kept clinging from the first minute at one end of their track, never moved and therefore were not taken into account. As expected, the "clinging problem" did not arise with infected gammarids injected with octopamine.

Although gammarids had to travel from one end of the tray to the other, the patterns of average photic performance and time-course were the same as those found using the fixed light source. In the hour following treatment, uninfected gammarids injected with serotonin were present in the half nearest to the light marginally more than saline injected animals, 3.5 times out of 6 vs 2.1 (Appendix IV-1, t-test, $p = 0.07$). Saline injected infected gammarids were present on average 4.0 times out of 6 in the half of the tray near the light source, while octopamine injected were present in this same half 4.3 times (Appendix IV-2). The performances of the two infected groups did not differ significantly from each other nor from the serotonin injected uninfected group, but both were significantly higher than that of uninfected controls (multiple t-tests, 0.05 level of significance). For serotonin injected infected animals, the curvilinear regression of the time course had the same characteristic shape as in experiments with a fixed light source, with a maximum 50 min post treatment, followed by a decline to a minimum (Fig. IV-5).

Distance covered

During the first 60 minutes, serotonin injected animals swam significantly longer distances (5.26 m) than all other groups; uninfected controls (3.56 m), infected controls (4.46 m), or octopamine injected infected animals (4.30 m) did not differ from each other (Table IV-6). During this same period, serotonin injected animals also swam significantly more towards the light (3.32 m) than saline uninfected controls (1.01 m), but did not differ significantly from the infected groups (about 3.8 m) (Table IV-6). Also, the movement towards the light source was a significantly higher proportion of the total distance covered in serotonin injected gammarids (65%), than in saline injected uninfected animals (31%), but again did not differ significantly from that of the infected groups (about 85%) (Table IV-6).

Testing the photic behavior with alternating light source showed that gammarids injected with 10 µg serotonin or octopamine retained their ability to swim. Although gammarids had to travel every 10 minutes to maintain themselves in the same photic environment, the patterns observed in experiments with a fixed light source and with alternating light source were similar. Thus, light was indeed the major factor involved when gammarids adopted a specific position during the experiments with a fixed light source.

Table IV-6. Photic behavior with alternate opposite light source, in uninfected gammarids injected with saline or 10 µg serotonin (5-HT), and in gammarids infected with the larval acanthocephalan Polymorphus paradoxus, injected with saline or with 10 µg octopamine (OA) (see details in text).
Average distances traveled per gammarid in one hour, and standard deviations (± s.d.); (a) represents the total distance traveled (in cm), (b) the distance traveled towards the light source (in cm), and (c) the proportion of the total distance traveled towards the light source (b / a)., expressed as a percentage.

	UNINFECTED		INFECTED		Significance 0.05; t-test †
Column #	1	2	3	4	
Treatment	Saline	5-HT	Saline	OA	
(n)	(12)	(12)	(9)	(12)	
a) Average Distance Swam Per Gammarid	356 ± 116	526 ± 176	446 ± 203	430 ± 186	1 ≠ 2; 1 = 3 = 4; 2 = 3 = 4;
b) Average Distance Swam Per Gammarid Towards Light Source	101 ± 114	332 ± 278	357 ± 193	393 ± 189	1 ≠ 2 = 3 = 4
c) Proportion of Distance Swam Towards Ligh Source (%)	31 ± 35	65 ± 45	83 ± 22	90 ± 12	1 ≠ 2 = 3 = 4 ††

† All possible t-tests per row (6) performed.
†† T-tests performed on arcsin transformed data.

D. Skimming behavior

It had become clear when studying photic behavior that infected and serotonin injected uninfected gammarids frequently skimmed along the water surface. This component of the altered escape behavior induced by *P. paradoxus* was studied further using finger bowls filled with pond water to a depth of 5 cm, under overhead lights. The water was agitated for 10 seconds, the number of gammarids skimming the surface of the water was counted and the skimming gammarids were re-immersed. This procedure was repeated every 5 minutes, for one hour. Individual gammarids were not marked, therefore observations could not be ascribed to individuals. The number of skimming responses observed in one hour was thus presented as a proportion of the total number of potential skimming responses for this hour [number of gammarids tested x number of tests (12)].

Uninfected untreated gammarids never skimmed the surface and saline injected gammarids skimmed the surface only once in the first hour and rarely thereafter, while serotonin injected animals skimmed the surface 41 times out of 144 (Table IV-7), significantly more than infected gammarids (61 times out of 420 in two different experiments) ($X^2 = 14.083$, $p = 0.0002$).

In uninfected gammarids injected with serotonin, the time-course of the behavior showed a rise, a maximum about one hour post-treatment, and a subsequent decline (Fig. IV-9). In infected gammarids the skimming behavior

Table IV-7. Skimming behavior in uninfected gammarids (untreated, injected with saline, or with serotonin), and in gammarids infected with the larval acanthocephalan Polymorphus paradoxus (untreated). The skimming behavior is expressed as the number of skimming responses observed during 12 assays in one hour divided by the potential number of skimming responses in that hour (12 x n).

Experiment	Treatment	n	# Skimming / Potential # Skimmig	%
UNINFECTED				
Jul22	Untreated	24	0 / 28	0
Sep10	Untreated	12	0 / 144	0
Jul25	Saline	12	1 / 144	0.5
Jul25	Serotonin, 10 µg	12	41 / 144	28
INFECTED				
Jul22	Untreated	24	48 / 288	17
Sep10	Untreated	11	13 / 132	10

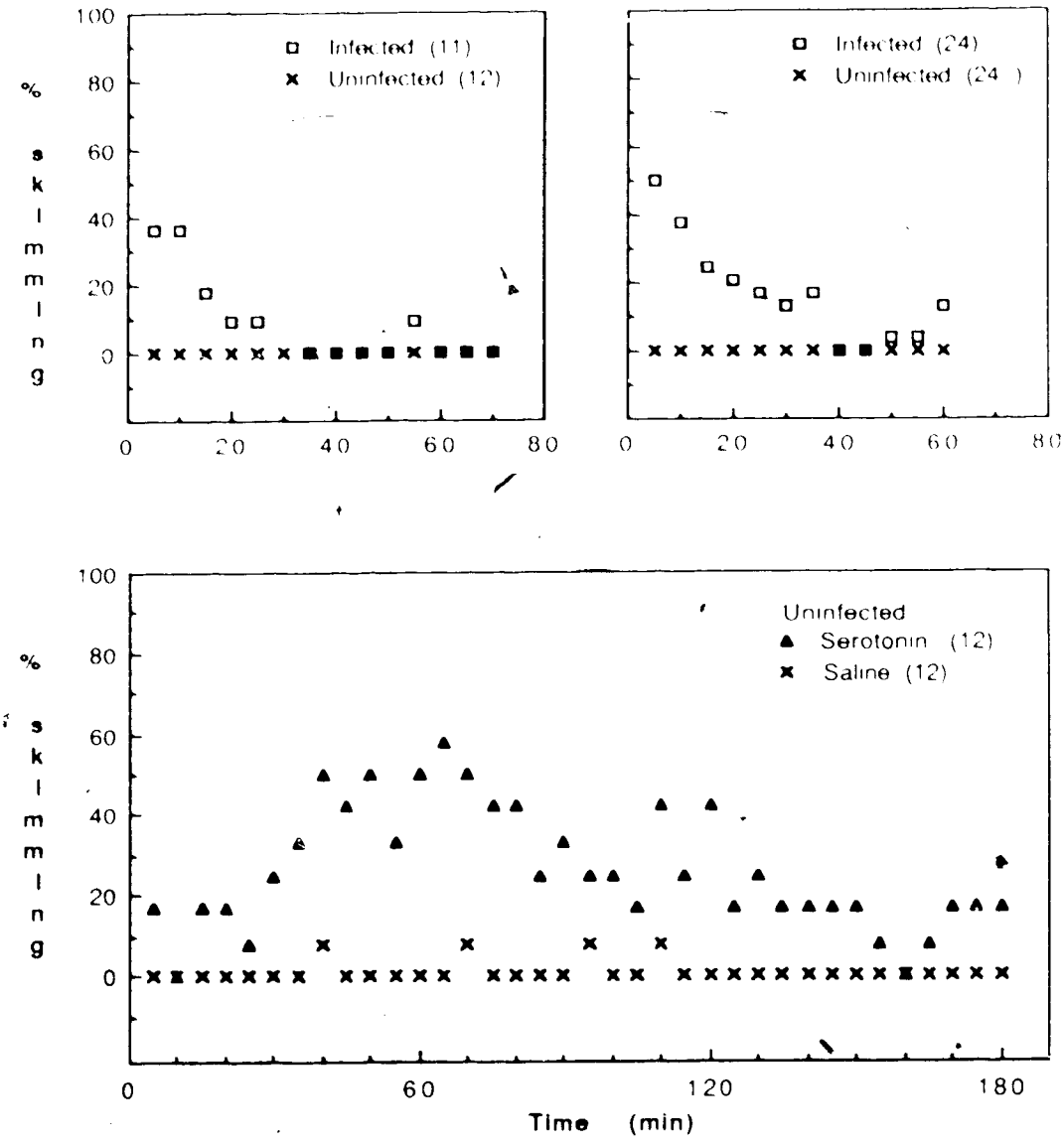


Fig. IV-9. Time-course of skimming behavior in gammarids infected with the larval acanthocephalan Polymorphus paradoxus (untreated), and in uninfected gammarids (untreated, injected with saline, or injected with 10 µg serotonin).

habituated very rapidly.

E. Summary of results

The vast majority of the parameters studied in this chapter on photic behavior showed a significant difference between the performance of uninfected and infected gammarids and between that of uninfected controls and serotonin injected animals. Depending on the parameter studied, the photic behavior of serotonin injected animals either did not differ significantly from that of infected controls (e.g., variability in average photic performance among experiments, distance covered towards light source, proportion of the total distance covered towards light source), or showed a similar trend to a lesser extent (average photic performance), or to a greater extent (skimming behavior). Serotonin injected animals differed from all other groups in the time-courses of photic and skimming behaviors, apparently due to the transitory nature of the action of the biogenic amine.

Octopamine, which antagonized the clinging behavior of infected gammarids, did not affect their photic behavior. For all the parameters studied the performance of octopamine injected infected animals was similar to the performance of infected controls.

V. COMPOUND EYE AND SCREENING PIGMENTS

A. The compound eye of gammarids - A qualitative study

There were no detectable qualitative differences between the eyes of infected and uninfected gammarids in histological sections (Fig. V-1; compare d and e with f and g). Thus, the following description applies to both kinds of animals. In adult *G. lacustris* each lateral compound eye consisted of some 50 ommatidia. Each ommatidium was composed of five retinular cells, one thinner than the others (Fig. V-1b), a crystalline cone, and accessory pigment cells surrounding the retinular cells. The intricate junction of the five retinular cells formed the rhabdom - the photosensitive structure. The nuclei of the retinular cells were located under the basement membrane. The axons of the retinular cells formed a bundle proximally.

Several structures showed marked differences in shape depending on whether the eyes had been light or dark adapted. In light adapted gammarids the crystalline cones assumed a more elongated shape than in dark adapted gammarids (Fig. V-1; compare d and f with e and g). The rhabdoms, extended and straight after exposure to light, became twisted after exposure to dark, and the base of the crystalline cones seemed nearer to the basement membrane. However, the most striking changes occurred in the distribution of the two sets of screening pigments, the retinular pigment in the retinular cells, and the white

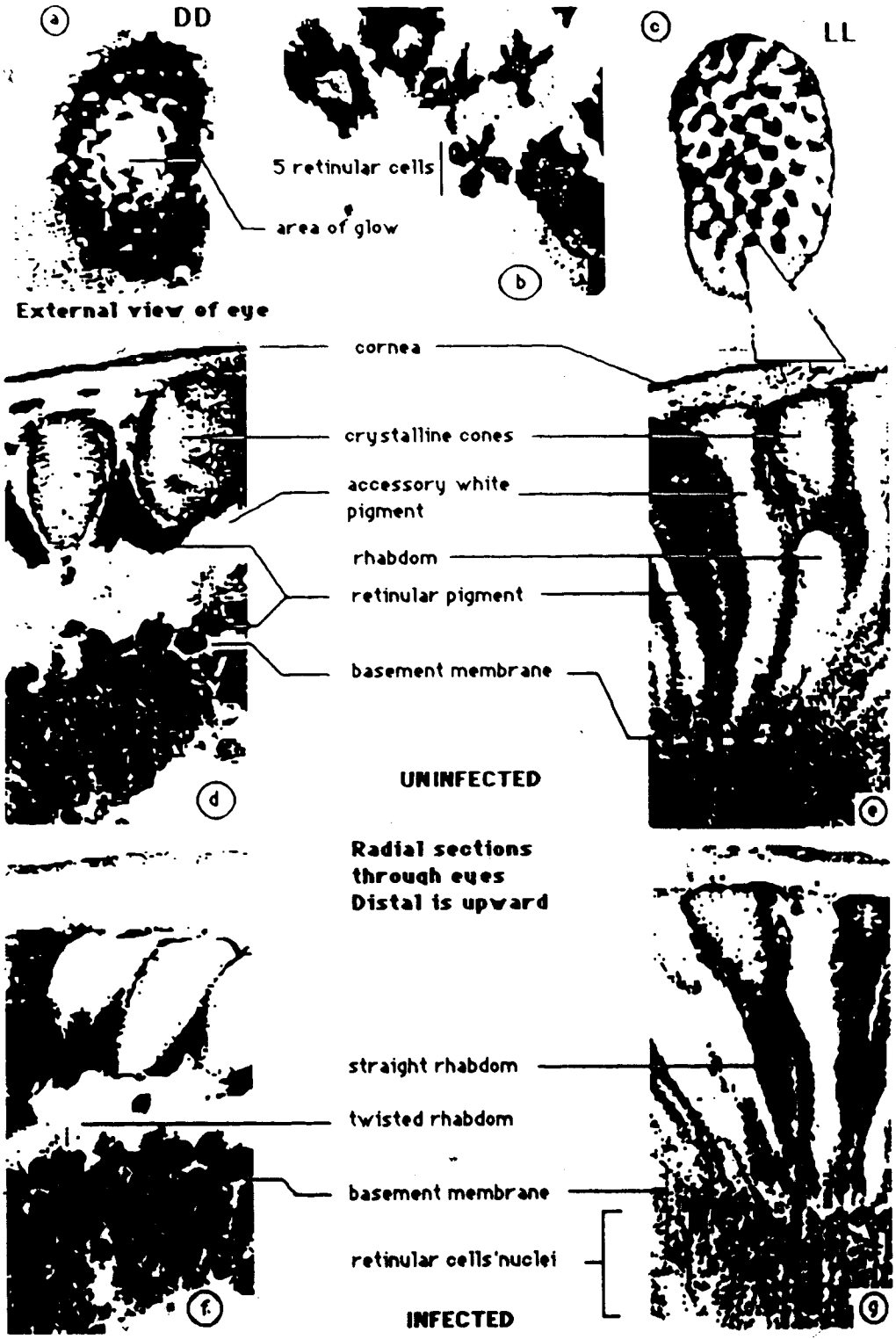
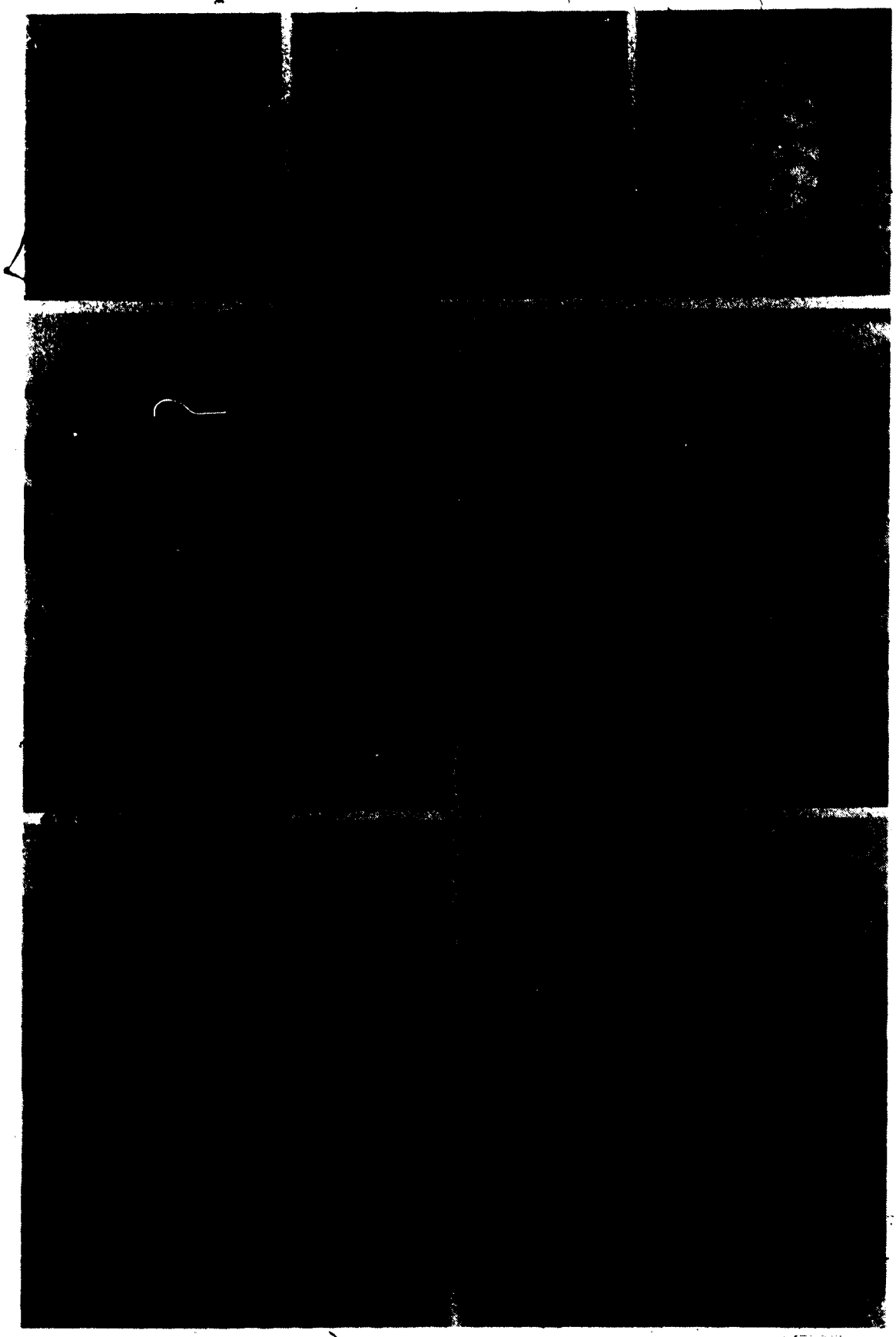


