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The Effects of Two Acanthocephalans, *Polymorphus paradoxus* and *P. marilis*, on the Reproduction of Their Intermediate Host, *Gammarus lacustris (Crustacea)*

by

Alexandra S. Zohar

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements
for the degree of DOCTOR OF PHILOSOPHY

Department of Zoology

EDMONTON, ALBERTA

FALL 1993

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **THE EFFECTS OF TWO ACANTHOCEPHALANS, *POLYMORPHUS PARADOXUS* AND *P. MARILIS*, ON THE REPRODUCTION OF THEIR INTERMEDIATE HOST, *GAMMARUS LACUSTRIS (CRUSTACEA)*** submitted by **ALEXANDRA S. ZOHAR** in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY.**

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To Greg

ABSTRACT

The effects of two acanthocephalans, *Polymorphus paradoxus* and *P. marilis*, on the reproduction of their intermediate host, *Gammarus lacustris*, were examined in the field (Cooking Lake, Alberta) and in the laboratory.

There was no evidence that either parasite castrated either female or male gammarids. Parasitized females possessed ovaries and bristled oostegites, and parasitized males had testes, mature sperm, calceoli, and enlarged gnathopods.

There was a positive relationship between brood size (number of eggs or immatures borne) and length for both *P. marilis*-infected and uninfected females. However, the brood sizes of *P. marilis*-infected females were reduced by 3-5 eggs compared to brood sizes of similar sized uninfected females. Few *P. paradoxus*-infected females were found during the brooding periods; therefore, their egg output could not be assessed.

Females infected with *P. marilis* were less attractive to males than uninfected ones in laboratory tests. Their pairing success in the lake was unaffected, except for small females, or during periods of female-biased sex ratios when males did prefer to pair with uninfected females. Pairing success of *P. paradoxus*-infected females was greatly reduced both in the laboratory and in the field. The pairing success of males infected with either parasite was lower than that of uninfected gammarids both in the field and laboratory tests. The reduced pairing success of infected males was the only aspect of male reproduction to be affected by parasitism.

Female gammarids were more susceptible to overwinter mortality than males. However, there was no evidence that *P. marilis* reduced the longevity of females, or that either parasite affected male longevity. In contrast, *P. paradoxus*-infected females appear to be susceptible to selective winter mortality.

Gammarus lacustris reproduction was more seriously affected by *P. paradoxus* than by *P. marilis*. The differential effects of these parasites may be due to the greater energetic demands by the larger parasite (*P. paradoxus*) or to the differences in the degree of behavioural alterations they induce in gammarids (Bethel and Holmes, 1973). Additionally, *P. paradoxus* altered the winter distribution of infected gammarids in the lake, potentially reducing their chances of encountering mates. Although parasitized individual male and female gammarids incur reproductive losses, it is unlikely that infections with *P. paradoxus* or *P. marilis* would influence the *G. lacustris* population at Cooking Lake.

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Chapter 1 - Introduction

Studies of parasite-induced alteration of host behaviour explore one or more of the following aspects: the nature of the altered behaviour, the mechanisms which precipitate these alterations, the consequences of behavioural alterations to individual hosts and to host populations, and the evolutionary history of the host-parasite system (Moore and Cotelli, 1990). Few host-parasite systems are studied at all of the aforementioned levels. Studies on the acanthocephalan *Polymorphus paradoxus* Connell and Corner, 1957 (Palaeacanthocephala: Polymorphidae) in its intermediate host, *Gammarus lacustris* Sars, 1846 (Amphipoda: Gammaridae) constitute a notable exception. Many aspects of this host-parasite system have been explored, including the altered behaviour, its consequences to the ecology of *G. lacustris* in summer, mechanisms of behavioural modifications, and theories on how this relationship may have evolved (Bethel and Holmes, 1973; 1974; 1977; Helluy and Holmes, 1990). In this field and laboratory-based study I compared some of the effects of this parasite, and of another acanthocephalan, *Polymorphus marilis* Van Cleave, 1939, on winter ecology and reproductive success of *G. lacustris*.

Holmes and Bethel (1972) categorized four means parasites employ in altering the behaviour of their hosts: (1) reduced stamina, (2) increased conspicuousness by modifications of morphological features, such as color or size, (3) disorientation, and (4) altered responses to environmental stimuli. These authors suggested that parasite-induced behavioural alterations of intermediate hosts constitute a parasite strategy to increase predation on intermediate by final hosts, thereby facilitating the parasite's transmission. This theory is supported by experimental evidence showing that intermediate hosts whose behaviour have been altered by a parasite are subject to greater predation by final hosts than are uninfected conspecifics (Bethel and Holmes, 1977; Helluy, 1984; Brown and Thompson, 1986). Altered host behaviour does not always result in increased parasite transmission (Moore and Gotelli, 1990) and parasitism may even increase the host's apparently cryptic behaviours (Moore and Gotelli, 1992). Thus, host behavioural alterations are not necessarily a parasite strategy and may be host responses to infection (host adaptations), or side effects of the infection with no direct adaptive significance (Minchella, 1985; Holmes and Zohar, 1990).

The consequences of parasite-induced behavioural changes are not restricted to increasing the susceptibility of intermediate hosts to predators. The reproductive fitness of parasitized hosts may be lowered by partial or complete host sterilization

(Reinhard, 1956; Baudoin, 1975), or by altered social behaviours, such as courtship (Hamilton and Zuk, 1982) and social ranking (Rau, 1983; 1984). Reduction in the survivorship and fitness of parasitized individuals may also affect intermediate host populations. Dobson (1988) used mathematical models to quantify the population dynamic consequences of parasite-induced behavioural modifications. Enhanced transmission, as a consequence of behavioural alterations, increased the basic rate of reproduction of the parasite, which decreased the size of the intermediate host population required to maintain the infection. Increased rates of parasite transmission were more advantageous to parasites colonizing small, ephemeral, or patchy host populations than for those continuously present in large host populations (Dobson, 1988).

Many parasites, including protozoa, helminths and arthropods can affect the behaviour of their intermediate hosts (Holmes and Bethel, 1972; Moore, 1984a; Holmes and Zohar, 1990). Parasites can affect host behaviour by causing host organ malfunction, or by localizing in, or migrating through sense organs, the central nervous system or musculature (Holmes and Zohar, 1990). Behavioural alterations can also result from reduced nutritional levels of infected hosts by parasites that are an energetic or nutrient drain, lower assimilation efficiency, anorexia, or reduced oxygen delivery by the circulatory and pulmonary systems. Parasites can also modulate host neuroendocrine control systems by either destroying hormone-producing organs (eg. gonads) or through parasite secretions of regulatory compounds (Holmes and Zohar, 1990).

The Host-Parasite Systems

Almost all arthropods infected with acanthocephalans exhibit some altered responses to environmental stimuli such as light, humidity, and mechanical disturbances, and/or are more active than their uninfected conspecifics (Moore, 1984b; Table 1.1). Both *P. marilis* and *P. paradoxus* induce some degree of behavioural pathology in their *Gammarus* host (Bethel and Holmes, 1973). *Polymorphus marilis*-infected gammarids are photophilic but negatively phototactic when disturbed. The behaviour of *P. paradoxus*-infected gammarids is more grossly altered; infected amphipods are photophilic, positively phototactic and, when disturbed, exhibit altered escape responses by skimming the water surface and clinging to floating material (Bethel and Holmes, 1973). Such altered evasive responses significantly increase the probability of infected gammarids being

Table 1.1: The effects of acanthocephalans on the behaviour of their intermediate hosts (modified from Table 1 in Moore, 1984a). Continued on next page.

Parasite & Host	Photophilia	Substrate Choice	Response to Disturbance	Activity	Habitat Shift	Predation	Reproduction		Source
							Female	Male	
Acanthocephalus dirus									
Caecidotea intermedius	I			I	X	I (x7.5)*	R	0	1, 2, 3
(isopod)									
A. jacksoni									
Lirceus lineatus	I			I			R	0	2, 4
(isopod)									
Plagiorhynchus cylindraceus									
Armadillidium vulgare	0	X	0	I		I (x1.6)	R	0	5
(isopod)									
Moniliformis moniliformis									
Blattela germanica	0	X		R					6
Diploptera punctata	0	0		0					7
Periplaneta americana	I	X		i			0		8, 9
P. brunnea	0	X		0					10
Supella longipalpa	R	X		0					11
(cockroaches)									
Corynosoma constrictum									
Hyalella azteca	I		X			I			12, 13
(amphipod)									
Polymorphus paradoxus									
Gammarus lacustris	I	X	X		X	I (x4)			12, 13
(amphipod)									

Table 1.1: **The effects of acanthocephalans on the behaviour of their intermediate hosts (continued)**

Parasite & Host	Photophilia	Substrate Choice	Response to Disturbance	Activity	Habitat Shift	Predation	Reproduction		Source
							Female	Male	
Polymorphus marilis *G. lacustris* (amphipod)	I		0			0			12, 13
Polymorphus minutus *G. lacustris* *(amphipod)*	I					I	R	0	15, 16
Pomphorhynchus laevis *G. pulex* (amphipod)	I	X			X	I (x4)	R	0	17, 18, 19, 20
Octospiniferoides chandleri									
Cypridopsis vidua	I				X				21
Physocypria pustutosa (ostracods)	I				X				21

Sources: 1. Camp and Huizinga, 1979; 2. Oetinger and Nickol, 1981; 3. Hechtel *et al.*, 1993; 4. Muzzal and Rabalais, 1975; 5. Moore, 1983a; 6. Gotelli and Moore, 1992; 7. Allely *et al.*, 1992; 8. Wilson and Edwards, 1986; 9. Moore, 1983b; 10. Carmichael and Moore, 1991; 11. Moore and Gotelli, 1992; 12. Bethel and Holmes, 1973; 13. Bethel and Holmes, 1977; 14. Holmes and Bethel, 1972; 15. Hindsbo, 1972; 16. Hynes, 1955; 17. Rumpus and Kennedy, 1974; 18. Brown and Thompson, 1986; 19. McMahon *et al.*, 1991; 20. Poulton and Thompson, 1987; 21. DeMont and Corkum, 1982.