Fig. V-1. Dark (DD; a,d,f) and light (LL; c,e,g) adaptations in the eye of Gammarus lacustris uninfected (d,e) or infected with the larval acanthocephalan Polymorphus paradoxus (f,g).



reflecting pigment in the accessory screening pigment cells.

Retinular pigment

In light adapted eyes (Fig. V-1e,g), the black pigment granules located in the retinular cells were distributed along the rhabdom, presumably to protect it from excess light. After dark adaptation, (Fig. V-1a,d,f), the black pigment had moved both distally, between the crystalline cones, and proximally, where it concentrated at the base of the rhabdom and under the basement membrane near the nuclei of the retinular cells.

Light and dark adapted eyes also differed in gross appearance, in apical view. When the black pigment was light adapted, the crystalline cones appeared as black circles (Fig. V-1c, and V-2). When dark adapted, and observed in the axis of the cone cell, the center of the circle appeared white, due to the exposed reflecting pigment (Fig. V-1a). Only a few cone cells could be seen in their axis, due to the slight curvature of the eye. They formed a white area, similar to that found in the dark adapted eyes of decapods, referred to as area of glow (Aréchiga, 1977). The retinular pigment extended along the rhabdom at very low light intensities and the circle of the crystalline cones always appeared black (Fig. V-1c and V-2), except when the gammarids were retrieved from an environment of total darkness (Fig. V-1a).

Accessory screening pigment

The accessory pigment cells extended from the basement membrane to the cornea, and were filled with a pigment that was very photolabile (Debaisieux, 1944). After a few hours, the white color observed in reflected light disappeared. The cell membranes of the accessory pigment cells were not clearly visible, but their nuclei were apparent, distal to the basement membrane. During light adaptation, the white pigment moved distally (Fig. V-1e and g), and the cone cells appeared to be pushed apart by the subcuticular position of the white pigment. Presumably, this reflected light back to the environment. During dark adaptation, the white pigment moved proximally, receding from between the crystalline cones. Light was then probably reflected towards the exposed rhabdoms (Fig. V-1d and f).

The state of the white pigment could also be assessed by external examination of the intact eye under the dissecting microscope (Fig. V-1a,c and V-2). The more light adapted, the whiter the eye, because the white pigment came to extend between the crystalline cones, in a graded fashion, during light adaptation. Qualitatively, the compound eye of infected and uninfected gammarids could not be differentiated by histological methods. However, preliminary observations under the dissecting microscope showed that the eyes of infected gammarids appeared generally whiter than the eyes of uninfected animals. This led to a quantitative assessment of the distribution of the

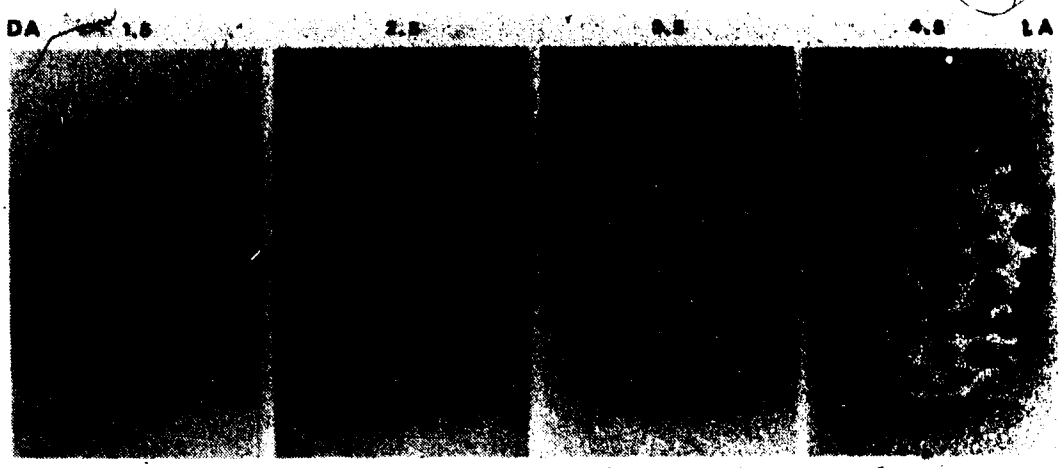


Fig. V-2. Position of the white accessory screening pigment in the compound eye of *Gammarus lacustris* - quantitative assessment (eye score). From left to right, the white pigment is more and more superficial; i.e., more and more light adapted. The whole scale ranges from 1 to 5.

white pigment in the eyes of infected versus uninfected amphipods.

B. Distribution and movements of accessory screening pigment - A quantitative study

Eye score in infected and uninfected gammarids

The distribution of the white pigment was assessed by grading the appearance of the eyes of living or freshly killed gammarids on a scale of 1 to 5 (with 0.5 unit steps); 1 represented the darkest eye (the most dark adapted distinguishable externally) and 5 the whitest eye (the most light adapted) (Fig. V-2). The average of two measurements, called eye score, was used (see details in Chapter II).

The eye scores of infected and uninfected gammarids were compared in twenty five samples spread from September 1986 to September 1987, with 6 infected gammarids and 6 uninfected gammarids in each sample. For the 25 samples, the average score of uninfected gammarids was 2.44 ($n = 147$) and that of infected gammarids was 2.58 ($n = 148$) which was significantly higher ($p = 0.047$, two way anova, Table V-1a). Although the anova showed no significant variance among experiments, a simple regression of the average difference in eye score between infected and uninfected animals for the 25 samples on the sampling date (Appendix V-1) was positive ($r = 0.360$) and marginally significant ($p = 0.077$), suggesting that a seasonal factor was involved, as in the

Table V-1. Two way analysis of variance of the average eye score of uninfected gammarids, and of those infected with the larval acanthocephalan Polymorphus paradoxus, throughout the open-water seasons from September 1986 through September 1987.

a) All samples (25)

Source	df	Sum of Squares	Mean Square	F-test	P value
Inf vs Uninf (A)	1	1.506	1.506	3.980	0.047
Samples (B)	24	10.304	0.429	1.134	0.307
AB	24	10.252	0.427	1.128	0.313
Error	245	92.74	0.379		

b) April through July samples (14)

Source	df	Sum of Squares	Mean Square	F-test	P value
Inf vs Uninf (A)	1	0.0004	0.0004	0.001	0.972
Samples (B)	13	7.216	0.555	1.558	0.105
AB	13	3.720	0.286	0.803	0.656
Error	136	48.455	0.356		

c) August through November samples (11)

Source	df	Sum of Squares	Mean Square	F-test	P value
Inf vs Uninf (A)	1	3.375	3.375	8.306	0.005
Samples (B)	10	3.078	0.308	0.757	0.669
AB	10	4.703	0.47	1.158	0.327
Error	109	44.285	0.406		

clinging and photic behaviors. A subsequent analysis of the results from April through July (14 samples), showed no significant difference in eye score between infected (n = 82) and uninfected (n = 82) gammarids, with an overall mean of about 2.5 ($p = 0.972$, two way anova, Table V-1b). In contrast, the results for August through November (11 experiments), showed a significant difference ($p = 0.005$, two way anova, Table V-1c), with a mean score of 2.34 for uninfected gammarids (n = 66) and a mean score of 2.67 for infected gammarids (n = 65).

Thus, the white pigment was on average more superficial in infected gammarids than in uninfected ones, but only in the latter part of the year.

Comparison of accessory pigment movements in infected and uninfected gammarids

The migration of the white pigment after dark and light adaptation was compared in infected and uninfected gammarids in several experiments, with slightly different protocols (see Chapter II, and Table V-2). The amplitude of pigment migration was obtained, for each individual, by substracting the eye score post adaptation from the eye score pre-adaptation. Distal movements of the white pigment (light adapting) and proximal movements (dark adapting) from experiments S19,20, N20,21 and S6,7 were examined in a two way anova testing the difference in amplitude of pigment migration between infected and uninfected gammarids. There

Table V-2. Migration of accessory (white) pigment in uninfected gammarids (U), and those infected with Polymorphus paradoxus (I), following light (LL) or dark (DD) adaptation, as measured by changes in eye score (Es).
The correlation coefficient (R) and its probability value for a regression of individual gammarid' s Es at t1 on its Es at t0 are also presented.

Exp.	Sample size	Conditions before t0	Conditions between t0 and t1	Amplitude of pigment migration	R Es t0 vs Es t1 p
		Es t0 Mean ± s.d.	Es t1 Mean ± s.d.	Es t1 - Es t0 Mean ± s.d.	
S19,20	24 12 I, 12 U	2 hr LL 2.71 ± 0.79	2 hr DD 1.98 ± 0.77	-0.73 ± 0.38	0.884 p < 0.001
S23,24	24 12 I, 12 U	Tank 2.50 ± 0.70	2 hr LL 2.60 ± 0.80	0.10 ± 0.35	0.899 p < 0.001
N20,21	24 11 I, 13 U	Tank 2.44 ± 0.69	2 hr DD 2.02 ± 0.57	-0.42 ± 0.34	0.875 p < 0.001
		2 hr DD 2.02 ± 0.57	2 hr LL 2.53 ± 0.56	0.51 ± 0.31	0.851 p < 0.001
N27,28	12 U	Tank 2.85 ± 0.76	24 hr DD 1.75 ± 0.68	-1.10 ± 0.23	0.956 p < 0.001
S6,7	11 6 I, 5 U	Tank 2.48 ± 0.43	24 hr DD 2.09 ± 0.39	-0.39 ± 0.360	0.614 p < 0.05
		24 hr DD 2.09 ± 0.39	7 hr LL 2.68 ± 0.39	0.59 ± 0.34	0.619 p < 0.05

was no significant variation between infected and uninfected gammarids, but a highly significant variation among experiments (Table V-3). The difference in amplitude was positive (indicating distal movements) for all light adapting tests (mean = 0.36, n = 59) and negative (indicating proximal movements) for all dark adapting tests (mean = -0.54, n = 59). That the significant variability between experiments was indeed due to the results of light adaptation versus dark adaptation was shown by partitioning the variance among experiments, comparing the results for those involving dark adapted versus those involving light adapted gammarids. There was a highly significant difference between the two groups of experiments, but no significant variation among experiments within groups (Table V-3).

Rate and range of accessory pigment migration

Results obtained with infected and uninfected gammarids were pooled to study the characteristics of white pigment migration. When tested every hour, the eye score of animals subjected to dark adaptation decreased slowly over time (Fig. V-3 a and b). The average amplitude of the migration after 24 hrs dark adaptation differed markedly between experiments (-1.10 in experiment N27,28, and -0.32 in experiment S6,7; Table V-2). For eyes subjected to light adaptation after 24 hours in the dark, the maximum amplitude (+ 0.59 units) was reached after 3 hours, with a plateau thereafter (S6,7; Fig. V-3c). The time-courses suggest that

Table V-3. Analysis of variance of the amplitude of migration of the accessory pigment in uninfected gammarids, and in those infected with the larval acanthocephalan Polymorphus paradoxus, with partitioning of the variance between experiments involving light (LL) and dark (DD) adaptation. Data from Table V-2.

Source	df	Sum of Squares	Mean Square	F-test	P value
Inf vs Uninf (A)	1	0.118	0.118	0.978	ns
Exps (B)	5	27.901	5.580	46.155	0.0001
LL vs DD	(1)	23.810	23.810	196.780	0.0001
within groups	(4)	4.091	1.023	0.040	ns
AB	5	0.253	0.051	0.418	ns
Error	106	12.815	0.121		

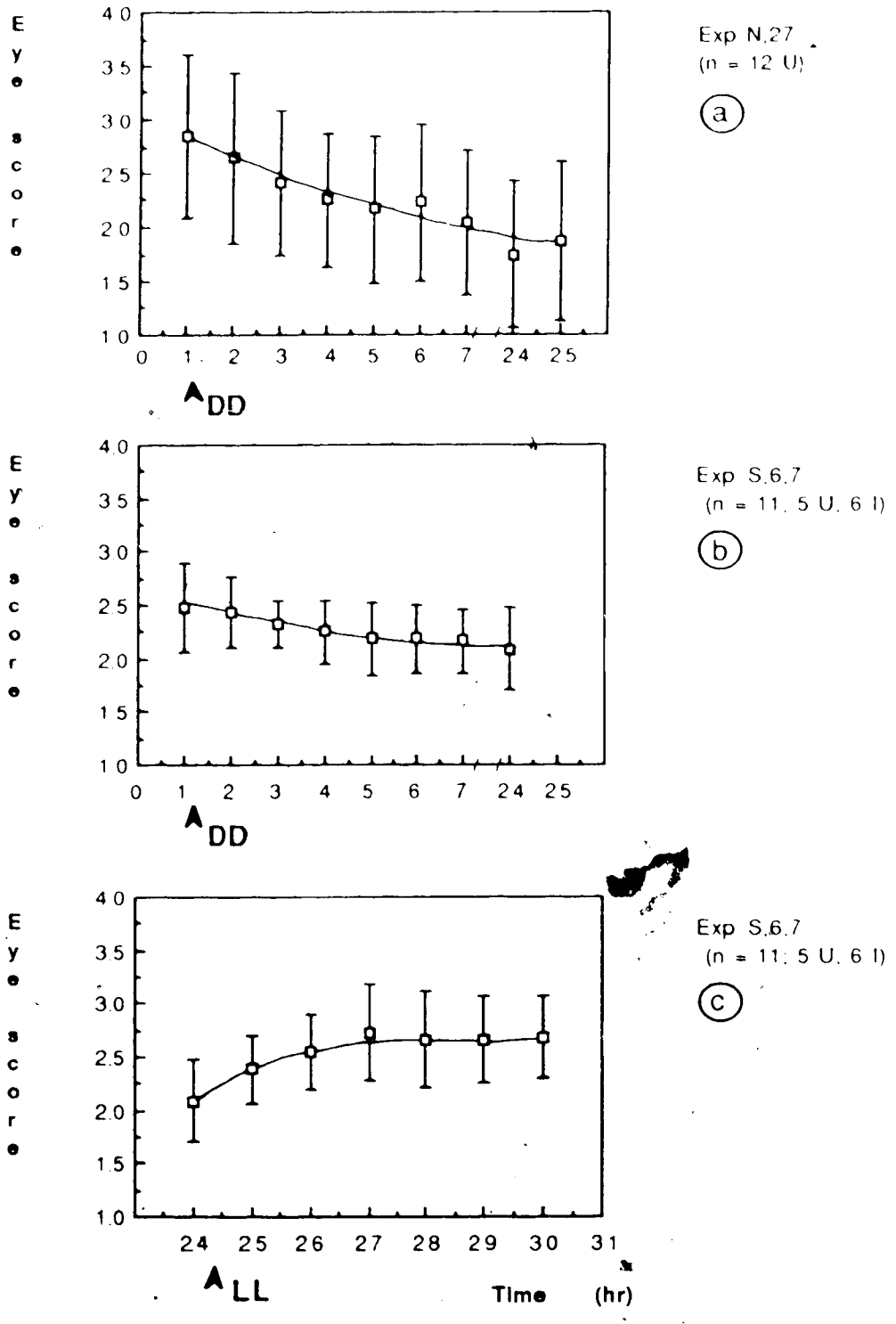


Fig V-3. Time-courses of average eye score in gammarids, during dark adaptation (DD, a and b), or light adaptation (LL, c).
Curve c follows up curve b, with changed conditions; U = uninfected gammarids, I = gammarids infected with the larval acanthocephalan Polymorphus paradoxus.

the proximal migration is slower than the distal migration. However, the two pulses of light every hour that were necessary to obtain an eye score were likely to modify the course of the white pigment migration under dark adaptation. As a matter of fact, in another experiment (N20,21; Table V-2), there was no significant difference between the amplitude of the migration proximally (- 0.417) and distally (0.510) after 2 hours conditioning without interruptions (t-test, $p < 0.05$).

As for the clinging and photic behavior, high interindividual and interexperimental variations were accompanied by a strong element of consistency within individuals. For each experiment, there was a significant correlation between individual gammarid's eye scores pre and post adaptation (Table V-2). In other words, an individual with a high light adapted eye score has a high dark adapted eye score, and an individual with a low light adapted eye score has a low dark adapted eye score. This is illustrated in Figure V-4 where the individual time-courses stay approximately parallel to each other throughout the experiment. Quite remarkable is the fact that the correlation is still present when light or dark adaptation in the sample has reached a plateau. This implies that individual eye scores vary within a given narrow range, about 1/5 or 1/10 of the potential range, and that this range is about the same in infected and uninfected gammarids but at different set points.

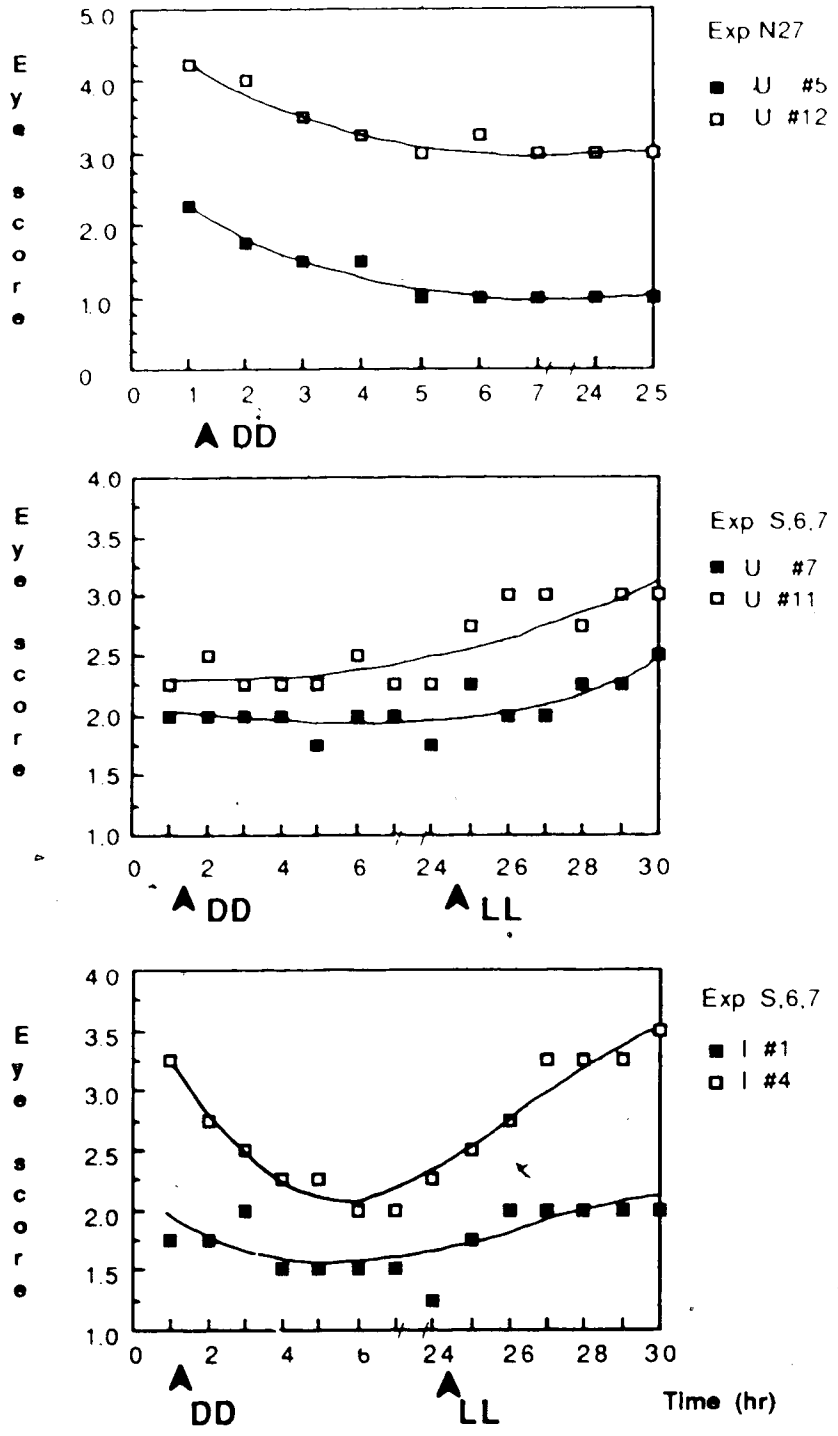


Fig V-4. Time-courses of eye score in individual gammarids, during dark adaptation (DD), or light adaptation (LL).
U #5, 12, 7, and 11 are individual uninfected gammarids, I #1, and 4 are individual gammarids infected with the larval acanthocephalan Polymorphus paradoxus.

Serotonin, accessory pigment migration and photic behavior

Serotonin is known to act as a releasing factor for various chromatophorotropic hormones controlling migration of pigments in tegumental chromatophores of crustaceans (see review in Fingerman, 1985). It is therefore plausible that the increased photopositivity induced by serotonin injected into uninfected gammarids would be mediated through distal movements of the white pigment. To test that hypothesis, 24 gammarids, 12 on each of two consecutive days, were tested for eye score, then immediately injected, half with saline, and half with 10 µg 5-HT HCl. Gammarids were then placed in individual tracks of the testing apparatus for photic behavior with a fixed light source (see Chapter II) and their position assessed every 5 minutes. Forty minutes post injection, eye scores were tested again. Serotonin injected animals were indeed more photopositive; they were present in the lighted compartment 47 times out of 66 observations (72%), while gammarids injected with saline were present in the same compartment only 8 times out of 66 observations (12%)(Table V-4). However, there was no significant difference in the amplitude of migration of the white pigment between serotonin and saline injected animals; the difference in eye score pre injection and 40 minutes post injection was 0.08 in saline injected animals, and -0.02 in serotonin injected ones (Table V-4; t-test, $p > 0.05$) There was once again a highly significant correlation between pre and post treatment individual eye scores in both control and

Table V-4. Effects of serotonin on photic behavior and pigment migration in uninfected gammarids. Pigment migration indicated by difference in Eye score (Es) before and after injection with saline or 10 µg serotonin; photic behavior indicated by number of times present in the lighted half of the testing apparatus.

	Saline	Serotonin
Sample size	12	12
Es before injection Es t0 ± s.d.	2.21 ± 0.74	2.62 ± 0.90
Es 40 min after injection Es t0+40 min ± s.d.	2.29 ± 0.87	2.60 ± 1.10
Es t0+40 - Es t0 ± s.d.	0.08 ± 0.27	- 0.02 ± 0.46
Regression (R) Es t0 vs Es t0+40 p	0.956 p < 0.001	0.915 p < 0.001
Presence in light between t0 and t0+40 # presences / # observations %	8 / 66 12	47 / 66 71

serotonin treated gammarids. The increase in photopositivity induced by serotonin injected into uninfected gammarids was not mediated through distal migration of the white pigment.

VI. DISCUSSION

A. Patterns of behavior

Holmes and Bethel (1972) and Bethel and Holmes (1973) investigated the altered behavior induced by different species of larval acanthocephalans on the behavior of amphipods. The authors discovered that several aspects of the altered behavior resulted from modified responses to environmental stimuli. *Polymorphus paradoxus* had the most dramatic effect on the behavior of its intermediate host. When mechanically disturbed, infected gammarids escaped towards a light source, towards the surface in natural conditions; skimmed the surface, sometimes as if they were caught by the surface tension; clung firmly to any suitable material; and remained immobile in a flexed posture. Uninfected animals, when disturbed, escaped away from a light source, towards the bottom, did not skim and did not cling. In addition, the habitat of infected gammarids was shifted towards zones of higher illumination.

The present study has provided some additional details about the behavior of infected and uninfected amphipods. Three recurrent trends were present in the results: 1) infected animals did not invariably show altered behavior; instead, there was a predictable decrease over time in the number responding; 2) a high interindividual variability was accompanied by elements of consistency within individuals; and 3) seasonal patterns could be linked to biotic or

abiotic factors.

1) The decrease in number of infected individuals exhibiting altered responses is well illustrated by changes in clinging behavior. After an initial mechanical stimulus, all infected gammarids clung for a few minutes to a few hours, with a high interindividual variability. If no further stimuli were delivered, they then resumed their activities. Given the appropriate repeated tactile stimulations, most infected gammarids continued to cling for up to several hours, but the proportion clinging gradually decreased. This clinging period was followed by a refractory period, during which no clinging could be elicited, then by a second clinging period, shorter than the first. These elongated periods of clinging following repeated stimulations could have an ecological significance. In a field situation, an infected gammarid clinging to some vegetation after an initial disturbance would return to normal activities relatively rapidly; however, if it was clinging to the fur of a muskrat or to the feathers of a duck, the movements of the vertebrate would probably stimulate further bouts of clinging in the infected amphipod. Eventually, the potential definitive host may groom or preen itself and ingest the infected gammarid.

The photic behavior of infected gammarids showed a slight decrease over time in photopositivity. It can be interpreted as the directional response to light subsiding and being gradually replaced by the graded response to

light. The skimming behavior was the quickest to habituate in infected gammarids. The skimming behavior is definitely part of the altered escape behavioral sequence, but appears to be best interpreted as the end product of the directional response to light, rather than as a distinct behavioral pattern. Infected gammarids escape towards the source of light and get trapped in the surface tension, while uninfected gammarids escape away from the source of light and end up digging in the mud.

2) A high interindividual variability was found in the length of the clinging response in infected gammarids. There was, however, a significant positive correlation between the length of the total clinging period and that of the refractory period, and between the length of the initial clinging period and that of the subsequent clinging period (Chapter III). The high interindividual variability in photic performance of infected and uninfected controls was accompanied by a significant positive correlation between performance before and after treatment with saline (Chapter IV). The high variability between individual position of the reflecting pigment, goes along with a significant positive correlation within individuals between eye score before and after light or dark adaptation (Chapter V).

If a method of study can reveal a coherent pattern (such as a significant correlation), it indicates that a lack of pattern (such as a high variability) revealed by that same method is genuine. Thus, the high variability

observed in the three variables cited above was an intrinsic characteristic of the population studied, and not an artefact.

The interindividual variability in the length of the clinging behavior is difficult to interpret. Differences in individual experience prior to the tests, and, therefore, in degrees of habituation, constitute one possible cause. However, the interindividual variability in photic performance (and in eye score) could participate in the dispersion of the gammarids in the water column (see section "Photobehavior and screening pigments"; present chapter).

3) There was a common seasonal pattern in the clinging performance and the position of the reflecting pigment in infected gammarids. The strength of the clinging behavior increased during the year, and was especially strong in late summer and fall. Also, the reflecting pigment was slightly more superficial in infected gammarids than in uninfected ones, but only during late summer and fall. These facts may be related to the life history of the host and parasite populations. The lesser action of the parasite on its host was associated with overwintered gammarids infected by overwintered acanthocephalans (found from April until June). The activity of the helminth was greater in overwintered gammarids infected by the spring generation of *P. paradoxus*, maturing in July, and still higher in the spring generation of gammarids infected by the spring generation of *P. paradoxus*, maturing in August. (Part of the life history of

host and parasite described above is speculative, and based on monthly observations rather than on a systematic study; see Table II-1). This explanation would require that a young cystacanth be more effective than an overwintered one (which could be due to long term habituation in the overwintered gammarid), and that a cystacanth have more effect on a smaller, juvenile gammarid than on a larger, older one.