I= increase, R = reduction, 0 = no effect, X = altered

* increased predation rates calculated by Helluy (1988)

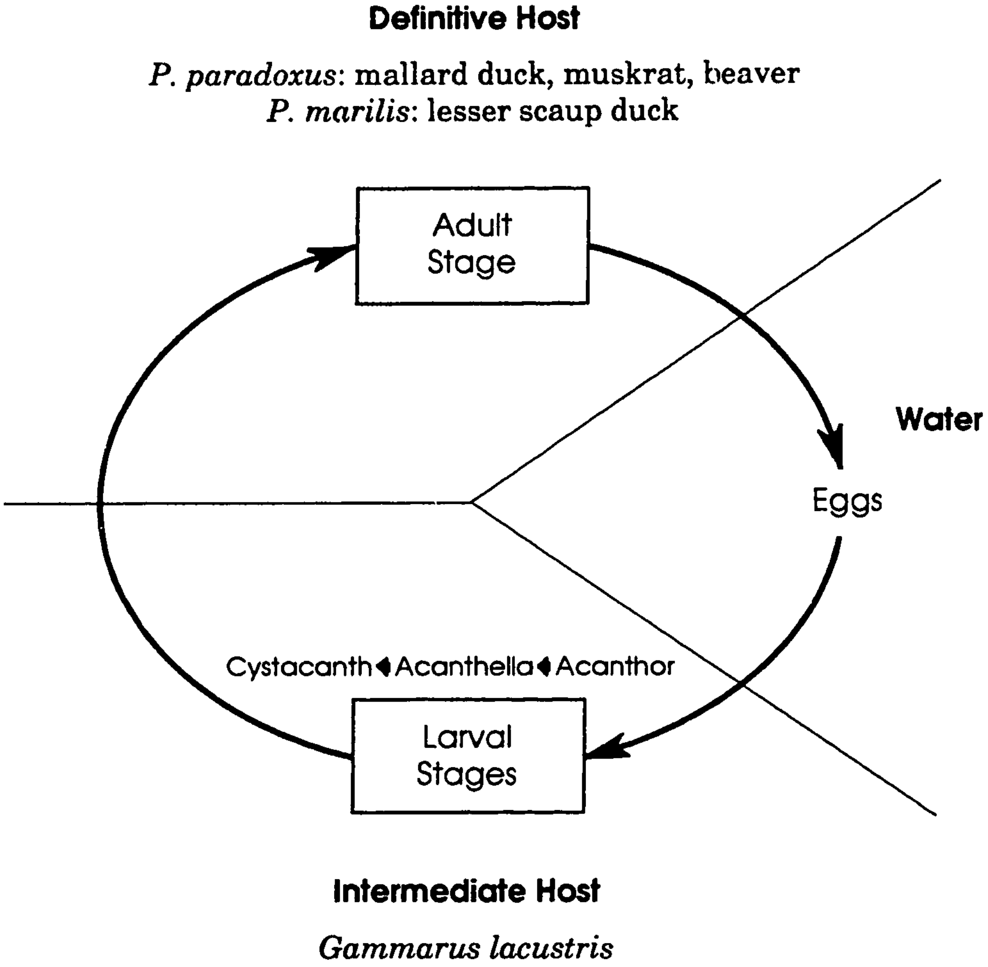
ingested by two of their final hosts, mallard ducks and muskrats (Bethel and Holmes, 1977). *Polymorphus paradoxus* cystacanths alter the photic and escape behaviour of gammarids by modulating certain neural pathways (Helluy and Holmes, 1990).

In the life cycle of *P. paradoxus* and *P. marilis* (Fig. 1.1), a gammarid becomes infected by ingesting an infective parasite egg. The egg hatches into an early larval stage, the acanthor, which exits the gut into the gammarid's haemocoel. The larval parasite undergoes additional developmental stages within the haemocoel: the acanthella and finally, the cystacanth, the infective stage. The cystacanths of *P. paradoxus* and *P. marilis* are bright orange and easily visible through the gammarid's cuticle. The final hosts for *P. paradoxus* are mallard ducks (*Anas platyrhynchos*), beavers (*Castor canadensis*) and muskrats (*Ondatra zibethica*); *P. marilis* develops in lesser scaup ducks (*Aythya affinis*) (Denny, 1969).

These two parasites have different transmission periods at Cooking Lake, Alberta (my study site). The prevalence of *P. marilis* cystacanths peaks in June; thereafter, the prevalence declines until the fall (Denny, 1967; Tokeson, 1971). The cystacanth prevalence is low and almost constant during the winter months (Tokeson, 1971). Tokeson and Holmes (1982) suggest that, although a small proportion of *P. marilis* overwinters in the cystacanth stage, the predominant overwintering mode of this parasite is to diapause in the fall, overwinter in the acanthor stage, and develop into cystacanths in the summer. There are no studies on the overwintering strategies of *P. paradoxus* in gammarids; however, researchers collecting gammarids at Cooking Lake note that parasitized gammarids are abundant in the fall, scarce during the winter, and abundant again as soon as the ice cover breaks (Denny, 1967; Helluy, 1988). Therefore, it appears that *P. paradoxus* overwinters as a cystacanth and can be transmitted in the spring. Potentially, *P. paradoxus* adult worms may also overwinter in their resident mammalian hosts, muskrats and beavers. New infections develop in June and July in summer-born gammarids, and *P. paradoxus* cystacanth prevalences peak in August (Denny, 1967; Helluy, 1988).

The intermediate host, *G. lacustris*, has a univoltine life cycle in Albertan lakes (Canada). Menon (1966) described the population ecology of *G. lacustris* in an Alberta lake (Big Island Lake). In many freshwater gammarids, including *G. lacustris*, females are receptive to mates for only a short period of time after moulting, and fertilization is possible only until the female's cuticle hardens. In such species there is a period of precopulatory guarding by males prior to mating to

Figure 1.1: Life cycle of *Polymorphus paradoxus* and *P. marilis*


Definitive Host
P. paradoxus: mallard duck, muskrat, beaver
P. marilis: lesser scaup duck
Adult Stage
Water
Eggs
Cystacanth ◀ Acanthella ◀ Acanthor
Larval Stages
Intermediate Host
Gammarus lacustris

ensure males a mating opportunity following the female's moult (Elwood *et al.*, 1987; Elwood and Dick, 1990). Thus, the reproductive cycle of these gammarids commences with precopulatory pairing under ice cover. The male inserts his gnathopods between the female's thoracic segments (Fig. 1.2) and carries her until she moults and is ready to oviposit (Conlan, 1991; Sutcliffe, 1992). Pairing activity in *G. lacustris* peaks in early spring after the ice thaw, coincident with the period of moult which results in ovulation (Menon, 1966; Helluy, 1988). Ovigerous females are found in the spring and early summer and juveniles are released in the early summer. Most of the adult population dies following mating and brooding, although some post-reproductive adults survive. Juvenile gammarids attain secondary sexual characteristics in the fall and form the breeding population during the following winter and spring (Menon, 1966).

I chose to study the *P. paradoxus-* and *P. marilis-G. lacustris* systems for several reasons. First, these acanthocephalans provide an excellent opportunity to compare the life-cycles and the adaptations of parasites of the same genus, *Polymorphus*, that utilize the same intermediate host, and are found in the same environment (Cooking Lake, Alberta). Despite these similarities, the two parasites differ in the extent of behavioural alterations they induce, their transmission periods, and their final hosts. Through comparative studies of different parasite species using the same host, or of same species using different hosts, the evolutionary history of host-parasite interactions and the potential evolution of host behavioural alterations can be derived (Moore and Gotelli, 1990). Second, some aspects of the life cycle, behavioural modifications, and neuroendocrine modulations caused by these parasites are known. Lastly, Cooking Lake, Alberta is an ideal location for the study of *P. paradoxus* and *P. marilis* because gammarids, aquatic mammals, and waterfowl (final hosts) are abundant, and infected gammarids can be collected easily.

Parasitism and Winter Distribution

Much of what is known about the effects of *P. paradoxus* and *P. marilis* on the behaviour of *G. lacustris*, and the ecological consequences of the gammarids' altered behaviour, has resulted from field and laboratory studies carried out during summer. Major changes in *P. paradoxus* infection between fall and spring sampling periods, and the fact that critical pre-reproductive activities of the host occur in late winter, suggest that winter is a significant period in the life cycles of both host and

Figure 1.2: *Gammarus lacustris* in precopula; the male (left) attaches to the female (right) with his gnathopods

parasite. Accordingly, one of the objectives of this study was to extend the scope of previous studies to the winter period. The aim of this study was to examine further the ecological consequences that may be associated with *P. paradoxus* and *P. marilis* infections. Bethel and Holmes (1973) hypothesized that the microdistribution of gammarids in the lake is affected by parasitism with both *P. paradoxus* and *P. marilis*. Gammarids infected with *P. paradoxus* should be distributed in the surface area (brightest region), and *P. marilis*-infected gammarids should be distributed throughout the lighted zones of lakes. This hypothesis was not tested in the lake and only anecdotal observations on the distribution of *P. paradoxus*-infected gammarids are available (Bethel and Holmes, 1973). Winter is an important period in the life cycles of both parasites and gammarids since the former must overwinter to be available for summer transmission, and the latter must survive winter to reproduce in the spring. Thus, the study of winter ecology of infected and uninfected gammarids is important to the understanding of biology and life cycles of the parasites and their amphipod hosts.

Both adult and larval parasites can modify the distribution of their intermediate or final hosts (see p. 52-53 in Holmes and Zohar, 1990). Such habitat shifts may be the outcome of a variety of behavioural alterations experienced by the parasitized host. Clams (*Macoma balthica*) infected with trematode larvae (*Parvatrema affinis*) crawl in high levels of the tidal zone leaving tracks in the mud, whereas most uninfected clams are buried in the mud (Swennen, 1969; Hulscher, 1973; Swennen and Ching, 1974). Infected clams may be closer to the mud surface, a more oxygen rich area than deeper in mud, because of greater oxygen demands imposed by the parasite (Swennen and Ching, 1974). A number of acanthocephalans alter the distribution of their arthropod hosts (Table 1.1). For example, *Acanthocephalus dirus*-infected isopods (*Caecidotea intermedius)* spend significantly more time in open areas away from shelter than uninfected isopods, thereby increasing their vulnerability to predation by fish definitive hosts (Hechtel *et al.*, 1993). Although these authors discount the possibility that the altered behaviour is the result of increased energy demands on the infected isopods, they do not identify the mechanism responsible for the modified predator avoidance behaviour.

Some parasites which alter the behaviour of their hosts are known to modify their hosts' winter distribution. Common periwinkles (*Littorina littorea*) infected with the trematode *Cryptocotyle lingua* are more common at high-tide zones, and migrate down the beach more slowly and to a lesser extent in winter than uninfected snails (Sinderman and Farrin, 1962; Lambert and Farley, 1968).

Although infected snails respond normally to cold stimuli (freezing air or water) their responses are slower and reduced in magnitude. The wintering microhabitat of the potato aphid (*Macrosiphum euphorbiae*) is changed by the endoparasitoid, *Aphidius nigripes*. Aphids infected with diapausing parasitoid larvae leave the host plant to mummify in concealed areas which protect the wasp-aphid mummy from hyperparasitism and adverse winter conditions (Brodeur and McNeil, 1989). The wasp larvae alter phototactic, thigmotactic responses, and substrate color preferences of parasitized aphids (Brodeur and McNeil, 1990). *Polymorphus paradoxus* and *P. marilis* also alter their gammarid hosts' responses to environmental stimuli (Bethel and Holmes, 1973), and may consequently affect the gammarids' winter distribution.

Effects of Parasitism on Reproductive Output

In 70 percent of the host-parasite systems reviewed by Dobson (1988) involving altered host behaviour, infection also reduced host fecundity. Few *P. paradoxus*-infected female gammarids are found with mature ovaries or broods (Bethel, 1972; Helluy, 1988). There is no evidence that *P. marilis* affects ovarian development or brood-bearing ability (Menon, 1966; Denny, 1969). However, the potential effects of these two parasites on the reproductive success of *G. lacustris* males and females have never been addressed. In this study the effects of infection with *P. paradoxus* and *P. marilis* on several aspects of *G. lacustris* reproductive success were examined. I studied reproductive parameters which may be affected by altered behaviour, such as mate choice and pairing success, and aspects that could be altered by the energetic demands of the parasites, including development of the gonads and secondary sexual characteristics, as well as egg production.