The clinging response of infected gammarids was related to a "normal" seasonal behavior, the precopulatory or mate-guarding behavior. The way male gammarids grasp the females for extended period of times during precopulation is indeed a clinging behavior, i.e., a firm grasp with the dactylopods of the gnathopods. Therefore, clinging is part of the normal behavioral repertoire of gammarids. Gammarids in precopula were only found from January until May. During that period, the decision to cling or not to cling to the female depends on subtle cues; for example, the presence of fertilized eggs or juveniles in the brood pouch of the female inhibits precopulation (Dunham, 1986). Thus, in uninfected gammarids, the clinging behavior is normally elicited by a specific set of stimuli, under precise circumstances. However, the field observations, and the clinging tests in the laboratory, suggested that at the right time of year, very strong stimuli may sometimes elicit the clinging behavior. In infected gammarids, at any time of year, a wide range of mechanical stimuli elicits this same behavior, under the wrong circumstances.

The third seasonal factor demonstrated in the results concerned the strength of the photopositivity in uninfected gammarids. The average photic performance changed by a factor of 6 over the year and was correlated with daylength and temperature (Chapter IV), as are many biological factors. This pattern corresponds with field observations. In spring and early summer, uninfected gammarids were very conspicuous near the shore at Cooking Lake, while in late summer and fall the gammarids seemed to migrate to deeper waters. When ice formed, the gammarids were hardly noticeable near the shore, although, on the basis of seasonal population size, they should have been more numerous than in the spring.

It has been shown in this section that the clinging behavior and the high photopositivity exhibited by infected gammarids may also be exhibited by uninfected gammarids, but only as responses to very specific stimuli and/or only during certain parts of the year. Therefore, the effects induced by the parasite are part of the normal behavioral repertoire of gammarids and are produced under inappropriate circumstances in infected hosts.

B. Altered escape behavior and amines

Three components of the altered behavior induced by *P. paradoxus* in infected gammarids, higher photopositivity, skimming and clinging behaviors, were reproduced in uninfected gammarids by injecting serotonin. The time-course

of the response to 10 µg serotonin was approximately the same for the three behavioral components, lasting no more than three hours and with a peak about one hour after injection (Fig. VI-1). It will be shown in the following section of this discussion (VI-C) that serotonin appears to participate in the directional response to light, which orients the escape behavior, rather than in the graded response to light, which is responsible for the light preferendum. Skimming and clinging behaviors are also parts of the altered escape behavior exhibited by infected hosts. Therefore serotonin injection mimicked all three components of the altered escape behavior.

Effects of 10 µg serotonin in a 50 mg gammarid: specific action or artefact

The previous sections have assumed that the responses noted were specific actions of serotonin and other neuroregulators. However, ten micrograms of biogenic amine in an animal weighing less than 100 mg is a very high dose. The concentration of serotonin in the haemolymph immediately after injection was in the low 10^{-3} M range (see calculation in Chapter II-B). Cooke and Sullivan (1982), in an article on hormones and neurosecretion in crustaceans, declare that "Current experience suggests that any effect requiring more than nanomolar concentration is suspect." However, for the following reasons, the responses obtained with 10 µg biogenic amine in gammarids are not likely to be just

artefacts emanating from a nervous system overwhelmed by a neurotransmitter.

1) While serotonin elicited clinging responses in uninfected gammarids, GABA, noradrenalin, dopamine, and octopamine (5 or 10 µg) did not. In infected gammarids, three biogenic amines, dopamine, noradrenalin and octopamine (10 µg), suppressed transiently the clinging behavior. However, octopamine was more potent than noradrenaline or dopamine and had a different, consistent time-course.

2) The clinging response was <u>not</u> a continuous response after injection of serotonin, but a response to a tactile stimulation. The effects of serotonin require another event (the tactile stimulus) to be expressed, which is certainly an element of specificity.

3) Neither octopamine nor serotonin prevented the gammarids from swimming (experiments with alternating light source), and a directional response to light is not likely to be a pharmacological artefact.

4) Serotonin injected into uninfected gammarids consistently produced three different behavioral components similar to those produced by the larval acanthocephalan *P. paradoxus* in infected hosts. Thus, serotonin elicited characteristic behavioral patterns rather than atypical disorganized responses.

5) Large quantities of serotonin and octopamine may be necessary to induce any effect if the two amines are metabolized quickly in the haemolymph of *Gammarus* after

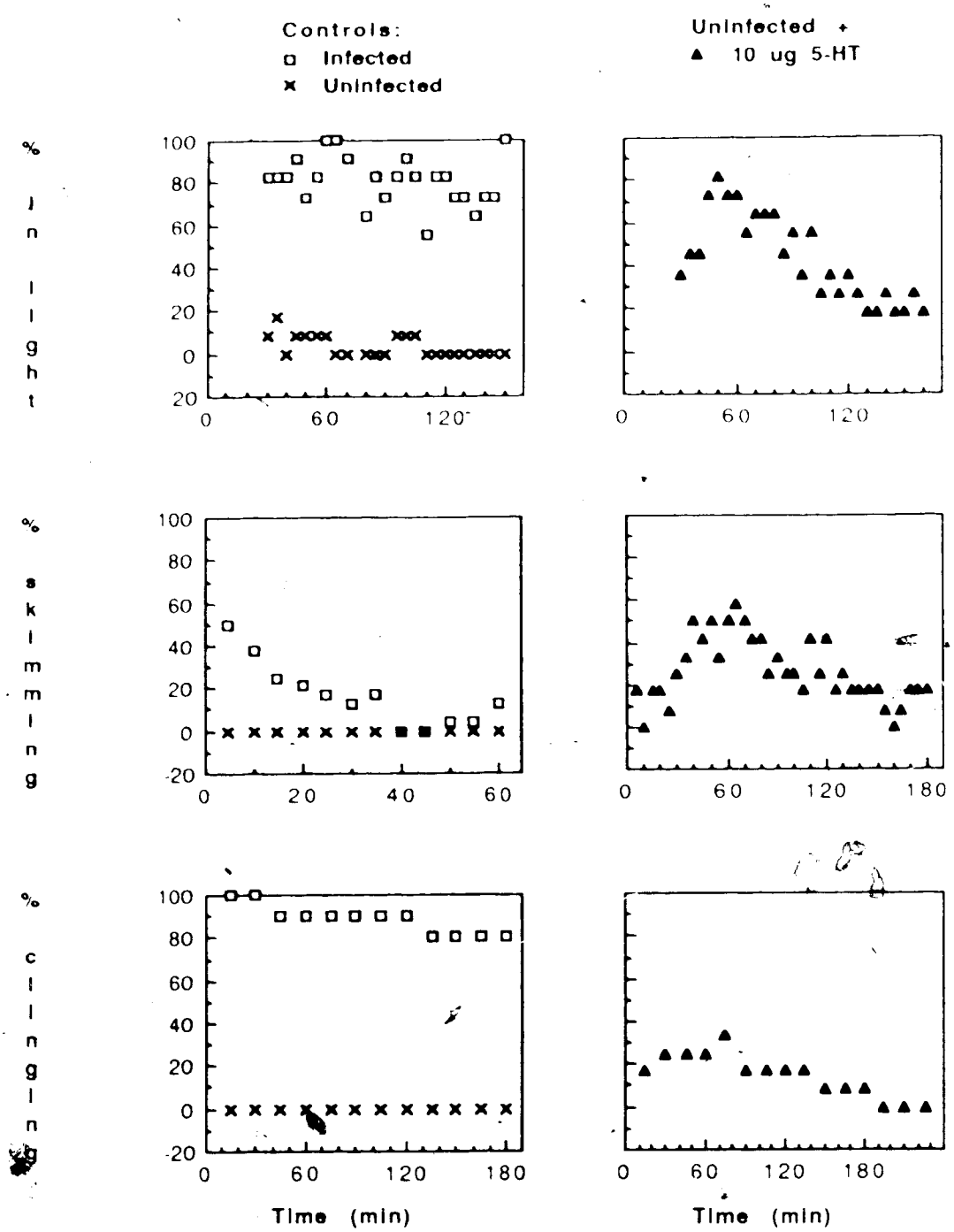


Fig VI-1. Time-courses of photic, skimming, and clinging behaviors in gammarids infected with the larval acanthocephalan Polymorphus paradoxus, in uninfected controls, and in uninfected gammarids injected with 10 µg serotonin at time 0 (see details in Chapters III and IV).

injection. Octopamine disappears rapidly from the haemolymph of insects. After a natural increase, like that produced by flight in the cockroach (Bailey et al., 1984) or after injection of high quantities in the locust (Goosey and Candy, 1982, in Bailey et al., 1984), levels of octopamine declined to near initial levels after only 5 minutes. If serotonin and octopamine act centrally in *Gammarus*, the conjunctive envelope surrounding brain and nerve cord might act as a "blood-brain" barrier, and most of the molecules of the amines could be metabolized before reaching the site of action. Possibly for these reasons, Berlind (1977) found that a pulse dose of serotonin needed to be 10 times more concentrated than a perfusion to induce a reversal of scaphognathite beating in the crab *Carcinus maenas*.

6) Furthermore, in the examples discussed in the next section, effects of serotonin on the central nervous system of decapods required concentrations in the range of 10^{-3} to 10^{-6} M (Glanzman and Krasne, 1983; Livingstone et al., 1980). The authors speculated that the physiological effects were normally achieved through the release of amines at central synapses rather than into the blood.

Mode of action

Serotonin and octopamine had opposite effects on the clinging behavior of gammarids, but not on their photic behavior, and one can infer that at least two distinct circuits are involved. Because of the more complete data

set, and more pertinent literature, the discussion in this section will focus on the clinging behavior.

Antagonistic serotonin - octopamine systems in crustaceans

The results obtained on the clinging behavior of gammarids (Chapter III) showed that 1) injected serotonin elicited clinging in uninfected gammarids, 2) injected octopamine, and to a lesser extent dopamine, noradrenalin, and the antagonist cinanserin, reduced or eliminated clinging in infected gammarids, and 3) injected extracts from cystacanths sometimes produced clinging in uninfected gammarids (although they frequently did not). These results strongly suggest that *P. paradoxus* produces a soluble chemical that elicits clinging through activating a serotonergic or serotonin sensitive pathway that acts in antagonism to an octopaminergic or octopamine sensitive pathway.

No information could be found regarding amines in amphipods. However, there are two other more extensively-studied crustacean systems in which high concentrations of octopamine and serotonin have opposite behavioral effects. Comparing and contrasting the three systems will give some indication as to the possible mechanism of action of the biogenic amines in the clinging behavior of infected gammarids. In one system, already mentioned (Chapter I), serotonin injected into crayfishes and lobsters induces a sustained flexion of the whole body while octopamine produces an opposite extended posture

(Livingstone et al., 1980; Livingstone et al., 1981; Kravitz et al., 1980; Kravitz et al., 1983; Beltz and Kravitz, 1983; Kravitz et al., 1984; Harris-Warrick and Kravitz, 1984; Beltz and Kravitz, 1986) (see details in Table VI-1). (This system will be referred to as the PO, POsture, system.) A long lasting postural action is achieved with 10 mg serotonin per 0.5 kg of lobster (Livingstone et al., 1980). The authors do not mention the blood concentration of serotonin after injection. If the haemolymph volume of the lobster is, like in *Gammarus*, approximately 25 % of its wet weight, the haemolymph volume of a 500 g animal is approximately 125 ml, and the concentration of serotonin in the lobster's haemolymph after injection of 10 mg is in the low 10^{-4} M range [$4.7 \times 10^{-5} \times (10^3 / 125)$]; see Chapter II-B).

In the other model, involving the crayfish, serotonin decreases the responsiveness of a pair of large neurons, the lateral giants (LGs), command neurons for one type of tailflip escape response; octopamine enhances the responsiveness of the LGs (Glanzman and Krasne, 1983) (see details in Table VI-2). Concentrations of 10^{-3} to 10^{-6} M are used to obtain differences in firing in the LGs. (This will be referred to as the LG system).

There are a number of similarities and differences between the three crustacean systems involving the antagonistic effects of serotonin and octopamine on behavior. In the HP system (Host-Parasite, the effects on

Table VI-1. Action of octopamine and serotonin on the lobster's postures.

In vitro, NEUROMUSCULAR JUNCTION

Both octopamine and serotonin (10^{-8}, 10^{-9} M) prime peripheral muscles (extensors and flexors) to respond more vigorously to normal stimuli;
both facilitate transmitter release in excitatory and inhibitory nerve endings;
both produce a contracture in muscle fibers and induce the appearance of Ca^{++} action potentials

In vitro, CENTRAL GANGLIA

Octopamine and serotonin (10^{-5}, 10^{-6} M) trigger the readout of opposite motor programs
Opposite actions

octopamine
increased firing in excitatory neurons to slow extensors,
decreased firing in inhibitory neurons to slow extensors,
increased firing in inhibitory neurons to slow flexors,
decreased firing in excitatory neurons to slow flexors,

serotonin
increased firing in excitatory neurons to slow flexors,
decreased firing in inhibitory neurons to slow flexors,
increased firing in inhibitory neurons to slow extensors,
decreased firing in excitatory neurons to slow extensors,

In vivo

Octopamine and serotonin induce opposite postures
(blood concentration after injection: 10^{-4}, 10^{-5} M) *

octopamine -- extended posture
serotonin ---- flexed posture

Livingstone et al., 1980
Kravitz et al., 1980

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* calculated from the authors' data.

Table VI-2. Action of octopamine and serotonin on the escape reaction of the crayfish.

A tactile stimulation on the abdomen of the crayfish elicits an abdominal flexion propelling the animal upward and forward within 0.1 second. This response is mediated by the lateral giants interneurons (LGs). The lateral giants are segmentally arranged and connect to giant motor neurons innervating fast flexor muscles.

(Wine and Krasne, 1982).

Octopamine (10^{-3}M to 10^{-6}M) enhances the lateral giants responsiveness.

Serotonin (10^{-3}M to 10^{-6}M) deppresses the lateral giants responsiveness.

(Glanzman and Krasne, 1983)

Serotonin mediates, at least in part, the crayfish' s restraint induced inhibition of the escape response: in crayfish with lowered levels of central 5-HT (obtained with the neurotoxin 5,7, DHT), the threshold for firing the LGs was lowered.

(Glanzman and Krasne, 1986)

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clinging behavior), serotonin produces a modulatory behavioral action. It elicits an effect, the clinging behavior, not by itself, but as a response to a tactile stimulus. The behavioral action of serotonin in the HP system possesses the characteristics of a modulatory synaptic action as defined by Kupfermann, 1979: "The most common features of modulatory synaptic effects are long-duration of action and contingent action. Contingent action refers to the property that modulatory transmitters often have little or no effects in themselves, but instead they alter the effects of other events." Octopamine injected gammarids did not cling, but there is no proof of a modulatory action; in addition, octopamine injected gammarids appeared more stretched than the controls. Thus, while there is evidence for a modulatory action of serotonin in the HP system, a command role of octopamine cannot be ruled out. In the LG system, both serotonin and octopamine produce modulatory actions. However, in the PO system, both subserve command roles; they elicit postures without any additional environmental stimulus.

In the PO and the HP systems, the end result of the action of octopamine and serotonin are similar: activity of extensors is obtained with octopamine, while activity of flexors is obtained with serotonin. In the LG system, the decrease in responsiveness of the Lateral Giants induced by serotonin reduces the chances of rapid abdominal flexion, leading to escape (Glanzman and Krasne, 1983). However, fast

(phasic) flexors are involved in this escape reaction, which lasts less than a second, while slow (tonic) flexors are involved in the postural action of serotonin in the lobster (and presumably in the gammarid), which lasts minutes to hours. The comments of Glanzman and Krasne (1983) on the differences between the LG and the PO systems might also apply to the difference between the LG and the HP systems: "The tonic and phasic motor systems do not necessarily function in parallel; in fact, tonic flexion is inhibited by LG-evoked phasic flexion of the abdomen (Kuwada and Wine, 1979). Hence, the contrapuntal effects of octopamine and 5-HT on the crayfish's LG escape response and on its posture, may be viewed as synergistic, and may imply that these monoamines have widespread effects on the neural circuitry mediating escape behavior." In addition, there are similarities between the stereotactic aspects of the restraint-induced inhibition of the crayfish escape behavior, mediated by serotonin (Glanzman and Krasne, 1986), and the stereotactic element in the gammarids' clinging behavior.

Level of action of serotonin and octopamine

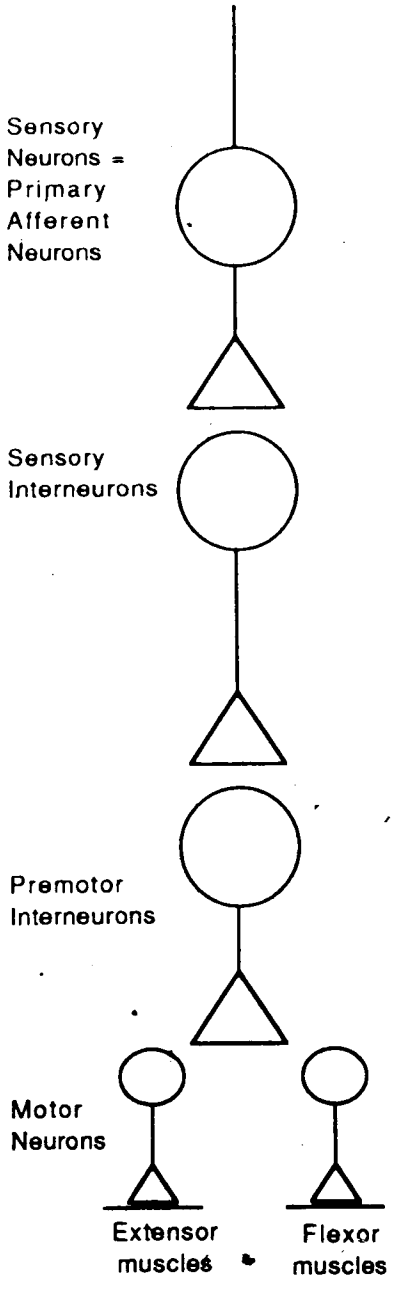
In infected gammarids, or in gammarids injected with serotonin, reflex pathways may be altered at the peripheral sensory level, at the central level, or at the peripheral effector level. I have no direct evidence, such as electrophysiological recordings, and there are no studies in the literature on the influence of serotonin and octopamine

on any reflex pathways in amphipods. However, an analogy with the PO system gives a strong argument against an action of serotonin and octopamine at the peripheral effector level in the clinging behavior. Concentrations of octopamine and serotonin in the range of 10^{-8} to 10^{-9} M have a common action at the neuromuscular junction in lobsters (Kravitz et al., 1980)(Table VI-1). Both amines prime peripheral muscles (extensors and flexors) to respond more vigorously to normal stimuli. However, the opposite postural action obtained at concentrations of amines 10,000 times superior is not peripheral. Flexion and extension, induced by serotonin and octopamine, respectively, are accompanied by increased and decreased firing in the expected motorneurons (Table VI-1).

In other invertebrate examples, serotonin influences the neural output at virtually all levels of reflex pathways involving mechanosensory receptors and muscular effectors (Fig. VI-2).

In the LG system, serotonin (10^{-3} to 10^{-6} M) may act to depress transmission to the Lateral Giant command neurons "at the synapse between sensory interneurons and the LGs or at the synapse between afferents and sensory interneurons other than A" (Glanzman and Krasne, 1983).

In the marine snail *Aplysia*, serotonin (10^{-4} M) acts at presynaptic terminals of mechanoreceptors in the sensitization of the gill-withdrawal reflex. When the tegument of *Aplysia* is stimulated at low frequency, the gill-withdrawal reflex decrements or habituates, while a



- Lobster (5-HT: threshold 10^{-10} M)
Action on sensory endings of stretch receptors
Depression of receptor potential and impulse discharge
Octopamine same effect
(Pasztor and Bush, 1987).

- Aplysia (5-HT: 10^{-4} M)
Mechanosensory terminals,
Presynaptic facilitation of transmitter release
(Kandel and Schwartz, 1982).

- Crayfish (5-HT: 10^{-3} to 10^{-6} M)
Synapse between afferent and sensory interneurons or synapse between sensory interneurons and premotor interneurons (LGs)
(Glanzman and Krasne, 1983)

- Lobster (5-HT: 10^{-5}, 10^{-6} M)
Premotor interneurons (at least in part)
Generation of coordinated motor patterns
(Harris-Warrick and Kravitz, 1984)

- Lobster, Crayfish (5-HT: 10^{-8}, 10^{-9} M)
Neuromuscular junctions
Enhancement of contraction in flexor and extensor muscles
Octopamine same effect
(Kravitz et al., 1980)

Fig. VI-2. Arbitrary reflex pathway between mechanoreceptors and muscular effectors illustrating different levels of action of serotonin (5-HT) in different invertebrate systems.

head shock causes the response to increase or sensitize. Either application of serotonin to, or direct stimulation of, a group of facilitatory interneurons (the L29 cluster in the abdominal ganglion) can simulate the effect of the head shock. It has been established that sensitization results from presynaptic facilitation of transmitter release by mechanoreceptor neurons in the abdominal ganglion (Brunelli et al., 1976; Klein et al., 1980; Klein, 1981; Klein et al., 1982; Kandel and Schwartz, 1982; Kandel et al., 1983; Kistler et al., 1985).

Finally, in the lobster, Pasztor and Bush (1987), looked at "non-synaptic modulation of membrane potentials in peripheral sensory endings". The authors used a stretch receptor, the oval organ, "which provides feedback from the ventilatory appendage to the nervous system". Serotonin, with a threshold of 10^{-10} M depresses receptor potential and impulse discharges in the dendrites of the sensory cells. Octopamine has the same effect.

The preceding studies suggest that peripheral effects of serotonin and octopamine are similar, and are induced by low concentrations of the amines. Because of the high concentrations of serotonin in the haemolymph required to obtain a significant effect (10^{-4} M), and because of the opposite effects of serotonin and octopamine in the host-parasite system, a non peripheral action of the amines is likely to be involved. However, by analogy with the regulation of the feeding behavior in the leech (Lent and

Dickinson, 1984; Lent, 1985), serotonin might also integrate, at different levels, the whole altered escape behavior of infected hosts. "The serotonergic neurons within the leech nervous system integrate feeding behavior by their selective modulation of certain central neurons and by their direct, powerful activation of several peripheral effectors." (Lent, 1985). In that system, serotonin functions as a neurotransmitter in some roles and as a neuromodulator in others. The modulatory role, already parallels that in the host-parasite system. In a hungry leech, and in a hungry leech only, a vibrational stimulus evokes the swimming phase of the feeding behavior, and a thermal stimulus induces biting. Thus, an animal in a definite physiological state associated with higher levels of serotonin exhibits distinct behavioral patterns evoked by transient stimuli. As Lent and Dickinson (1984) put it: "The organization of a specific recognizable behavior by serotonin is reminiscent of some of the varied mechanisms by which hormones are able to establish the physiological conditions required for more general behavioral states". Indeed, the control of the feeding behavior of the leech and that of the altered escape behavior of infected gammarids seems related to the modifier effects of hormones described by Truman and Riddiford (1977) (see Chapter I).

Actual involvement of serotonin in the host-parasite system

Serotonin does reproduce qualitatively several components of the altered behavior induced by the acanthocephalan *P. paradoxus* in its host. Thus, the acanthocephalan probably affects serotonergic or serotonin sensitive pathways in its host. However, there is as yet no proof of the actual involvement of serotonin or serotonergic pathways. Injected serotonin could in fact mimic another substance. It is known for example that 5-HT sometimes reproduces the action of neurohormones in insects (Raabe, 1982). Also, in the gill-withdrawal reflex of *Aplysia*, serotonin reproduces the effects of the stimulation of the L29 cluster of interneurons; however, it has been shown by immunocytochemistry that these interneurons are not serotonergic (Kistler et al., 1985). Furthermore, the parasite may influence some inhibitory pathways acting in parallel with serotonin sensitive pathways. For example, dopamine did not elicit clinging in uninfected gammarids, but did suppress this response in infected animals (Chapter III). There was some indication that dopamine might also reduce the photopositivity in infected gammarids (Chapter IV). In uninfected animals, with normal levels of dopamine, the clinging response and the escape towards the light source would be inhibited, whereas, in infected gammarids, with lower levels of dopamine, the inhibition would be removed. All in all though, the data presented in this thesis and the literature suggest a genuine participation of

serotonin, but as an intermediate biochemical step, and not as a substance likely to be released in sufficient quantity at the host-parasite interface.

Although serotonin was not mentioned in a study concerning the biogenic amines of another adult acanthocephalan *Moniliformis dubius* (Budziakowski et al., 1983), serotonin has been found in acanthocephalans. The Falck Hillarp histochemical fluorescence technique revealed 11 neurons with serotonergic properties out of 86 neurons in the cerebral ganglion of adult *Macracanthorhynchus hirudinaceus* (Wang, 1976). [1] It is therefore conceivable that cerebral neurons of *P. paradoxus* cystacanths are capable of synthesizing serotonin and releasing it through the tegument. However, it is unlikely that the high concentrations required to induce the clinging behavior and the increased photopositivity in uninfected gammarids would be reached. The smallest quantity of serotonin injected was 1 µg, which induced only a feeble clinging response. One microgram of serotonin gives a concentration of 10^{-5} - 10^{-4} M in the haemolymph, whereas circulating serotonin levels determined in the lobster are in the 10^{-9} M range

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[1] The cerebral ganglion of acanthocephalans is located in the proboscis sac, the muscular envelop into which the proboscis can be retracted (and is in larval acanthocephalans). This cerebral ganglion consisted of 78 cells in both adults and cystacanths of *P. paradoxus* (unpublished data).

(Livingstone et al., 1980). If serotonin blood levels are similar in gammarids to those in lobsters, then, the parasite would have to increase serotonin blood concentration by a factor of 10,000, which seems highly unlikely. If serotonin is indeed involved, it is more probably released at central synapses rather than in the haemolymph by the parasite. In the two other crustacean systems in which large quantities of serotonin and octopamine have opposite behavioral effects, the authors do not raise doubts as to the actual involvement of the two amines; they do suggest that, in physiological conditions, octopamine and serotonin are released at central synaptic sites (Beltz and Kravitz 1983; Glanzman and Krasne, 1983).

A number of simple experiments can be performed to test whether or not serotonin is actually involved in the system *G. lacustris* / *P. paradoxus* and in other host-parasite pairs. High pressure liquid chromatography (HPLC) should be used to compare serotonin levels in the haemolymph of uninfected gammarids and in that of *P. paradoxus* infected gammarids. More promising, according to the previous assumptions, serotonin-like immunoreactivity should be studied comparatively in the nervous system of uninfected gammarids and those infected by *P. paradoxus*. This experiment could lead to even more fruitful results in another system. The metacercariae of the trematode *Microphallus papillorobustus* are located mostly in the forebrain in *Gammarus insensibilis* and *Gammarus aequicauda*

(Helluy, 1982), and modify the gammarids' responses to disturbance, eliciting a "startle" response (but no clinging) (see Chapter I). Looking for serotonin-like immunoreactivity in vibratome sections of the brain of gammarids with and without the parasite in situ, could reveal differences in the intensity of staining (as in Glanzman and Krasne, 1986), and/or in the shape or number of processes stained. Also, one could attempt to suppress serotonin activity in infected hosts to "cure" them. 5,7-dihydroxytryptamine is a neurotoxin causing the degeneration of serotonergic neurons (see references in Glanzman and Krasne, 1986). Injecting 5,7 DHT into infected *G. lacustris* was tried during the course of my studies in an ill-prepared and inconclusive experiment that deserves to be repeated. Two experiments I did perform, also deserve to be repeated. Single injection of 10 µg PCPA (p-chlorophenylalanine), an inhibitor of the synthesis of serotonin, did not influence the long term clinging performance of infected gammarids. These negative results could be due to low sample sizes, or to dosage related reasons. The results obtained with cinanserin (SQ 10,643), an antagonist of serotonin, are more promising. Cinanserin (5 to 20 µg) decreased significantly and in a dose-dependent manner the clinging performance in infected gammarids. However, the absence of a clear pattern in the time-courses is more suggestive of a general pathological effect of the drug than of a specific action on the clinging behavior.

Serotonin is a well documented neurotransmitter of cestodes (Gustafsson, 1985), which are able to synthesize this biogenic amine from tryptophan and 5-hydroxytryptophan (Ribeiro and Webb, 1983), as well as absorbing it in large quantities from the host intestine (Cyr et al., 1983). There is another report of parasitism being associated with elevated levels of serotonin. Serotonin contents were higher in the intestine of rats infected by *Hymenolepis diminuta* than in the intestine of uninfected controls (Cho and Mettrick, 1982). In addition, a circadian periodicity in 5-HT levels in intestinal mucosa and lumen, in blood, and in *H. diminuta* tissues was demonstrated. Cho and Mettrick (1982) suggested that the increased levels of 5-HT in the intestinal lumen was due to an increased release of the amine from the enterochromaffin cells of the intestinal mucosa. Another explanation involves the intestinal bacterial flora in parasitized guts. Luminal bacteria release monoamine oxidases, which decrease luminal 5-HT levels through catabolism (Mettrick, 1982). Thus, the increased serotonin level could result from decreased degradation rather than from increased production. The conclusions to draw from the comparison of a system involving an adult cestode in the intestine of a vertebrate, and a system involving a larval acanthocephalan in the body cavity of an invertebrate, are necessarily limited. Nevertheless, in both cases, an helminth is associated with elevated levels of serotonin in its environment. Also, the

example of the cestode suggests that the larval acanthocephalan could act on the nervous system of its host by preventing the degradation of serotonin rather than activating its release.

It has been argued that, if serotonin is indeed involved in the system *P. paradoxus / G. lacustris*, it is more likely to be released at central synapses in the host than in the haemolymph by the parasite. The question of how *P. paradoxus* may alter the serotonergic activity in the CNS of gammarids is left open. A priori, any kind of molecule could be released by the parasite. "The evolution of allelochemic agents must depend on a balance between metabolic cost and natural selection" (Whittaker and Feeny, 1971). The ecological advantage of the altered host behavior for the larval *P. paradoxus* seems striking, and natural selection could have favored the production of an "expensive" molecule like a protein. A cestode larva (sparganum of *Spirometra*) found in vertebrates is known to release, at the host-parasite interface, a protein of a molecular weight circa 50,000 that has the properties of mammalian growth hormone (Mueller, 1974).