It has been long recognized that host reproduction can be reduced by parasitic infections of both vertebrate (eg. Hamilton and Zuk, 1982; Schall, 1983) and invertebrate hosts (Baudoin, 1975; Hurd, 1990). The destruction or alteration of host gonadal tissues is referred to as parasitic castration (Baudoin, 1975). However, gonadal destruction is not the only result of parasitic castration. Castrating parasites can have direct effects on their hosts' secondary sexual characteristics, internal organs, internal secretions, viability and growth, behaviour, reproduction, and sex reversal (Baudoin, 1975). Baudoin (1975) noted that the nature of parasitic castration depends on the host's sex. He suggested that since females expend more calories per individual gamete than do males, ovaries are more likely to be affected than testes. Females may also invest more time and energy in parental care than males. In such cases, the secondary sexual characteristics (eg. brood sacs) and maternal behaviours may be altered by parasitism. Conversely, males spend more time and energy defending territories, fighting other males, and courting females. Therefore, secondary sexual characteristics involved in defense, fighting, and courtship (eg. chelae of crustaceans) may be reduced in castrated males. Thus, due to the potential differences in parasite effects on reproduction in females and males, it is best to discuss the two sexes separately.

Parasites can alter reproduction of female arthropods by inducing a variety of physical and/or behavioural pathologies. Hurd (1990) reviewed the effects of the cestode *Hymenolepis diminuta* on the fecundity of the beetle, *Tenebrio molitor*. Infected female beetles lay fewer eggs under crowded conditions, the egg viability and its yolk content are reduced in parasitized females, and the ovaries of infected females contain about 50% less protein than those of uninfected ones. Parasitic barnacles of the family Sacculinidae alter the appearance and behaviour of both male and female brachyuran crabs so that they resemble ovigerous females (Reinhard, 1956). Among the modifications in the external appearance of infected female crabs are the pratical appearance of an adult abdomen and some other secondary sex characteristics (hyper-feminization) and, simultaneously, small pleopods (hypo-feminization; Reinhard, 1956). The cestode, *Anomotaenia brevis*, alters the morphology, brood size and behaviour of colonial ants, *Leptothorax nylanderi*. Infected ants are smaller and differ in colour and some aspects of body shape from uninfected ants, and are usually modified queens that rarely produce eggs, do not leave the nest and spend most of their time begging food from normal workers (Plateaux, 1972; Gabrion *et al.*, 1976).

In six out of seven studies involving acanthocephalans in arthroopds, reproduction of female hosts was adversely affected by parasitism (Table 1.1). Acanthocephalan-infected female hosts may be smaller than uninfected ones (eg. Moore, 1983a), exhibit modified secondary sexual characteristics (eg. Hynes, 1955), possess smaller or no ovaries (eg. Oetinger and Nickol, 1981), and may be unattractive to males (eg. Ward, 1986). Thus, both the female host's energetic investments in reproduction (eg. gonadal development), and reproductive behavioural aspects (attractiveness to mates), appear to be affected by acanthocephalans. Based on the available literature, it appears that acanthocephalans may affect reproduction of female arthropods through direct gonadal destruction. There was no evidence or suggestion that these parasites act through altering the host's internal secretions (eg. hormonal modulation).

Most of the literature on parasitic castration of male invertebrates deals with insects and crustaceans (eg. reviews by Wülker, 1964; Reinhard, 1956). Parasites, including protozoans, helminths and arthropods, can precipitate a wide array of negative effects on the reproductive physiology and behaviour of infected male insects and crustaceans. For example, the parasitic barnacle, *Loxothylacus panopei* alters the testicular germinative zone of the mud crab, *Rhithropanopeus harrissii*, resulting in diminished spermatogenesis (Rubiliani, 1985). This parasite also modifies the sinus glands of the central nervous system, resulting in sinus glands with few or no secretions. These organ alterations are induced by the parasite through direct contact with the internal organs or the release of toxic substances (Rubiliani, 1985). Another barnacle, *Sacculina granifera*, alters the behaviour of parasitized male shore crabs to resemble that of ovigerous females. Infected crabs do not engage in courtship or aggressive displays as do normal males; instead they exhibit the same behaviour as ovigerous females, including seaward migration, digging, the occupation of depressions, and the performance of brood-sac grooming (Bishop and Cannon, 1979). Secondary sexual characteristics are altered in chironomids infected by some nematodes. Infected male chironomids possess structurally less complex antennae and tarsi than uninfected males (Rempel, 1940). This may affect their mate-searching behaviour (Barnard, 1990). Parasites can also affect the hormonal secretions of their hosts. For example, Hartnoll (1967) found evidence that *Sacculina* spp. suppress the endocrine activity of crabs.

There is little evidence that acanthocephalans affect reproduction in male arthropods. None of the acanthocephalan-arthropod studies listed in Table 1.1 report any adverse effects of parasitism on testicular development or spermatogenesis. There is evidence, however, that acanthocephalans may affect the development of secondary sexual characteristics (LeRoux, 1933) and the pairing success (Ward, 1986) of gammarids infected with *P. minutus*. Additionally, male cockroaches (*Periplaneta americana*) infected with *Moniliformis moniliformis* are less responsive to female phermone than uninfected cockroaches (Carmichael *et al*, 1993). It appears that acanthocephalan infections are either less pathogenic to male than female hosts, or that these parasites affect aspects of male reproduction that have not been explored (eg. courtship, pairing and mating success).

Barnard (1990) listed five means by which parasites may reduce the reproductive output of their hosts: (1) parasitic castration (the alteration or destruction of gonads and indirectly, of secondary sexual characteristics); (2) brood loss (reduced number of eggs per female); (3) reduced attractiveness to mates, or reduced inclination to mate; (4) reduced longevity, leading to fewer reproductive episodes; and (5) the costs of counter-adaptations to parasites (eg. immunity). The first four are direct measures of reduced reproduction; the fifth is not. In addition, the energetic costs of mounting an immune response (or any other counter-adaptation) would affect one or more of the others. Therefore, the cost of counter-adaptations was not addressed in this study. I do address the consequences of infection with *P. paradoxus* and *P. marilis* on the reproduction of *G. lacustris* based on the first four of Barnard's (1990) means: parasitic castration, brood loss (reduced number of eggs or immatures in the brood pouch), reduced attractiveness or mating success, and reduced longevity (overwinter survival to the breeding period).

The influence of infection with *P. paradoxus* and *P. marilis* on the lake distribution of gammarids in winter is examined in Chapter 2. The winter habitat of parasitized gammarids was compared to that of uninfected male and female adults and juvenile gammarids. The effects of the two parasites on several aspects of female reproduction including parasitic castration, brood losses, reduced attractiveness to males in the field and in laboratory tests, and winter survivorship are examined in Chapter 3. The influence of *P. paradoxus* and *P. marilis* infections on reproduction of male gammarids, including parasitic castration, their ability to compete with uninfected males for females and their inclination to pair with females in both the field and laboratory, and on their winter survivorship are reported in and and female gammarids on the *G. lacustris* population is discussed in Chapter 5.

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Chapter 2 - The effects of two acanthocephalans, *Polymorphus paradoxus* and *P. marilis*, on the winter distribution of their intermediate host, *Gammarus lacustris*

Parasite-induced behavioural changes in intermediate hosts may be a parasitic strategy to increase predation on parasitized intermediate hosts, thereby increasing the probability of parasite transmission (Holmes and Bethel, 1972; Bethel and Holmes, 1973; Moore, 1984; but see Moore and Gotelli, 1990). One mechanism is to increase the overlap between the habitats occupied by the infected intermediate hosts and the feeding niches of the definitive hosts (Holmes and Bethel, 1972). Behavioural alterations resulting in a habitat change are produced by a variety of parasites and can be precipitated by various mechanisms, ranging from physical pathology to modulation of the host's endocrine system (Holmes and Bethel, 1972; Holmes and Zohar, 1990).

Several acanthocephalan-infected arthropods exhibit altered habitat choices as a result of modified responses to environmental stimuli. In laboratory experiments, terrestrial isopods (*Armadillidium vulgare*) infected by *Plagiorhynchus cylindraceus* are found more frequently in less humid areas, light-colored substrates, and less sheltered areas than uninfected isopods (Moore, 1983). Such behavioural alterations result in greater predation on infected isopods by the starling definitive host (*Sturnus vulgaris*). Bethel and Holmes (1973) compared the effects of *Polymorphus paradoxus* and *P. marilis* on the behaviour of their amphipod intermediate host, *Gammarus lacustris*. Both parasites alter the response of gammarids to light. Uninfected *G. lacustris* are photophobic and negatively phototactic when disturbed. *Polymorphus marilis*-infected amphipods are photophilic but do not respond differently to different light intensities, and are negatively phototactic when disturbed. In contrast, *P. paradoxus*-infected gammarids are strongly photophilic, are attracted to regions of highest illumination, and are positively phototactic when disturbed. In response to disturbance of water surface or to direct touch, uninfected and *P. marilis*-infected gammarids dive to the bottom; however, whereas uninfected gammarids burrow in the mud, *P. marilis*-infected gammarids soon return to the lighted zone. The evasive responses (response to disturbance) of *P. paradoxus*-infected gammarids are grossly modified; infected gammarids swim to the water surface, skim the surface and cling to floating material. Based on these observations, Bethel and Holmes (1973) predict that *P. marilis*-infected gammarids will be distributed throughout the lighted zones of lakes, and *P. paradoxus*-infected gammarids will be distributed in the surface area (the regions of highest illumination).

In the field, gammarids infected with *P. paradoxus* are often found firmly clinging to surface material such as vegetation or dead waterfowl (Bethel and Holmes, 1973). They are also found more often in surface samples than in random dip-netted samples. The distribution of *P. marilis*-infected gammarids does not appear to be affected by the parasite since its summer prevalence is the same in near-shore (15 yards offshore) and open-water sites (200 yards offshore; Tokeson, 1971). There are no observations on the distribution of *P. marilis*-infected amphipods in the water column.

Bethel's (1972) studies on behavioural alterations were conducted at 20°C, providing evidence on the effects of parasitism at summer temperatures only. Transmission of *P. marilis* occurs only during the open-water seasons, coincident with the residence period of migratory waterfowl, the definitive hosts of these parasites. Transmission of *P. paradoxus* may occur both during the open-water season, and during the ice-cover period to resident mammalian definitive hosts, beavers and muskrats. Winter (the ice-covered period), however, is important in the life cycle of both the parasites and their amphipod intermediate hosts. The parasites must overwinter in the amphipods to ensure their transmission in the following summer (Denny, 1967; Tokeson, 1971; Bethel, 1972). The gammarids spend about half of their life span in ice-covered lakes, mature sexually, and start forming precopulatory pairs during the winter (Menon, 1966). Overwintering amphipods in shallow winterkill lakes of the north temperate zone, such as Cooking Lake, experience low water temperatures, anoxia, and hydrogen sulfide accumulation, resulting in some *G. lacustris* mortality. Thus, winter is a period associated with environmental stress which may result in amphipod mortality and potential parasite losses.

Although both *P. paradoxus* and *P. marilis* are exposed to the same physiological and environmental conditions in the gammarid host, the two parasites appear to employ different overwintering strategies, possibly associated with differences in the probablility of winter transmission to their definitive hosts. Only a small proportion of *P. marilis* overwinter as cystacanths, while the predominant overwintering mode of this parasite is to diapause in the fall, overwinter in the acanthor stage, and develop into cystacanths in the spring (Tokeson, 1971; Tokeson and Holmes, 1982). There are no studies on the overwintering strategies of *P. paradoxus* in gammarids; however, researchers who have collected gammarids at

Cooking Lake noted that cystacanth-bearing gammarids were abundant in the fall, scarce during the winter, and abundant again as soon as the ice cover broke (Denny, 1967; Helluy, 1988). Therefore, it appears that *P. paradoxus* overwinters as a cystacanth. An alternate hypothesis for the overwintering of *P. paradoxus* is that it develops into a cystacanth during the winter. This alternate hypothesis is unlikely since *P. marilis*, and other polymorphid acanthocephalans, require a threshold temperature of 7°C in order to initiate development (Tokeson and Holmes, 1982). However, there are no studies on the temperature requirements of *P. paradoxus* or its developmental rates at low temperatures, and thus the possibility of its winter development cannot be entirely discounted.

In this study I examined whether the behavioural alterations caused by *P. paradoxus* and *P. marilis* infections, which affect the summer distribution of parasitized gammarids, also alter the distribution of gammarids during the winter. Additionally, I explored how differential distributions may affect the parasitized hosts and the transmission strategies of the parasites. The specific objectives of my study were: (1) to examine whether *P. paradoxus* or *P. marilis* cause habitat shifts in infected gammarids during winter by comparing their winter distributions with that of uninfected gammarids; and (2) to explore some potential factors (eg. aggregative tendencies and substrate choices) which may affect the winter distribution of infected and uninfected gammarids.

Materials and Methods

Field Collections

Gammarus lacustris were collected from Cooking Lake, 53°25'N 113°03'W, located 30 km southeast of Edmonton, Alberta. Mitchell and Prepas (1990) provide detailed information on the limnology of Cooking Lake. The lake is eutrophic and shallow, with a mean depth of 1.7 m and a maximal depth of 4.6 m. The water is highly turbid and the bottom is soft and covered by a layer of ooze over most of the lake (Bethel, 1972). Therefore, the rooted aquatic vegetation is sparse and limited to the edge of the shoreline and shallow regions. The aquatic macrophytes include a variety of emergent vegetation, including bulerush (*Scripus* spp.) and cattail (*Typha latifolia*), free-floating duckweed (*Lemna minor* and *L. trisulca)* and submergent pondweeds (*Potamogeton pectinatus*, *P. vaginatus* and *Zannichellia palustris*; Mitchell and Prepas, 1990). The most abundant macroinvertebrates are chironomids, amphipods, including *Gammarus lacustris* and *Hyalella azteca*, leeches, and oligochaetes (Kerekes, 1965). There are a few brook sticklebacks in the lake, but no other fish are present due to anoxia and winterkill (Kerekes, 1965). Cooking Lake has large populations of amphipods; due to the turbidity and absence of fish predators, the amphipods are distributed throughout the water column. The abundant amphipods are fed upon by waterfowl which use the lake as a moulting and breeding area. Mallards (*Anas platyrhynchos*), definitive hosts for *P. paradoxus*, and lesser scaup ducks (*Aythya affinis*), final hosts for *P. marilis*, are common. Muskrats (*Ondatra zibethica*) and beavers (*Castor canadensis*), additional hosts for *P. paradoxus*, are also common year round (Mitchell and Prepas, 1990). The high *Gammarus* densities and the availability of potential final hosts facilitate extensive transmission of *P. paradoxus* and *P. marilis* at Cooking Lake.

All sampling was conducted at Lakeview, a man-made bay with a sandy shore and emergent vegetation. Both the west and east sides of the bay were heavily vegetated with emergent plants and had a soft mud bottom. In the summer of 1988, the South Cooking Lake Sail Club was established at Lakeview. The Sail Club removed some of the vegetation along the shore line, and removed the eastern arm of the bay in March 1992. The increased human activity and the changes in the bay structure did not appear to adversely affect the *Gammarus* population as thousands of amphipods were collected after the establishment of the Sail Club. However, the prevalence of *P. paradoxus* decreased following the establishment of the club, indicating that the mammalian or avian populations of definitive hosts may have dwindled. Samples were collected from September, 1987 to May, 1992.

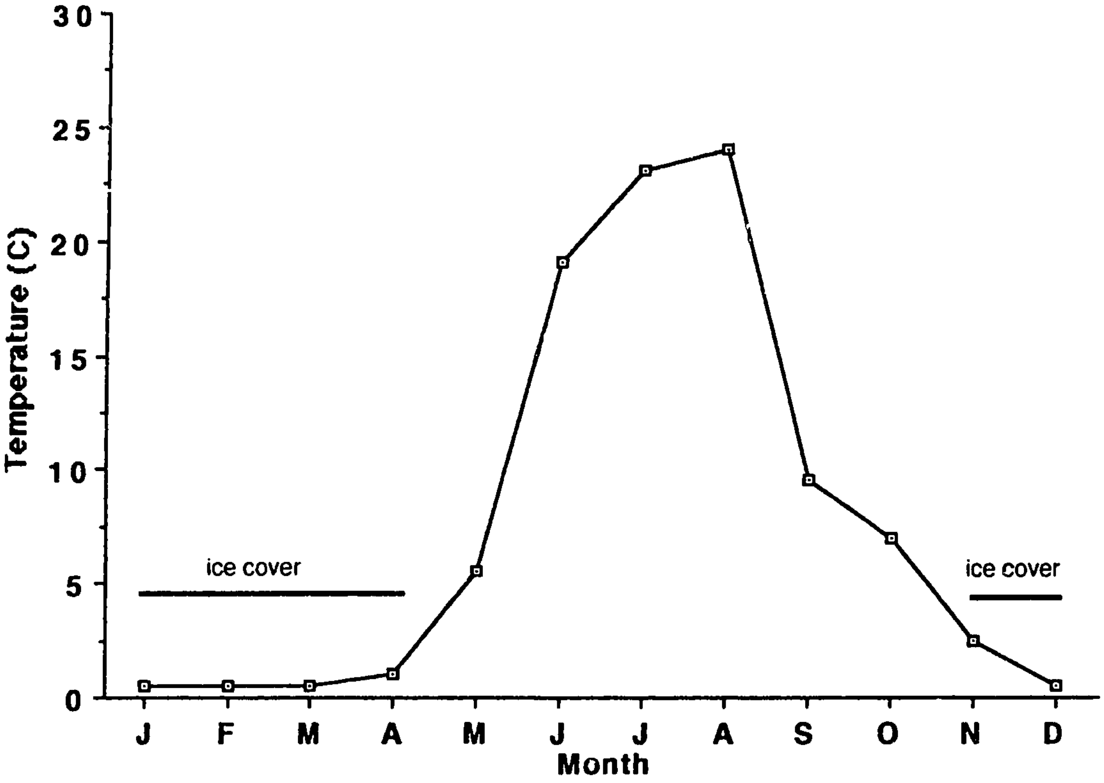
During open water periods gammarids were collected by random dip-netting (using a net with 1 mm mesh). The net was swirled five times before its contents were placed in a bucket with lake water. Dip-netting usually yielded several hundred amphipods per sample. The continuous swirling created a mixing of the water column and prevented uninfected gammarids from escaping, resulting in a representative sample of gammarids from the entire water column. Samples were taken from shallow water sites close to shore (<30 cm of water) and from deeper water further from shore (>60 cm of water).

In winter, holes were cut through the ice cover using an 8 inch power auger and/or ice chisel. Gammarids were frequently ejected by water pressure after the ice cover was penetrated, and these gammarids were collected. Additional gammarids were obtained by rotating the auger blade inside the hole shaft and thus ejecting more water containing gammarids. I attempted to collect gammarids from both shallow and deep water sites. However, as the ice formed and thickened during the winter, I had to collect progressively further from the location of the summer shoreline. Therefore, the water volumes in shallow and deep water sites were smaller than those of open-water samples and were dependent on distance from shore. Holes dug in winter are referred to as shallow water when close to shore and deep water when further from the shoreline. The water temperature at 5-10 cm below the water surface (or ice surface in winter) was recorded at each sampling date (Fig. 2.1).

Samples were collected at least once a month, and two to ten random samples were taken at each collection date. Data from different samples were not combined except as follows. In October 1991 (open water), I took eight samples from shallow water sites and five from deep water sites; they were combined into single shallow and deep samples for statistical analysis. Additionally, in November and December 1991 and in January 1992, a different sampling programme was conducted. Six holes, five meters apart, were cut along the arm of the bay as a shallow sample. Deep samples were collected from holes cut in a line parallel to the shallow samples, but five meters towards the centre of the bay. These samples were taken to determine the distribution patterns of gammarids under the ice. Data obtained from these replicate samples were considered separately and then combined for statistical analysis. This set of data (October 1991-January, 1992) was considered independently from the other monthly data because of the replicated sampling procedures and because all gammarids collected were counted. This independent set of data was used to test some of the trends observed from the other monthly data, namely parasite prevalences and the proportion of juvenile gammarids in shallow and deep water sites.

The field-collected gammarids were examined in the laboratory soon after collection, to minimize the chances of *Gammarus* mortality and the development of acanthors into acanthellae. One to three hundred gammarids from each sample were killed in 70% alcohol, straightened and their lengths were measured from the base of the antennae to the tip of the telson. Gammarids collected from September, 1987 to November, 1988, were measured to the nearest mm using a ruler. These amphipods were classified as juveniles if their length was less than one cm, and as adults if greater than one cm (Menon, 1966). In samples collected after November, 1988, the gammarids were measured using an ocular micrometer (1 ocular unit=1.5 mm). These measurements were less precise than the ruler measurements but were more replicable than the former measurements. The sex of adult amphipods was determined based on the presence of ovaries or testes (primary sexual

Figure 2.1: Water temperatures at Cooking Lake, Alberta, 1989


30
25
20
15
10
5
0
Temperature (C)
ice cover
ice cover
J
F
M
A
M
J
J
A
S
O
N
D
Month

characteristics). The sex of smaller gammarids with immature ovaries or testes was based on the presence of rudimentary secondary sexual characteristics, oostegites in the females, second antennae calceoli and modified gnathopods in males (Menon, 1966). Gammarids lacking distinct sexual characteristics were considered to be juveniles. All amphipods were dissected and examined microscopically for the presence of parasites.

The distribution patterns of parasitized and uninfected gammarids from shallow and deep waters were compared among three seasons: open water in the fall (September-October), the winter under ice cover (November-March), and the spring open water period (April). The data for the ice free period were restricted to those collected in April because increasing water temperatures in spring trigger an increase in the prevalence of *P. marilis* infections due to the development of overwintering acanthors (Tokeson and Holmes, 1982). The seasonal (fall, winter, and spring) prevalences were based on data pooled from monthly collections (ie. pooled by date).