C. Photobehavior and screening pigments

The results obtained on the photic behavior of gammarids and on their photomechanical characteristics (Chapter IV and V respectively) showed that 1) injected serotonin increased the photopositivity of uninfected

gammarids, 2) injected octopamine had no effect on the photopositivity of infected gammarids, 3) the reflecting pigment was, on average, in a slightly more superficial position in infected than in uninfected gammarids; 4) in individual gammarids, the migration of the white pigment covered only a fraction of the potential range of migration, and that fraction had approximately the same amplitude but a different set point in different individuals, and 5) the photopositivity induced in uninfected gammarids by injected serotonin was not accompanied by a migration of the reflecting pigment.

These seemingly contradictory results can be integrated by the following set of assumptions. There are two distinct components in the photic behavior of *G. lacustris*: a directional response to the position of the light source, and a graded response to light intensity. The directional response, modulatory in nature, is part of (and orients) the escape behavior; the graded response, non modulatory, is equivalent to the light preferendum and contributes to the positioning of the gammarids in the water column. The light preferendum is mediated at least in part through the position of the reflecting pigment. The results indicate that injected serotonin affected the directional response to light; however, at least in the short term, serotonin did not affect the position of the reflecting pigment, therefore probably had no effect on the light preferendum of the gammarid. The plausibility of these assumptions is examined

in the following discussion.

Dual nature of responses to light

The distinction between responses of animals to direction or gradient of a stimulus is included in the definition of a "Taxis" by Burr (1984). "Taxis. Migration oriented with respect to the stimulus direction or gradient (Note 8) which is established and maintained by direct turns..." In note 8, Burr points out that graded and directional responses are indeed distinct, but that the distinction has not always been acknowledged. However, the difference is clear in a field study on the larvae of the crab *Rhithropanopeus harrisii* (Forward, 1985). The distinction is between long term positioning along a gradient of light intensity, and rapid orientation upon a sudden stimulus. During the day, the larvae are associated with an isolume. At night, a slow decrease in light intensity initiates an upward migration. However, a rapid decrease in light intensity triggers a shadow response - a reflex to avoid predators - and the larvae descend in the water column.

Bethel and Holmes (1973) explored two types of responses to light in *Gammarus lacustris* infected by different larval acanthocephalans: 1) upon disturbance, the orientation of the escape with respect to light; and 2) the choice of zone in an aquarium offering a darkened and a lighted zone. Uninfected gammarids swam away from the light

source upon disturbance and most of the time were present in the darkened zone. *Polymorphus paradoxus* infected gammarids swam towards the light source upon disturbance and were present mostly in the lighted zone. *Polymorphus marilis* infected gammarids swam away from the light source when disturbed, but were present mostly in the lighted zone. The response to disturbance can be interpreted as a response to the direction of light, and the choice of zone of illumination as a response to a gradient of light intensity. It is remarkable that *P. paradoxus* altered both directional and graded responses to light in gammarids, whereas *P. marilis* altered the graded response without affecting the directional response.

Which type of phototaxis was measured in this study? Pardi and Papi (1961) mention that, in laboratory situations, light fields often have both directionality and gradient and that it is difficult to assess whether experimental animals are responding to one or to the other. The setting used to study the photic behavior of gammarids (Chapter IV) did have both directionality and gradient. (In experiments with a fixed light source, the tray offered a gradient of light intensity covering 3 log units from approximately 10^{-2} foot-candles (10^{-1} lux) to 10 foot-candles (10^{2} lux).) It did not clearly distinguish between directional and graded responses.

Polymorphus paradoxus infected *Gammarus lacustris* appear to be more sensitive to disturbance than their

uninfected counterparts (Bethel and Holmes, 1973). In experiments with a fixed light source (Chapter IV), a plexiglass partition was fitted down in the middle of the tray, for a brief moment every 10 minutes, to count the gammarids in the lighted part of their track. This slight disturbance could have triggered the escape reaction of infected gammarids and thus their directional response to light, leaving the uninfected gammarids undisturbed. The latter thus would have selected their light preferendum in the tray. Also, because the clinging behavior can last up to four hours after an initial disturbance, it is reasonable to suspect that the response of swimming towards the origin of light after disturbance may also last for extended periods of time. Thus, it is likely that, in the testing apparatus, the responses to light of infected gammarids were largely directional, habituating over time, and those of uninfected gammarids essentially graded.

Light preferendum and screening pigments

The idea that light responses in crustaceans are determined at least in part by the movements of screening pigments is not new, as attested by the title of a 1905 article by Smith: "The effect of pigment migration on the phototropism of *Gammarus annulatus* S.I. Smith". Although Smith studied the photobehavior of gammarids in relation to the position of the retinal pigment in histological preparation, it is the position of the accessory screening

pigment that is more likely to be associated with the light preferendum in gammarids.

There are only two sets of screening pigments in amphipods but three sets in the better known decapods (Autrum, 1981; Shaw and Stowe, 1982). The screening pigment present in the visual retinular cells is called the proximal, retinal, retinular, or dark pigment. The white pigment in the accessory cells is referred to as the "tapetum", white, or reflecting pigment (Henkes, 1952). Amphipods apparently lack the second accessory pigment found in decapods, the distal, dark, also called "iris", pigment. In this discussion, I will use "retinular" ("retinal"), "reflecting" and "distal" respectively, with "accessory screening pigments" used as a generic term for the last two.

The dark and light adapted positions of the retinular pigment in *G. lacustris* are very similar to those found in *Gammarus ornatus* (Parker, 1905; Smith, 1905), *Gammarus locusta*, *Talorchestia longicornis* (Bennitt, 1924) and *Gammarus oceanicus* (Ali and Steele, 1961), [2] but slightly differ from the situation in *Gammarus pulex* and *Echinogammarus berilloni* (Debaisieux, 1944). In the last two species, the black pigment granules were less dense around the rhabdom in the dark adapted eye than in the light adapted eye, but the median part of the rhabdom was never

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[2] According to Ali and Steele (1961), the *Gammarus ornatus* of Parker (1899) and Smith (1905) is the *Gammarus oceanicus* of their own study, due to synonymy.

totally exposed. The bidirectional, divisive migration of the black retinular pigment granules, found in *G. lacustris*, is generally not present in decapods except in the crab *Libinia* (Eguchi and Waterman, 1967). Neither Parker (1899), Smith (1905), Bennitt (1924), nor Ali and Steele (1961) mentioned changes in the position of the reflecting pigment or in the shape of the crystalline cones in gammarids. In that respect, however, the situation in *G. lacustris* closely resembles the one described in *E. berilloni* by Debaisieux (1944). The subcuticular position of the light adapted reflecting pigment is unusual in crustaceans but was found in the horseshoe crab *Limulus* (Fahrenbach, 1975). Generally, in decapods, the reflecting pigment migrates under the basement membrane during light adaptation.

The physiologies of the retinular and the accessory screening pigments are very different in the time-course of migration, the light intensity that triggers migration, and the controlling mechanisms. The difference with respect to time course and light intensity triggering migration in the crayfish are perfectly summarized by Aréchiga (1977:394). "The proximal [retinular] pigment operates with a low threshold and short time-course, enabling the visual system to work at low levels of intensity. The distal pigment migration in turn shows a high threshold and a slow time-course as though its normal function is that of permitting the visual system to be desaturated at high intensities of illumination by bringing the amount of light

admitted in the rhabdom within the operation range of the retinal photoreceptors". (The reflecting pigment does not move significantly in the crayfish, but does undergo migrations in other decapods.) Similarly, in gammarids, the retinular pigment is the fast acting pigment, migrating from its divided dark adapted position towards the light adapted position in a matter of minutes and at very low light intensities, 0.0012 meter-candles (about 10^{-3} lux) in *G. locusta* (Bennitt, 1924), and 10^{-3} to 10^{-4} foot-candles (10^{-2} to 10^{-3}) lux in *G. oceanicus* (Ali and Steele, 1961). The reflecting pigment appears to function as the slow acting pigment. Although, to my knowledge, the factors affecting the migration of the reflecting pigment in gammarids have not been documented in the literature, the results in Chapter V show that the migration of the accessory screening pigment from a dark to a light adapted position took a matter of hours.

Mechanisms controlling the movements of screening pigments belong to three categories: the cells containing screening pigments may be 1) independent effectors responding directly to changes in ambient light intensity, 2) under neural control, or 3) under hormonal control with either an endogenous, or an exogenous, determinism. Mechanisms controlling the movements of the retinular pigment are of type 1) or 2), those controlling the movements of accessory screening pigments of type 3).

In the crayfish "retinal photoreceptors are intrinsically independent pigmentary effectors where the migrations can occur as a direct response to the presence or absence of light" (Frixione et al., 1979). However, neural control of the migration of the retinal pigment has also been reported in some species (Autrum, 1981).

Turning to the accessory screening pigments, the migration of the distal pigment in decapods is under control of at least one hormone, an octodecapeptide, the Light Adapting Hormone [(LAH), also called light adapting Distal Retinal Pigment Hormone (DRPH)]. This hormone also disperses black, red and white pigments in the tegumental chromatophores of crustaceans. The sinus gland appears to be the major source of LAH (see reviews in Cooke and Sullivan, 1982; Keller, 1983; Rao, 1985). More than one hormone may be involved. A dark adapting DRPH has been separated from the light adapting DRPH in *Palaemonetes* sp. (Fingerman et al., 1971). Movements of the reflecting pigment, although less studied, are also under hormonal control (Kleinholz, 1985). In isopods, extracts of sinus glands and supraesophageal ganglia produce dark adaptation of the reflecting pigment (Fingerman and Oguro, 1963).

Furthermore, a range of concentration of eyestalk extracts containing LAH, when injected into *Palaemon* spp., produces a graded response in the distal pigment, similar to that produced by a range of light intensities (Kleinholz, 1938; Kleinholz and Knowles, 1938). Rodríguez-Sosa and

Aréchiga (1982) showed that the distal pigment in the crayfish modulates light responsiveness over 1 log unit of light intensity, at levels at which the proximal retinal pigment is fully light adapted. In Cooking Lake for example, light intensity drops by approximately 1 log unit at 2 meters depth according to the formula of Riley (1957)[3].

Based on the above, it is reasonable to conclude that, in *G. lacustris*, the retinular pigment is responsible for short term, rapid responses to sudden changes in light intensity. The reflecting pigment is responsible for longer term, slower adjustments to general levels of ambient light, and may respond in a graded fashion to the length of dark adaptation, the light intensity (over about 1 log unit), or the concentration of eyestalk extract and Light Adapting Hormone (Fig. VI-3).

Although the influence of different factors in the migration of screening pigments has been studied extensively, there are, to my knowledge, no studies that relate the light preferendum of an animal with the position of its screening pigment. It seems logical that the relationship between accessory screening pigment position

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[3] $I_z = I_0 (1 - e^{-Kz}) / (Kz)$ with K = attenuation coefficient, z = depth (in meters), I_0 = light intensity reaching the surface, I_z = light intensity reaching depth z. In Cooking Lake the average attenuation coefficient is 4.7 (Dave Trew, personal communication) and I_2 is about $100 / (4.7 \times 2) = 10.64$.

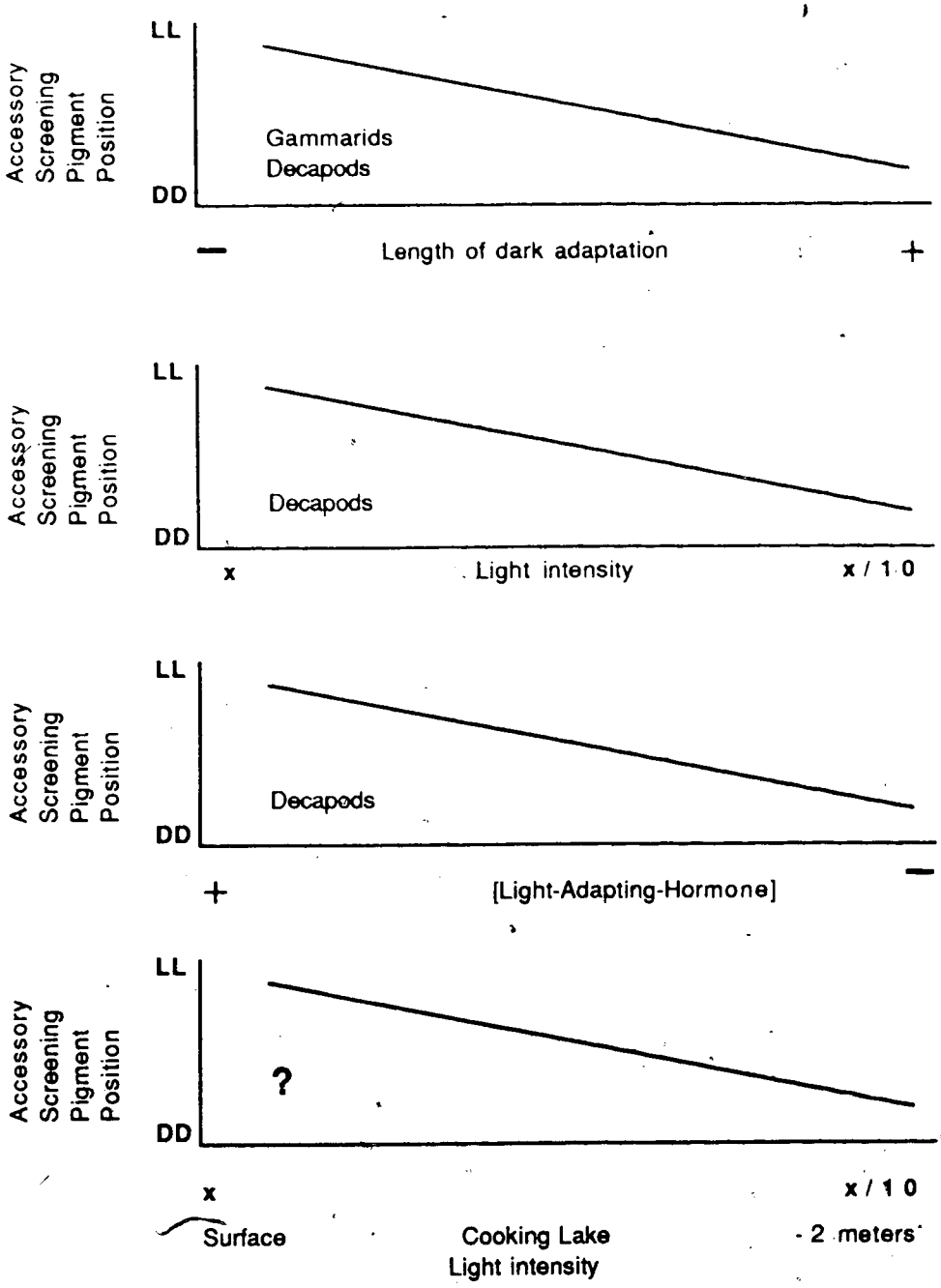

Fig. VI-3. Influence of different factors on accessory screening pigment position in the lateral eyes of crustaceans (see explanation in text).

and light intensity (Fig. VI-3) should be true regardless of whether the former or the latter is the dependent variable. Thus, in an environment offering a light gradient (as a natural aquatic environment does), an animal should dwell at the optimal light intensity defined by the position of its accessory screening pigment.