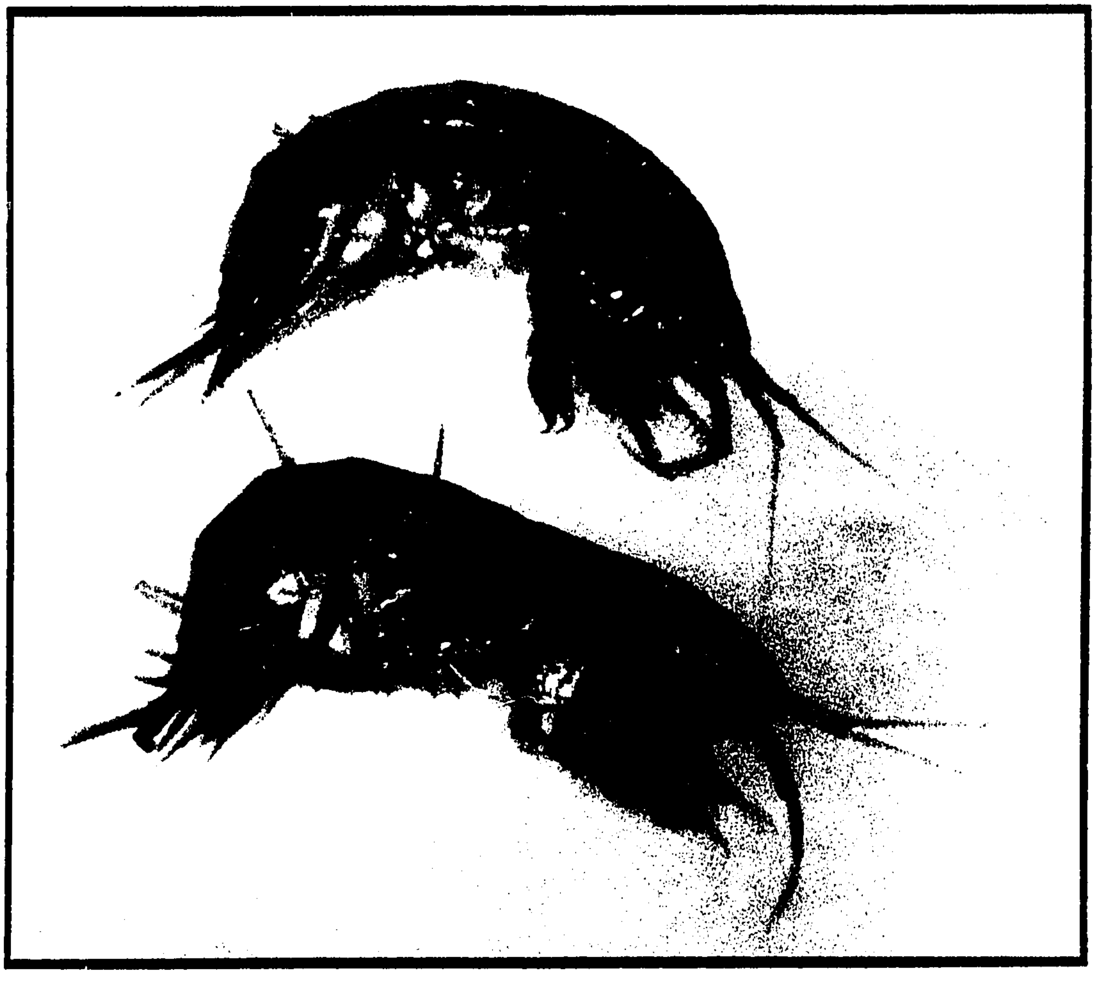
Laboratory Experiments

Several laboratory experiments were conducted in order to compare aggregative tendencies, and potential differences in habitat choices of infected and uninfected gammarids. Because there were differences in the lake distributions of *P. paradoxus*-infected, *P. marilis*-infected, or uninfected adults, and uninfected juveniles (see Field Observations results), the experimental groups were composed of either infected or uninfected adult males or of uninfected juveniles. Parasites are readily identified in live gammarids since the cystacanths of both parasites are bright orange and visible through the gammarids' cuticle. The cystacanths of *P. paradoxus* are larger than those of *P. marilis*, thereby allowing the distinction between parasites (Fig. 2.2). A subsample of half the uninfected gammarids used in each test was dissected at the end of each experiment to ensure their parasite-free status.

Aggregation Experiment

The tendency to aggregate was compared among four groups of gammarids: uninfected juveniles, uninfected adults, *P. paradoxus*-, and *P. marilis*-infected adults. All gammarids were field-collected in August, 1990 and all infections were naturally acquired. Twenty gammarids from each experimental group were allowed to acclimate in a shallow tray (21 x 33 x 5.5 cm) containing white sand (Burrico) and

Figure 2.2: *Gammarus lacustris* infected with a *Polymorphus marilis* cystacanth (top) and with a *P. paradoxus* cystacanth (bottom). Note the difference in cystacanth sizes.



three cm of water. The experiments were conducted in environmental chambers maintained at 18°C and 18L:6D photoperiod. The trays were photographed twice during the light hours, at 19 hours and 24 hours after the start of each experiment, to record the location of the gammarids. There were five to twelve replicate trays for each group.

Two types of data were obtained from the photographs and used for analysis of the spatial distribution of gammarids. The first was to examine the radial distribution in the trays. A measuring grid was constructed by drawing concentric rectangles that divided the tray into six zones of equal area on an acetate sheet. Full-size prints were made and the photographs were analysed by superimposing the acetate on each individual photograph and counting the number of gammarids contained in each zone. This initial analysis showed that most gammarids were located on the periphery of the trays (see the Results section). The second type of data, recorded only for gammarids found in the outermost zone (near the tray's edge), was the point on the tray's margin closest to the midpoint of each gammarid. Some photographs differed slightly in magnification, so the distance around the periphery of each tray was standardized to 360 units. An arbitrary origin was chosen and used in all analyses, and the distance in units around the margin of the tray from the origin to each gammarid was recorded using a digitizing tablet.

Analysis to detect aggregation, and the spatial scale at which it occurs, was modified by Dr. A. Shostak (University of Alberta) from a procedure in Pielou (1977). The procedure as described in Pielou (1977) repeatedly combines adjacent quadrats in a 2-dimension grid and calculates an index of aggregation (Morisita's I_δ) for each area of
1, 2, 4, 8 ...n quadrats:

$I_\delta = \delta / \delta_{RAN}$

where δ = the probability that two individuals chosen at random from the observed distribution belong to the same quadrat

δ_{RAN} = the probability that two individuals chosen at random from a random distribution belong to the same quadrat.

The value of this index is unaffected by quadrat size. In the absence of sampling error, if the observations are randomly distributed at a particular spatial scale, then $I_\delta = 1$, uniform distribution results in $I_\delta < 1$, and aggregation results in $I_\delta > 1$.

This procedure was applied to the gammarid observations in the following manner. First, for each photograph, I_δ was calculated for a range of spatial scales (1, 2, 3, ... 90, 120, 180 of the 360 radial units). Then, a randomization procedure was done to determine if I_δ values were significantly greater than 1. For each spatial scale, a sample of n hypothetical gammarid locations was drawn from a random distribution (where n = the number of gammarids around the tray's margin), and I_δ was calculated. This was repeated 10,000 times, and the I_δ values which excluded the upper and lower 2.5% of values were considered to represent 95% confidence limits. It was concluded that there was a significant degree of aggregation for a particular spatial scale if the observed I_δ value exceeded the upper 95% confidence limit.

Attraction/Avoidance Experiment

Many shallow water samples taken in winter (under ice) that contained *P. paradoxus*-infected gammarids, contained few uninfected adults. Such segregation between infected and uninfected gammarids may be the outcome of uninfected animals avoiding infected ones. To examine whether uninfected gammarids avoid parasitized amphipods, the distances maintained between pairs of uninfected gammarids, infected gammarids, and infected-uninfected animals were compared. This experiment was carried out in a modified gammarodrome box (Helluy, 1988), a plexiglass box (140 x 31.5 x 11 cm) divided using internal partitions to form 24 tracks (70 x 2.5 cm per track). The interior of the box and the partitions were painted white to increase the contrast between the gammarids and their background. The water depth was three cm. A pair of gammarids was introduced into the centre of each track and allowed to acclimate for three hours. After the acclimation period the tracks were photographed to record the position of each animal. Five combinations of pairs were used: two *P. paradoxus*-infecteds, two *P. marilis*-infecteds, two uninfected gammarids, one *P. paradoxus*-infected and one uninfected gammarid, and one *P. marilis*-infected and one uninfected amphipod. Single infected and uninfected gammarids were also tested to determine whether there were any biases in the distributions of individuals in the tracks. All gammarids used were field-collected in early April, 1992. Only males were used to eliminate the effects of sexual attraction, and because in the spring most of the *P. paradoxus*-infected gammarids collected were males. This experiment was conducted in an environmental chamber maintained at 2°C and 8L:16D, similar to under ice environmental conditions at Cooking Lake. There were 22 to 30 replicates for each combination, and 10 to 13 replicates for the single gammarids.

Substrate Choices

The preferences of infected and uninfected gammarids for different substrates were examined. Shallow trays (23.5 x 36 x 6 cm) were divided in half, each half containing substrate one cm deep and covered by three cm of water. The substrates used were fine sand, aquarium gravel (1-2 mm in diameter; Hagen), and small rocks (3-5 cm in length). The sand and gravel were similar to the substrate types existing at Lakeview, and the rocks were collected at Cooking Lake. Ten each of *P. paradoxus*-infected, *P. marilis*-infected, and uninfected adults and juvenile gammarids were tested in trays containing two substrate choices: rocks and sand, sand and gravel, or gravel and rocks. The gammarids were allowed to acclimate for four hours. The number of gammarids in each half of the tray (ie. on each substrate type) was recorded, as well as the proportion of gammarids that were swimming versus those that had settled on the substrate. All gammarids were collected in August, 1990. This experiment was conducted in an environmental chamber maintained at 18°C. There were six to nine replicates for each group but swimming-settlement observations were done for only four to eight replicates. Occasionally, dead gammarids were found at the end of the experiment, these animals were excluded from the analysis.

Depth Preferences

Water depth preferences of *P. paradoxus*-infected and uninfected male gammarids were examined in a tank (27 x 51 x 30 cm) with a gradient of increasing water depths. The tank was tilted so that the water level was one cm in the raised edge of the tank and 20 cm in the opposite end. The tank was divided into four zones of equal length and varying depths: 1-5, 5-10, 10-15, and 15-20 cm of water (A, B, C, and D zones, respectively). Single gammarids were placed in the centre of the tank and one cm below the water surface. The zone and the position in the water column of each gammarid were determined once an hour for a three hour period. The experiment was carried out at 2°C because all experimental animals were winter collected (November, 1992) and maintained in an environmental chamber kept at 2°C. There were 20 replicates for each experimental group.