Let us then assume that indeed there is a correlation between accessory pigment position and light preferendum. The next question is a question of causality. Is the position of the screening pigment a consequence of the light preferendum, or is the light preferendum a consequence of the position of the pigment? If the determinism of the position of the pigment can be shown to be endogenous, at least in part, then one can reasonably assume that the light preferendum is a consequence of the position of the pigment, at least in part. Both endogenous and exogenous hormonal mediated control of pigment migration have been found in crustaceans. In some species, migration of pigment depends entirely on endogenous rhythms, which persist in constant illumination or constant darkness; in other species, migrations of pigment are responsive to ambient light intensity (see review in Autrum, 1981). In the former, the light preferendum is likely to be a function of the endogenous rhythm, in the latter, pigment position is likely to be a function of the light preferendum.

In *G. lacustris*, migration of the reflecting pigment towards a dark adapted position can be triggered during

daytime (Chapter V). This implies that the reflecting pigment can migrate as a result of variations in ambient light intensity. However, the range of migration in an individual covers only a fraction of the total range seen in different individuals. The data are consistent with the hypothesis that migration triggered by exogenous factors is superimposed on an endogenous set point, with both exogenous and endogenous determinisms under hormonal control. Circulating levels of light and/or dark adapting hormone would determine a given position of the accessory screening pigment. This circulating level would vary from individual to individual and thus be responsible for individual variations in light preferendum. Seasonal rhythms of circulating levels of light and dark adapting hormones could also control seasonal variations in pigment position and thus mediate seasonal variations in light preferendum. However, some light and dark adapting hormone - approximately the same amount in each individual - is apparently stored in a readily available form, the release of which is apparently controlled by exogenous factors.

In such a system, the parasite could alter the set point, the basic level of circulating hormone, but not the amount or release of stored hormone. This hypothesis is consistent with the observations that infected gammarids have, on average, whiter eyes, and that the responses of their white pigment to variations in ambient light intensities are normal.

A certain number of predictions should be verified if these conclusions are valid. In a long apparatus offering a precise gradient of light intensities, a correlation should be found between eye score and light preferendum in G. *lacustris*. Gammarids represent the perfect material to perform this kind of experiment. They are probably the only animals known to present concurrently two convenient characteristics. The position of their accessory screening pigment can be assessed quantitatively by simple external examination of the eye, and their photobehavior can be tested easily because of their small size.

Uninfected G. *lacustris* in summer are quite photophilic (Chapter IV), and infected and uninfected animals do not dwell in widely separated habitats. Thus, a large difference in average eye score between uninfected and infected G. *lacustris* cannot be expected. In another system, a larger difference should be found. In brackish water in the south of France, *Gammarus insensibilis* has a bimodal distribution with a peak in the upper 10 cm of the water column, and another one below 110 cm (Janssen et al., 1979). Helluy (1983b) suggested that the superficial position of the gammarids was probably due to the extremely prevalent cerebral metacercaria of the trematode *Microphallus papillorobustus*, which alters the photic behavior of its host. In this system, a large difference in average eye score between infected and uninfected animals would be anticipated.

Light sensitivity in the eyes of invertebrates is controlled for a large part by photomechanical changes (Autrum, 1981). The graded position adopted by the accessory screening pigment may be the explanation in yet another host-parasite system. The chaetognaths belonging to the species *Sagitta friderici* infected by a larval hemiurid (Trematoda) were found, on average, about 5 meters above uninfected animals, whether these uninfected animals were present at average depths of 17 or 25 meters (Pearre, 1979). Light is known to be an important cue in chaetognaths' behavior.

The responses induced by injected serotonin in uninfected gammarids did not include a migration of the accessory screening pigment (Chapter V). However, serotonin did increase photopositivity in uninfected gammarids, by overriding individual pretreatment photic patterns. This overriding, rather than additive, action of serotonin on the photic performance is consistent with the interpretation that serotonin does not influence the light preferendum, but does influence the directional response to light.

Nevertheless, the potential long term influence of serotonin on the position of the accessory screening pigment cannot be completely discarded, because serotonin has been linked in many circumstances to the control of pigment movements. Serotonin is involved in producing exocytosis in the sinus gland of a crayfish (Strolenberg and Van Herp, 1977) and of an isopod (Martin, 1978). The sinus gland is

known to store hormones controlling pigment movements (Keller, 1983). Serotonin elicits erythrophore pigment dispersion by stimulating the release of RPDH (red pigment dispersing hormone) in the crab. The amine induced color changes are probably mediated by the neuroendocrine system since serotonin induces the pigmentary changes in vivo and in vitro but not in isolated legs in vitro (see review in Rao, 1985). In addition, there is evidence that norepinephrine stimulates the release of light adapting DRPH in the crab *Uca pugilator*, while dopamine stimulates the release of dark adapting DRPH (Kulkarni and Fingerman, 1986). In some isopod / helminth systems, the pigmentation of the host is altered (see review in Oetinger and Nickol, 1981), and it would be interesting to test serotonin in an isopod / helminth system. (Unlike isopods *G. lacustris* does not possess chromatophores.)

VII. CONCLUSION

More than a dozen cases are reported where a larval helminth alters the behavior of its invertebrate intermediate host. In many of these cases, the altered behavior consists of modified responses to environmental stimuli (Holmes and Bethel, 1972). Experimentally, the altered behavior of the intermediate host makes it more vulnerable to predation by the definitive host of the parasite, and thus, probably, represents an adaptation to enhance the transmission of the worm. Case studies and ecological aspects of the "altered behavior" have been covered in a number of papers (see reviews in Holmes, 1976; Helluy, 1983; Moore, 1984). However, one question is not, to my knowledge, documented in the literature: what are the mechanisms by which the parasites modify the behavior of their intermediate hosts? This question underlies the whole thesis, and was addressed using a host-parasite system common in Alberta, the acanthocephalan *Polymorphus paradoxus* Connell and Corner, 1957 in its crustacean intermediate host *Gammarus lacustris* Sars, 1864. In infected gammarids the entire escape behavioral sequence is modified. When disturbed, infected gammarids swim towards a light source (in natural conditions towards the surface), skim the surface, cling to any suitable material, and remain immobile in a flexed posture. Uninfected gammarids escape away from the surface and hide in the mud. In addition, the habitat of infected gammarids is shifted toward zones of higher

illumination (Bethel and Holmes, 1973).

Two principal lines of research were followed. The first one emerged from an analogy: the flexed posture induced in lobsters after injection of the biogenic amine serotonin (5-hydroxytryptamine, 5-HT) was similar to the flexed posture of clinging infected gammarids. In lobsters also, octopamine produced an opposite extended posture (Livingstone et al., 1980). Thus, the effects of different amines on the behavior of gammarids was tested. The second line of research was based on the assumption that, if the responses to light in infected hosts are so deeply modified, their photoreceptors might be altered in some perceptible way.

To study the clinging behavior, the gammarids were placed in petri dishes lined with cloth, and subjected to a tactile stimulation. Whether or not the gammarids grasped the tissue fibers with the claws of the two first pairs of thoracic legs in a typical clinging response was noted. For the photic behavior, the gammarids were allowed to swim in individual tracks of a long plexiglass tray provided with one lamp (fixed light source), or two lamps (alternating light source). In experiments with fixed light source, the presence or absence of the gammarids in the lighted half of the tray was recorded every 5 minutes. The average number of times the gammarids were present in the lighted half in one hour was calculated. Also, the regression of the time-course of the response in gammarid samples was analysed. In

experiments with alternate light source, the lamps were switched on alternately at each end of the tray every 10 minutes; the distance covered by the gammarids away from or towards the light source was determined.

With these methods, some additional details were obtained on the behavior of infected and uninfected gammarids. Given the appropriate repeated stimulations, infected gammarids clung from a few minutes to several hours with a high interindividual variability. The clinging period was followed by a refractory period, the length of which was positively correlated with the length of the clinging period. Also, the similarities between the clinging response of infected gammarids and the way male gammarids grasp the females for extended period of times during precopulation were documented. This suggested that clinging is part of the normal behavioral repertoire of gammarids, but that it is elicited in the wrong circumstances and as a response to the wrong stimuli in infected gammarids. The photic behavior of uninfected gammarids, studied as controls, showed a seasonal pattern that was positively correlated with duration of day light and lake water temperature.

Serotonin (1, 10, 20 µg) injected into uninfected gammarids elicited qualitatively the clinging behavior typical of infected gammarids. However, serotonin did not reproduce the quantitative effects of the parasite. Not all serotonin injected gammarids responded by clinging, and those that did, responded significantly fewer times than did

infected gammarids. The clinging response of serotonin injected gammarids lasted only from a few seconds to a few minutes. Control uninfected gammarids hardly ever clung to the cheese cloth. Infected gammarids, and uninfected ones injected with 10 µg serotonin, showed a significantly higher photopositivity and a more predictable photic behavior than uninfected controls. Injected serotonin (10 µg) also elicited the skimming behavior in uninfected animals. The time-course of the effect of 10 ug serotonin was similar for the three behavioral patterns, extending over two to three hours with a peak about one hour post treatment. Octopamine (5, 10 µg), antagonized transiently (several hours) in a dose dependent manner the clinging behavior in infected gammarids, but did not affect their photic behavior. Dopamine and noradrenalin had lesser antagonistic effects on clinging.

Histological examination of the compound eyes of infected and uninfected gammarids, after light and dark adaptation, did not reveal any qualitative difference linked to the presence of the parasite. The position of the accessory white screening pigment was studied quantitatively by external examination of the eyes of living or freshly killed gammarids under a dissecting microscope. Arbitrary units were used with a scale from 1 to 5, 1 representing the darkest eye, the most dark adapted, and 5 the whitest eye, the most light adapted. The position of the accessory screening pigment was highly variable in both infected and

uninfected animals, but on average, this pigment was significantly more light adapted in the eyes of infected gammarids than in those of uninfected gammarids. The migration of the accessory screening pigment was studied during light and dark adaptation. In each individual, the range of migration of the pigment covered only a fraction of the potential range; the amplitude of the migration was similar in different individuals, but the set point was different. This was true for both infected and uninfected gammarids. However, the increased in photopositivity induced by serotonin in uninfected gammarids was not accompanied by a migration of the accessory screening pigment towards a more light adapted position.

The apparently contradictory results of the experiments on photic behavior and the screening pigment studies suggest that there are two distinct components in the photic behavior of gammarids, a directional response to the position of the light source, and a graded response to light intensity. The directional response is part of the escape behavior; the graded response is equivalent to the light preferendum and contributes to the positioning of the gammarids in the water column. The light preferendum is mediated at least in part through the position of the accessory screening pigment. Injected serotonin influences the directional response to light, but, at least in the short term, does not affect the light preferendum.

As in other crustacean systems (Livingstone et al., 1980; Glanzman and Krasne, 1983), large quantities of amine were necessary to obtain a behavioral effect in gammarids. (The concentration of serotonin in the haemolymph of the gammarids after injection of 10 µg was in the low 10^{-3} M range .) It is concluded that serotonin is unlikely to be released by the parasite into the host's haemolymph in sufficient quantity to affect the behavior of the gammarid. It is, however, reasonable to suppose that an increased serotonergic activity in the central nervous system of *G. lacustris* is induced, by an unknown mechanism, as a consequence of the presence of the larval acanthocephalan.

In the introduction, a number of questions were raised concerning the mechanisms whereby larval helminths alter the responses to environmental stimuli of their invertebrate intermediate hosts. These questions were used as a framework to the present study, and some partial answers emerged throughout the discussion. The behavioral patterns induced by *P. paradoxus* occur, in uninfected gammarids, only under specific circumstances and as a response to specific stimuli. The mechanism of action of the parasite appears to depend on whether responses to transient or persistent stimuli are involved. The acanthocephalan *P. paradoxus* probably acts on serotonergic or serotonin sensitive pathways in its gammarid host, and modulates the different components of the altered escape behavior (a response to a transient stimulus). In this case, the parasite is involved

in a neural action at the central level. The parasite is also suspected of altering the level of light adapting hormone, thus changing the position of the accessory screening pigment, and hence the response to light intensity (persistent stimulus), in infected gammarids. There, the action of the parasite would be hormonal, and at the sensory receptor level.

This thesis represents a first attempt at understanding the mode of action of parasites altering the responses to environmental stimuli in their invertebrate intermediate hosts. Pathology has often lead to significant discoveries about the normal state of a biological system. The simple behavioral pathology induced by *P. paradoxus*, or several other species of larval helminths, in invertebrates may provide a wealth of readily accessible information for the neuroethologist.

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APPENDICES

Appendix II-1. Molecular weight, and number of moles for 10 µg substance. All chemicals are from Sigma Cie except cinanserin (Squibb).

Chemical Substance	Molecular Weight	10 µg in moles
γ - Amino - n - butyric acid (GABA, piperidic acid)	103.1	9.71×10^{-8}
(±) Arterenol Hydrochloride (Norepinephrine, Noradrenalin)	205.6	4.86×10^{-8}
DL - p - Chlorophenylalanine	199.6	5.01×10^{-8}
5, 7 - Dihydroxytryptamine Creatinine Sulfate Salt (5, 7 - DHT)	403.4	2.47×10^{-8}
5 - Hydroxytryptamine Creatinine Sulfate Complex (5 - HT, Serotonin)	387.4	2.58×10^{-8}
5 - Hydroxytryptamine Hydrochloride (5 - HT, Serotonin)	212.7	4.70×10^{-8}
3 - Hydroxytyramine Hydrochloride (Dopamine)	189.6	5.27×10^{-8}
DL - p - Hydroxyphenylethanolamine Hydrochloride (Octopamine)	189.6	5.27×10^{-8}
SQ 10,643 Hydrochloride (Cinanserin)	mean: 358.7	2.79×10^{-8}

Appendix IV-1.

July 29, 30	Dark-cabinet, 60 min	12					
	Serotonin	12	19 - 29	5.17 ± 4.13	43 ± 34	0.66* (-0.2333)	0.82*
July 29, 30	Dark-cabinet, 60 min	12					
	Saline	12	31 - 41	5.83 ± 4.45	49 ± 37	0.66* (-0.2062)	0.83*
July 31,	No treatment	12		3.75 ± 2.93	31 ± 24		
Aug 1	(continued)	12		5.25 ± 3. 70	44 ± 31	0.37 (-0.1090)	0.63*
Aug 8	Serotonin	12	22 - 31	8.08 ± 4.17	67 ± 35	0.61* (-0.3965)	0.87*
Nov 11, 12	Serotonin	12	24 - 33	4.92 ± 4.76	41 ± 40	0.57* (-0.2600)	0.74*
Nov 11, 12	Saline	11	13 - 22	0.636 ± 1.50	5 ± 13	0.12 (-0.0172)	0.40
March 13, 1987	Serotonin	6	12 - 22	6.67 ± 4.32	56 ± 36	0.27 (-0.1123)	0.34
March 13	Saline	6	25 - 37	2.50 ± 3.56	21 ± 30	0.52* (0.3201)	0.59
March 17, 18**	Serotonin	12	23 - 35	3.50 ± 2.24	58 ± 37	0.81* (-0.3806)	0.92*
March 17, 18**	Saline	12	24 - 35	2.08 ± 1.31	35 ± 22	0.26 (0.1503)	0.73

* $p < 0.05$

**Light on alternately every 10 minutes at each end of the experimental tray; gammarids observed 6 times in one hour instead of 12.