Statistical analyses for all experiments were conducted using Statview 512 on a Macintosh computer. Probabilities of 0.05 or less were considered significant.

Results

Field Observations

The differential distribution of *P. paradoxus*-infected gammarids in shallow and deep water sites was compared seasonally (fall, winter, and spring) by pooling data from monthly collections (ie. pooled by date). In 1988-1989, the winter prevalence of *P. paradoxus* from both shallow and deep water sites varied among the monthly collections (X^2=442.76, df=8, *p*=0.0001 and X^2=86.48, df=8, *p*=0.0001). The data were pooled by season despite the heterogeneity among the collection dates because in all cases the prevalence of *P. paradoxus* was significantly greater in shallow (2.6-43.8%) than in deep (0.0-10.1%) water sites, and to increase sample sizes. The prevalence of this parasite was significantly greater in shallow than in deep water sites during fall, winter and spring 1988-1989, and during fall 1989-1990 (Fig. 2.3). Very few *P. paradoxus*-infected gammarids were found in the winter of the latter year, and the infection prevalences were indistinguishable between the shallow and deep waters. Infected gammarids were found only in shallow waters in both 1990-1991 and 1991-1992. In all study years, including those in which few infected gammarids were found in the winter, hundreds of *P. paradoxus*-infected gammarids were found clinging to vegetation and in the mud immediately following ice break. Parasitized gammarids were seldom found in deeper waters during spring. Thus, gammarids infected with *P. paradoxus* exhibit a preference for shallow water sites. Only cystacanths of *P. paradoxus* were found, and the parasite intensity was one.

The prevalence of *P. marilis* in shallow and deep sites was also compared seasonally (in fall, winter, and spring) by pooling data from monthly collection dates. Unlike the *P. paradoxus* prevalences, there were no statistical differences in *P. marilis* monthly prevalences within seasons, except among shallow samples from winter 1988-1989. Both acanthellae and cystacanths of *P. marilis* stages were found during the study periods, and the parasite intensity was one. Within each season, the *P. marilis* prevalence (combined prevalences of acanthellae and cystacanths) did not differ between shallow and deep waters (Fig. 2.4a and b). The prevalences of acanthellae and cystacanths were considered separately in 1990-1991 and 1991-1992 (Fig. 2.4c and d), and again, there were no differences in the prevalences between shallow and deep water sites. Thus, *P. marilis*-infected gammarids were equally distributed between shallow and deep water sites.

Figure 2.3: The prevalence of *Polymorphus paradoxus* infections in adult gammarids from shallow and deep water sites

* Significant differences between sites (X^2 test, $p<0.05$)

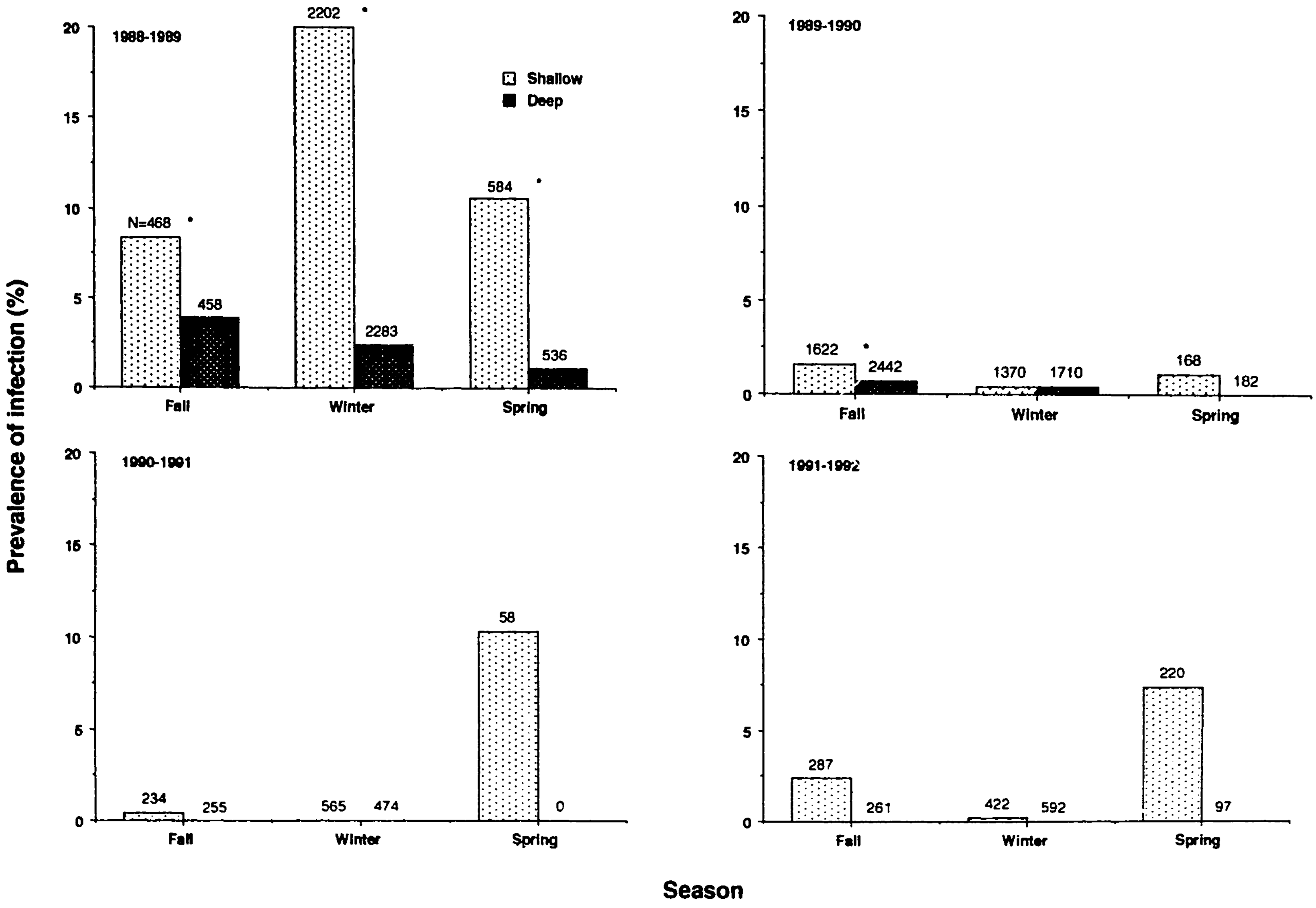
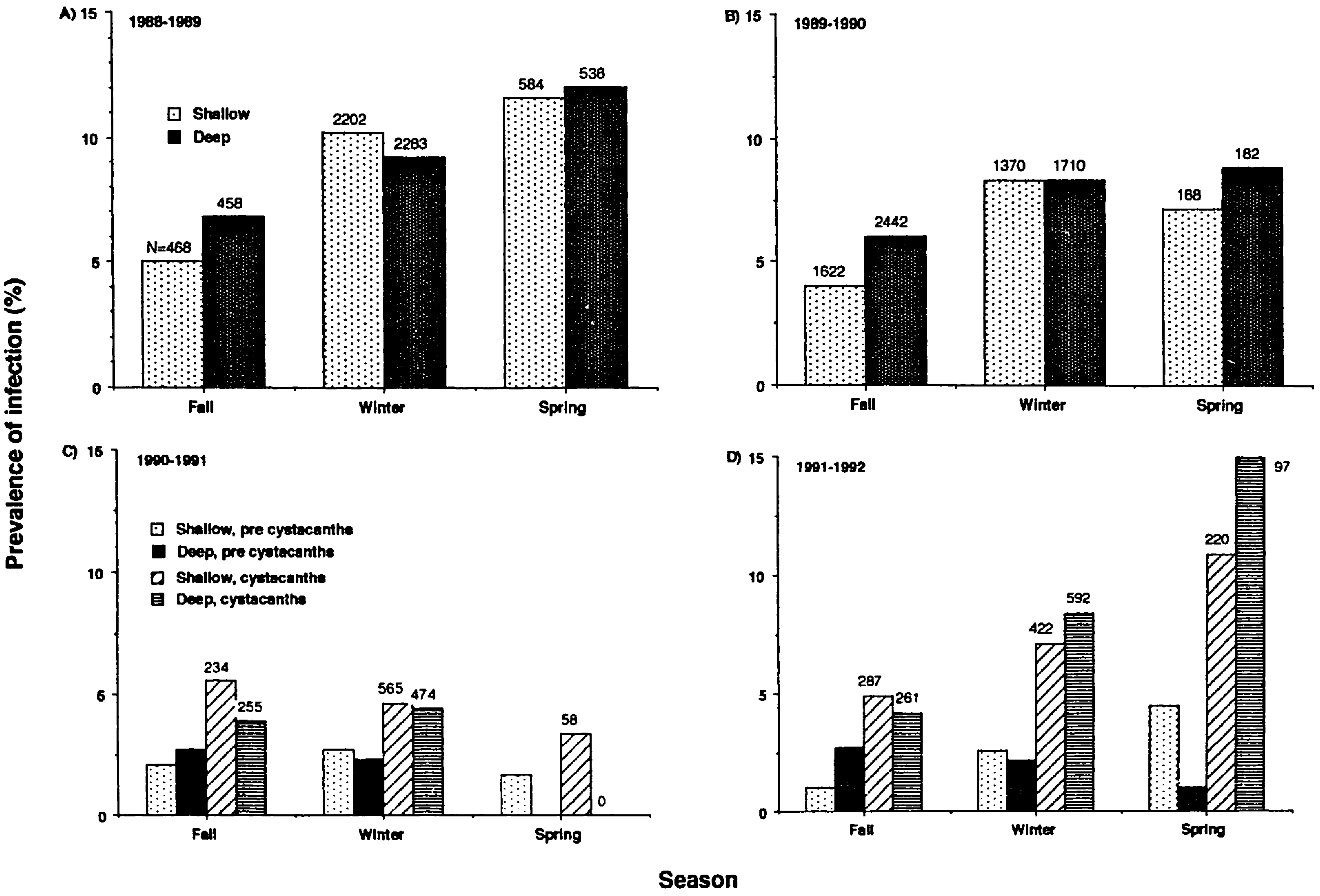

Prevalence of infection (%)
1988-1989
1989-1990
1990-1991
1991-1992
☐ Shallow
■ Deep
N=468
458
2202
2283
584
536
1622
2442
1370
1710
168
182
234
255
565
474
58
0
287
261
422
592
220
97
Fall
Winter
Spring
0
5
10
15
20
Season

Figure 2.4: The prevalence of *Polymorphus marilis* infections in adult gammarids from shallow and deep water sites (A and B are combined prevalences of acathellae and cystacanths)


Prevalence of infection (%)
A) 15
10
5
0
1988-1989
Shallow
Deep
N=468
458
2202
2283
584
536
Fall
Winter
Spring
B) 15
10
5
0
1989-1990
1622
2442
1370
1710
168
182
Fall
Winter
Spring
C) 15
10
5
0
1990-1991
Shallow, pre cystacanths
Deep, pre cystacanths
Shallow, cystacanths
Deep, cystacanths
234
255
565
474
58
0
Fall
Winter
Spring
D) 15
10
5
0
1991-1992
287
261
422
592
220
97
Fall
Winter
Spring
Season

The proportion of juveniles in the samples of *G. lacustris* population varied among sampling dates in all study years. However, data were pooled by season to compare the *Gammarus* age compositions in the shallow and deep water sites from which the parasite prevalence data were obtained. In the fall (except 1988-1989), significantly greater proportions of juveniles were found in shallow than in deeper waters (Fig. 2.5). Juveniles were also more abundant in shallow water during the winters of 1990-1991 and 1991-1992. The spring observations varied among the years, in 1988-1989 there were fewer juveniles in shallow water sites, but in spring 1991-1992, there were significantly more juveniles in shallow than in deep water. The data indicate that greater proportions of juveniles occur in shallow than deep water sites during fall and winter periods, but that this trend may reverse in spring.

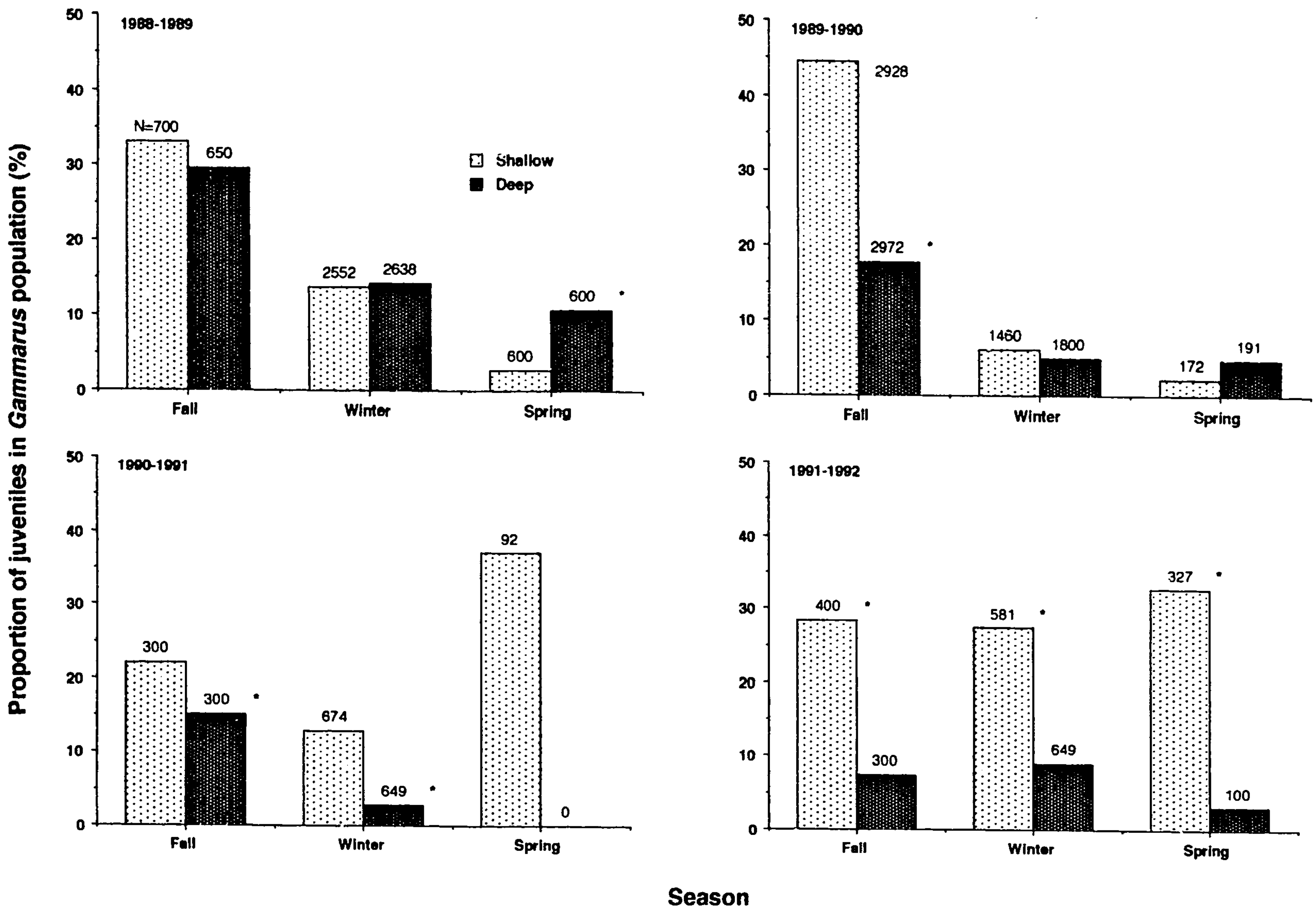
The sex ratios of the adult *Gammarus* populations from shallow and deep water sites were determined by comparing the proportions of females between the two sites for the 1990-1991 and 1991-1992 collections. The proportion of females was significantly greater in shallow than deep water in four out of five collections (Fig. 2.6); no deep water specimens were collected in the sixth (spring 1990-1991). Therefore, female gammarids tended to be distributed in shallower water than males.

The male to female length ratios of gammarids in precopula were compared between shallow and deep water sites (1990). These observations are based on relatively small samples (5-34 pairs of gammarids per sample) and obtained during a single year. The following results are based on the general trends found in these data. The length ratios were consistently greater in shallow than in deep water (Table 2.1). These differences were not significant within sampling dates or when the data from all sampling dates were pooled by depth (ANOVA, $p>0.05$); however, the trends were consistent during all sampling dates. Additionally, there was a significant correlation between the male and female lengths in three of four winter samples from deep water, but no such correlations in pairs collected from shallow sites (Table 2.1). There were significant correlations between the lengths of paired gammarids from both shallow and deep waters collected during the spring. Thus, males in shallow water may pair with smaller females than in deep water, and pairing may be random in shallow water but size assortative in deep sites.

The number of gammarids in holes under the ice was found to be highly variable, ranging from zero to more than a thousand animals. This indicated that

Figure 2.5: The proportion of juveniles in the *Gammarus lacustris* populations from shallow and deep water sites

* Significant differences between sites (X^2 test, $p<0.05$)



Proportion of juveniles in *Gammarus* population (%)
1988-1989
N=700
650
2552
2638
600
600
*
Shallow
Deep
Fall
Winter
Spring
1989-1990
2928
2972
*
1460
1800
172
191
Fall
Winter
Spring
1990-1991
300
300
*
674
649
*
92
0
Fall
Winter
Spring
1991-1992
400
*
300
581
*
649
327
*
100
Fall
Winter
Spring
0
10
20
30
40
50
Season

Figure 2.6: The proportion of females in the adult *Gammarus lacustris* populations from shallow and deep water sites

* Significant differences between sites (X^2 test, $p<0.05$)

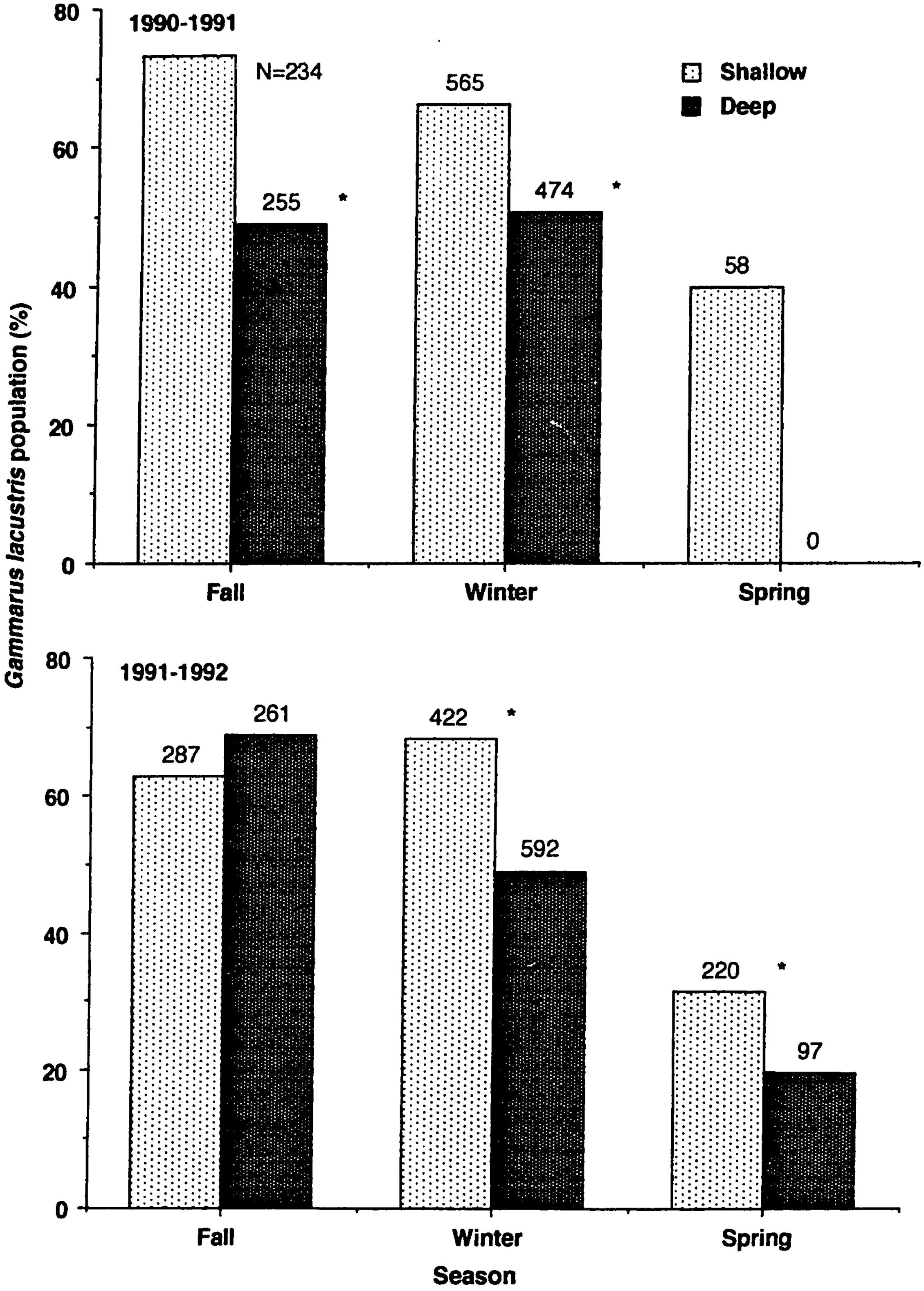

1990-1991
N=234
255
*
565
474
*
58
0
Shallow
Deep
Fall
Winter
Spring
1991-1992
287
261
422
*
592
220
*
97
Fall
Winter
Spring
Season
Gammarus lacustris population (%)
0
20
40
60
80

Table 2.1: Male to female length ratios, and the correlation between male and female lengths of paired gammarids from shallow and deep water sites (1990).