Appendix III-1. Monthly assessment of clinging behavior in uninfected gammarids. Values are number of gammarids clinging / number tested; gammarids were single individuals (S), and those separated during precopulation (P, sex ratio 1/1).

Date of collection	Tested on Day of collection		Tested 5 Days after collection	
	S	P	S	P
Sept 11, 1985	0 / 30	-	0 / 30	-
Oct 12	0 / 30	-	0 / 30	-
Nov 14	0 / 30	-	0 / 30	-
Dec 12	0 / 30	-	0 / 30	-
Jan 18, 1986	0 / 30	0 / 6	0 / 30	1 / 30
Feb 15	0 / 30	0 / 6	0 / 30	1 / 30
Mar 15	0 / 30	0 / 30	0 / 30	0 / 30
Apr 12	0 / 30	0 / 30	0 / 30	0 / 30
May 17	1 / 30	0 / 30	0 / 30	1 / 30
Jun 14	0 / 30	-	0 / 30	-
Jul 19	0 / 30	-	0 / 30	-
Aug 11	0 / 30	-	0 / 30	-

Appendix III-2. Average clinging performance of *P. paradoxus*-infected gammarids injected with different substances. The means and their corresponding standard deviations represent the number of clinging responses per gammarid for one hour (4 assays). Up to June 6, 1985, the experimental gammarids were collected in Polar Pond; from June 13 on, they came from Cooking Lake. Up to August 6, 1985, the gammarids born during the Spring 1984 were used in the experiments; from September 1985 on, the gammarids born during the Spring 1985 were used

Date	Treatment	Number of gammarids		Clinging responses	
		In sample	Clinging at least once	Mean ± s.d	%
May 15, 1985	Octopamine, 5 ug	8	3	0.38 ± 0.52	9 ± 13
May 27	Sham injected	5	5	4.00 ± 0.00	100 ± 0.0
May 27	Saline	9	9	3.33 ± 1.12	83 ± 28
	Longitudinal experiment, Saline and PCPA				
June 3	t0, Saline	9	9	3.00 ± 1.23	75 ± 31
	t0 + 6:30	9	5	0.78 ± 0.83	19 ± 21
June 4	t0 + 24:00	9	6	1.33 ± 1.41	33 ± 35
	t0 + 34:00	9	7	2.33 ± 1.66	58 ± 41
June 5	t0 + 48:00	9	7	2.67 ± 1.73	67 ± 43
June 6	t0 + 72:00	9	7	1.78 ± 1.48	44 ± 37
June 3	t0, PCPA, 10 ug	7	7	3.57 ± 0.79	89 ± 20
	t0 + 6:30	7	6	2.71 ± 1.50	68 ± 37
June 4	t0 + 24:00	7	5	2.00 ± 1.73	50 ± 43
	t0 + 34:00	7	7	3.43 ± 0.98	86 ± 24
June 5	t0 + 48:00	7	7	2.71 ± 1.38	68 ± 34
June 6	t0 +72:00	7	6	2.57 ± 1.51	64 ± 37
	End of PCPA experiment				

Appendix III-2.

		Longitudinal experiment, Untreated, Saline, Dopamine.			
June 13	No treatment	5	5	3.20 ± 1.10	80 ± 27
June 14	No treatment	5	3	2.40 ± 2.18	60 ± 55
June 13	Pre-treatment	10	10	3.70 ± 0.68	93 ± 17
June 14	Saline	10	9	2.80 ± 1.40	70 ± 35
June 13	Pre-treatment	9	9	3.67 ± 0.71	92 ± 18
June 14	Dopamine, 10 ug	9	8	2.11 ± 1.17	53 ± 29
		End of Dopamine experiment			
July 2	Saline	10	10	3.10 ± 0.99	78 ± 25
July 2	Noradrenaline, 10 ug	10	3	0.70 ± 1.25	18 ± 31
July 22	Saline	10	10	3.40 ± 0.97	85 ± 24
July 22	Cinanserin, 10 ug	10	8	2.30 ± 1.42	58 ± 35
July 23	Octopamine, 10 ug	7	0	0	0
Aug 6	Saline	10	10	3.80 ± 0.63	95 ± 16
Aug 6	Cinanserin, 5ug	10	10	3.80 ± 0.42	95 ± 11
Sept 15	Saline	10	10	4.00 ± 0.00	100 ± 0.0
Sept 15	Cinanserin, 20 ug	6	4	1.33 ± 1.51	33 ± 38
		Longitudinal experiment, Saline and Cinanserin.			
Nov 3	Pre-treatment	10	10	4.00 ± 0.00	100 ± 0.0
Nov 4	Saline	10	10	3.60 ± 0.97	90 ± 24
Nov 3	Pre-treatment	9	9	3.89 ± 0.33	97 ± 8
Nov 4	Cinanserin, 10 ug	9	8	2.33 ± 1.50	58 ± 38

Appendix III-3. Average clinging performance of uninfected gammarids injected with different chemical substances. The means and their corresponding standard deviations represent the number of clinging responses per gammarid for one hour (4 assays). Up to June 10, 1985, the experimental gammarids were collected in Polar Pond; from June 17 on, they came from Cooking Lake. Up to July 3, 1985, the gammarids born during the Spring 1984 were used in the experiments; from August 31, 1985 on, gammarids born during the Spring 1985 were used.

Date	Treatment	Number of gammarids		Clinging responses	
		In sample	Clinging at least once	Mean ± s.d.	%
Dec 19, 1984	No treatment	5	0	0	0
Dec 19	Serotonin, HCl, 10 ug	5	4	1.60 ± 1.14	40 ± 29
Dec 19	Serotonin, HCl, 1 ug	5	2	0.40 ± 0.55	10 ± 14
Jan 11, 1985	No treatment	5	0	0	0
Jan 11	Serotonin, 10 ug	4	3	2.25 ± 1.71	56 ± 43
Jan 11	Serotonin, HCl, 20 ug	4	3	1.00 ± 0.82	25 ± 20
Jan 17	GABA, 1 ug	5	0	0	0
Jan 17	GABA, 10 ug *	5	0	0	0
Jan 25	Saline	5	0	0	0
Jan 25	Octopamine, 5 ug	5	0	0	0

Appendix III-3.

May 1	Dopamine, 10 ug	4	0	0	0
June 10	Saline	10	0	0	0
June 10	Serotonin, CS, 10 ug	10	8	2.00 ± 1.33	50 ± 33
June 17	Cystacanth extract 20 cysts/70 ul saline	10	1	0.10 ± 0.32	3 ± 8
June 18	Serotonin, CS, 5 ug + Dopamine, 5 ug	9	4	0.78 ± 0.97	19 ± 24
July 3	Saline	10	0	0	0
July 3	Noradrenaline, 10 ug	10	0	0	0
Aug 31	Saline	10	0	0	0
Aug 31	Cystacanth extract 40 cysts/40 ul saline	10	2	0.20 ± 0.42	5 ± 11
Sept 22	Serotonin, CS, 10 ug	9	7	2.22 ± 1.30	56 ± 33
Aug 14, 1986	Serotonin, HCl, 10 ug	12	5	0.92 ± 1.51	23 ± 38

*The gammarids appeared dead and were totally immobile, for up to one hour after injection of 10 ug GABA.

Appendix IV-1. Average photic performance of uninfected *G. lacustris* injected with 10 ug serotonin (5-HT, HCl) or with saline. The two figures in the lag-time column represent the range of time between the first and last injections in the sample of n gammarids and the first assay. The average photic performance represents the number of times gammarids were present in the half of the tray nearest to the light source out of 12 observations in one hour; it is expressed in absolute numbers and in percentages with corresponding standard deviations. There were 12 observations per hour for all experiments except March 17, 18 (6 observations). The slope of the linear regression of the time-course, the correlation coefficients of the linear and third order regressions and their statistical significance are also listed.

Date	Treatment	n	Lag-time (min)	Presence in lighted zone average ± s.d.	Presence in lighted zone average ± s.d. %	Linear Regression R (Slope)	Curvi linear Regr. Order 3 R
Feb.18, 1986	Pre-treatment	12		5.25 ± 3.31	43 ± 27		
	Serotonin	12	24 - 37	5.92 ± 3.83	49 ± 32	0.94* (-0.5922)	0.97*
Feb.19	Pre-treatment	12		6.92 ± 3.18	57 ± 25		
	Saline	12	27 - 39	3.42 ± 4.46	28 ± 37	0.57* (0.1764)	0.65*
Feb.20	Pre-treatment	11		3.55 ± 2.81			
	Serotonin	11	26 - 39	7.09 ± 3.75	59 ± 32	0.77* (-0.3883)	0.91*
Feb.21	Pre-treatment	12		1.75 ± 2.34	14 ± 19		
	Saline	12	27 - 39	0.58 ± 1.00	5 ± 8	0.60* (-0.0738)	0.64*
July 11, 12	Dark-cabinet, 60 min	12					
	Serotonin	12	28 - 39	5.58 ± 4.30	47 ± 36	0.74* (-0.3788)	0.87*
July 11, 12	Dark-cabinet, 60 min	12					
	Saline	12	15 - 26	2.92 ± 3.92	24 ± 33	0.00 (-0.0003)	0.52

Appendix IV-2. Average photic performance of *P. paradoxus*-infected *G. lacustris* injected with different substances. The two figures in the "lag-time" column represent the range of time between the first and last injection in the sample of n gammarids, and the first assay. The average photic performance represents the number of times gammarids were present in the half of the tray nearest to the light source in one hour; it is expressed in absolute numbers and in percentages with corresponding standard deviations. There were 12 observations per hour for all experiments but May 19, 20 (6 observations). The slope of the linear regression of the time course, the correlation coefficients of the linear and third order regressions and their statistical significance are also listed.

Date	Treatment	n	Lag-time (min.)	Presence in lighted zone average ± s.d.	Presence in lighted zone average ± s.d. %	Linear Regression R (Slope)	Curvi linear Regr. Order 3 R
Dec.15, 1985	Pre-treatment	5		8.00 ± 3.54	67 ± 29		
	Saline	5	25 - 35	10.00 ± 4.47	83 ± 37	0.21 (0.0504)	0.71*
Dec.15	Pre-treatment	6		9.83 ± 3.55	82 ± 30		
	Octopamine, 10 ug	6	24 - 33	8.33 ± 3.72	69 ± 31	0.63* (-0.3983)	0.67*
May 22, 1986	Pre-treatment	12		7.67 ± 3.92	64 ± 33		
	Dopamine, 10 ug	12	21 - 36	7.33 ± 4.19	61 ± 35	0.29 (0.0969)	0.46
May 23	Pre-treatment	12		9.08 ± 3.29	76 ± 27		
	Saline	12	21 - 36	9.33 ± 4.01	78 ± 33	0.58* (-0.1649)	0.65*
May 29	Pre-treatment	11		9.64 ± 3.38	80 ± 28		
	Octopamine, 10 ug	11	24 - 35	9.46 ± 1.92	78 ± 16	0.48* (-0.1443)	0.55
May 30	Pre-treatment	11		9.64 ± 1.43	80 ± 12		
	Saline	11	24 - 34	10.00 ± 1.84	83 ± 15	0.28 (-0.0872)	0.50

Appendix IV-2.

June 25	Pre-treatment	12		11.17 ± 1.34	93 ± 11		
	Morphine, 5 ug	12	22 - 35	9.67 ± 3.26	81 ± 27	0.69* (-0.2150)	0.80*
June 26	Pre-treatment	12		10.92 ± 2.39	91 ± 20		
	Saline	12	24 - 35	9.67 ± 2.96	81 ± 25	0.36 (-0.1339)	0.76*
July 31,	No treatment	12		10.67 ± 2.57	89 ± 21		
Aug. 1	(continued)	12		10.33 ± 3.68	86 ± 31	0.66* (-0.1830)	0.74*
Aug. 6, 7	Pre-treatment	12		9.25 ± 4.45	77 ± 37		
	Enkephalin, 10 ug	12	16 - 25	9.00 ± 3.36	75 ± 28	0.48* (-0.1592)	0.70*
Aug. 6, 7	Pre-treatment	12		10.58 ± 2.11	88 ± 18		
	Saline	12	24 - 37	9.83 ± 2.86	82 ± 24	0.76* (-0.3002)	0.76*
1987							
May 19, 20**	Saline	9	26 - 35	3.89 ± 0.78	67 ± 17	0.29 (0.1499)	0.49
May 19, 20**	Octopamine, 10 ug	12	25 - 35	4.33 ± 0.78	72 ± 13	0.25 (0.0697)	0.62
May 21	t0, No treatment	12		7.75 ± 4.52	65 ± 38		
	t0 + 24, with no disturbance	12		5.66 ± 3.65	47 ± 30	0.17 (-0.1559)	0.23

* $p < 0.05$

** Light on alternately every 10 minutes at each end of the experimental tray; gammarids observed 6 times in one hour instead of 12.

Appendix V-1. Average eye score in uninfected gammarids and in those infected with the larval acanthocephalan Polymorphus paradoxus, for 25 samples spread from September 1986 to September 1987 (listed in chronological order.). The range of possible values for eye score extends from 1, darkest eye (= most dark adapted position of white pigment), to 5, whitest eye (= most light adapted position of white pigment).

	Infected			Uninfected			Eye score	Day of year
	n	Eye score		n	Eye score		Inf. - Uninf.	
Date		Mean	s.d.		Mean	s.d.		
S19	6	3.417	.516	6	2.208	.813	1.209	262
S20	6	2.625	.848	6	2.583	.516	0.042	263
S23	6	2.583	.957	6	2.5	.671	0.083	266
S24	6	2.625	.666	6	2.292	.6	0.333	267
N20	6	2.667	.917	6	2.417	.342	0.250	324
N21	5	2.55	.542	7	2.179	.826	0.371	325
Ap20	6	2.5	.447	6	2.292	.579	0.208	110
Ap21	6	2.208	.246	6	2.458	.781	-0.250	111
Ap27	6	2.875	.607	6	2.75	.612	0.125	117
Ap28	6	2.458	.459	6	2.292	.431	0.166	118
My6	6	2.458	.368	6	2.083	.516	0.375	126
My7	6	2.167	.606	4	2.188	.315	-0.021	127
My14	6	2.333	.492	6	2.208	.292	0.125	134
My15	5	2.65	.783	6	3.042	.534	-0.392	135
My18am	6	2.333	.438	6	2.667	.492	-0.334	138
My18pm	5	2.75	.5	6	2.875	.737	-0.125	138
Jun15	6	2.792	.66	6	2.583	.376	0.209	166
Jul14	6	2.375	.306	6	2.625	1.068	-0.250	195
Jul20	6	2.875	.862	6	2.25	.524	0.625	201
Jul28	6	2.458	.813	6	2.875	.862	-0.417	209
A18	6	3.125	.627	6	2.25	.387	0.875	230
A20	6	2.208	.292	6	2.333	.303	-0.125	232
A24	6	2.625	.647	6	2.292	.9	0.333	236
A28	6	2.417	.376	6	2.208	.557	0.209	240
S6	6	2.458	.485	5	2.5	.395	-0.042	249