Date	Mean M:F ratio (N)		r, p	
	Shallow	Deep	Shallow	Deep
Under ice				
Feb. 2	N/A	1.22 (34)	N/A	0.37, 0.03*
Mar. 3	1.25 (30)	1.22 (10)	0.30, 0.10	0.69, 0.03*
Mar. 17	1.25 (33)	1.20 (9)	0.34, 0.06	0.14, 0.74
Mar. 31	1.28 (5)	1.24 (23)	0.09, 0.89	0.50, 0.02*
Apr. 4	1.29 (11)	N/A	0.58, 0.07	N/A
Open water				
Apr. 22	1.31 (7)	1.21 (20)	0.78, 0.04*	0.56, 0.01*

N/A No sample available

* Statistically significant correlation between male and female lengths

the gammarids were not randomly distributed in the study area. The distribution of *P. paradoxus*-infected gammarids was also found to be highly variable. Large numbers of infected gammarids were found only in the winters of 1988-1989 and 1991-1992. In both winters, hundreds of *P. paradoxus*-infected gammarids were found clinging to single roots of decaying vegetation, usually in areas very close to shore (areas which are vegetated in summer). It appeared that both infected and uninfected gammarids tended to have a non-random distribution in the winter.

In order to investigate clumped distributions, the total number of gammarids collected from each hole in the ice was determined. Collections from different holes in the ice yielded either very few gammarids (< 50) or over one hundred gammarids (Fig. 2.7). Table 2.2 is an example of the number of gammarids obtained from different holes from a single sampling date. The variance to mean ratio, as an index of dispersion (Elliott, 1971), was calculated for three sampling dates (November, December, 1991, and January, 1992) in which all the gammarids collected from each hole were counted; the distribution in all three sampling dates was clumped (Table 2.3). There was no correlation between water depth and the number of gammarids found per hole (November, $r^2 = 0.31, p = 0.06$, December, $r^2 = 0.005, p = 0.83$, and January, $r^2 = 0.09, p = 0.48$).

The data collected under the replicated sampling protocol (October, 1991-January, 1992) were considered independently from the other monthly collections and were used to test some of the patterns observed based on monthly collections (parasite prevalences and proportion of juvenile gammarids in shallow and deep water sites). The prevalence of *P. marilis* and the proportions of juveniles did not vary significantly among samples taken from shallow or deep water sites; very few *P. paradoxus*-infected gammarids were found, so that it was not possible to compare parasite prevalences among the replicate samples. Therefore, the data for each species were pooled for shallow and for deep samples. The results of the replicated sampling are shown in Table 2.4. The prevalence of *P. paradoxus* varied among the three collection dates; the prevalence was significantly greater in shallow than in deep water in the November sample, but not on the other two sampling dates. Therefore, these results are inconclusive. Gammarids infected with *P. marilis* were equally distributed in shallow and deep water sites. The proportions of juveniles in the samples were significantly greater in shallow than in deep waters, during both open water and ice cover periods. The distribution of *P. marilis*-infected gammarids

Figure 2.7: Aggregation of gammarids as indicated by number of gammarids collected per hole cut in the ice during winter

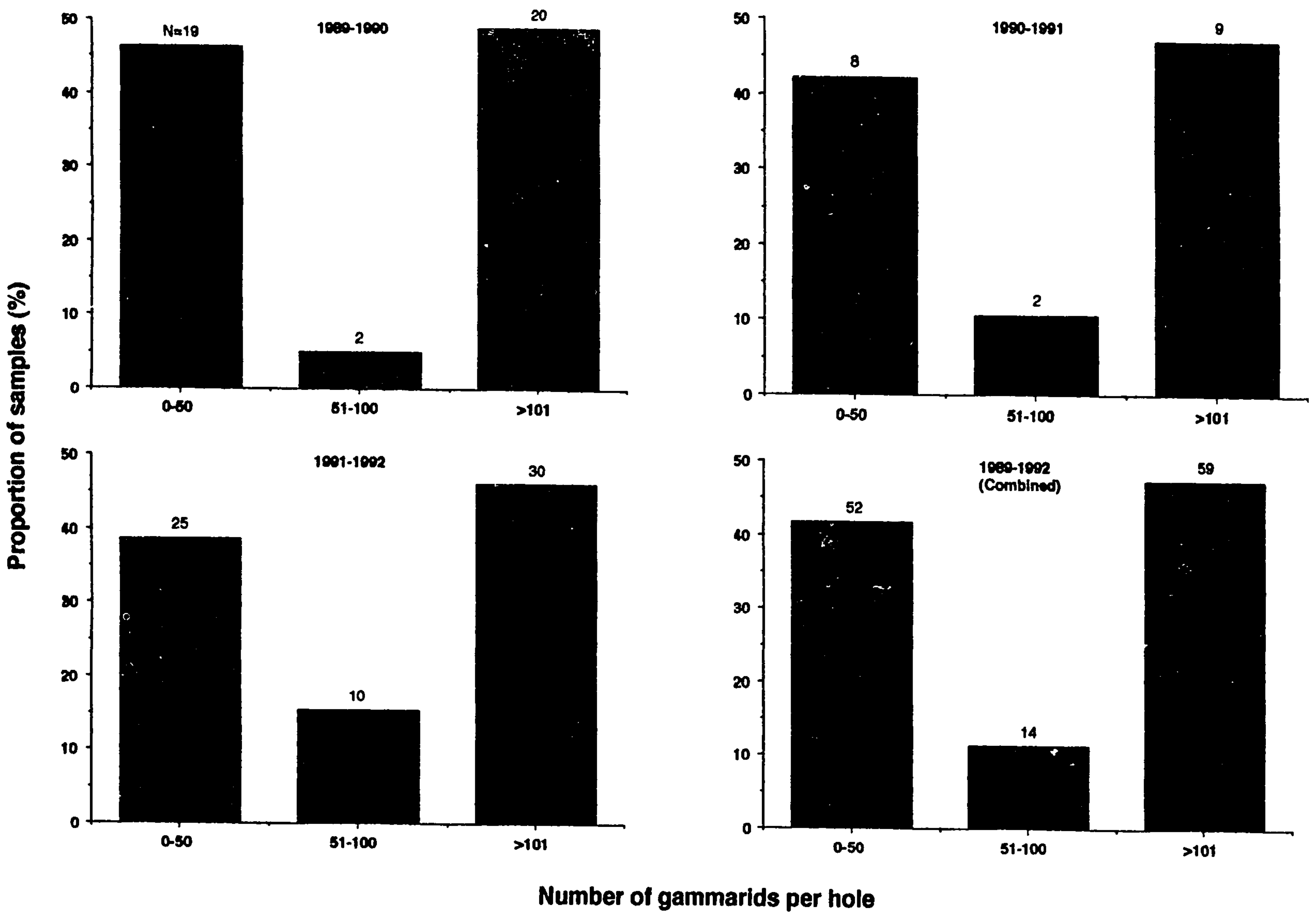

Proportion of samples (%)
Number of gammarids per hole
1989-1990
N=19
2
20
1990-1991
8
2
9
1991-1992
25
10
30
1989-1992
(Combined)
52
14
59
0-50
51-100
>101
0
10
20
30
40
50

Table 2.2: Example of clumped distribution of gammarids among different sites (holes in the ice) in winter (December, 1991).

Shallow sites			Deep sites*		
Ice depth (cm)	Water depth (cm)	Total number of gammarids	Ice depth (cm)	Water depth (cm)	Total number of gammarids
39	0.5	0	21	21	43
38	2	367	25	20	390
29	3	8	38	21	124
34	8	31	30	25	14
35	15	6	31	24	158
30	10	182	32	38	156

* Shallow and deep sites refer to near shore and further from shore collections, respectively. The deep holes were cut in a line parallel to the shallow ones.

Table 2.3: The clumped winter distribution of *Gammarus lacustris* under ice cover (based on variance to mean ratio as an index of dispersion)

Date	Variance / mean	X^2+	df	p
November, 1991	1,768.24 / 35.67	545.33	11	<0.005*
December, 1991	18,705.30 / 123.75	1,662.69	11	<0.005*
January, 1992	277,825.14 / 372	5,227.90	7	<0.005*

\+ $X^2 = S^2 (n-1) / \bar{x}$ (index of dispersion; Elliot, 1971)

* Clumped distribution

Table 2.4: Parasite prevalences and the proportion of juvenile gammarids in shallow and deep water sites (each date based on five to eight shallow and deep samples)

Date (condition)	**Parasite prevalence Number infected / total collected (%)**				**Proportion juveniles Number juveniles / total collected (%)**	
	P. paradoxus		***P. marilis***			
	Shallow	**Deep**	**Shallow**	**Deep**	**Shallow**	**Deep**
October, 1991 (open water)					233/559 (41.7)	18/250 (7.2)
					X^2=16.17, p=0.0001*	
November, 1991 (ice cover)	10/87 (11.5)	1/320 (0.3)	2/87 (2.3)	8/320 (2.5)		
	X^2=250.54, p=0.0001*		X^2=0.04, p=0.92			
December, 1991 (ice cover)	6/590 (1.0)	5/861 (0.6)	10/580 (1.7)	22/861 (2.5)	215/580 (37.1)	135/861 (15.7)
	X^2=0.93, p=0.34		X^2=1.10, p=0.30		X^2=89.29, p=0.0001*	
January, 1992 (ice cover)	4/1022 (0.2)	2/1766 (0.1)	36/1022 (3.5)	78/1766 (4.4)	501/1022 (49.0)	534/1766 (30.2)
	X^2=2.32, p=0.13		X^2=1.21, p=0.27		X^2=97.85, p=0.0001*	

* Significant differences between shallow and deep water sites

and of juveniles between shallow and deep water sites were similar to those obtained from the 1988-1992 monthly data.

Experimental Results

In the aggregation experiment, gammarids from all four experimental groups, *P. paradoxus*-, *P. marilis*-infected adults, uninfected adults and uninfected juveniles, showed a marked preference for the outer [illegible]6) of the tray (Table 2.5). The number of gammarids found in zone 6 was [illegible] greater than expected for 1/6 of the total tray area (X^2 tests, $p < 0.01$). N[illegible] gammarids used per trial could be identified in the photographs, resulting [illegible]er observations than the actual number of gammarids used.

The distribution of gammarids along the tray's edge was analyzed by comparing observed I_δ values to the upper 95% confidence limit for each quadrat size (see details in Materials and Methods). Such analysis examined general clumping patterns across a series of replicate tests. Clumped distributions were found in a high proportion of the observations (photographs), in 60.0% (9/15) of the photographs of *P. paradoxus*-infected, 47.8% (11/23) of *P. marilis*-infected, 56.3% (9/16) of uninfected adults, and 70.0% (7/10) of uninfected juveniles. There was little tendency to clump at a scale of 1-2 units (the size of a gammarid was about one unit); therefore, the gammarids were clumped but did not generally touch each other. I found no pattern in the aggregative tendencies of the four groups, clumped distributions were present at a variety of spatial scales, and no clear patterns of scale were found (Fig. 2.8). Some of the groups were found clumped in one photograph, but were randomly distributed in the other, indicating that temporal changes in the distributions of the same group of gammarids can equal the variation in patterns among trays of different categories of gammarids. Therefore, this analysis provided an indication of general clumping tendencies, but not of the spatial scale or spatial patterns at which clumping may occur. Given the variation within replicates, no difference in pattern of aggregation could be attributed to age or infection status of the host.

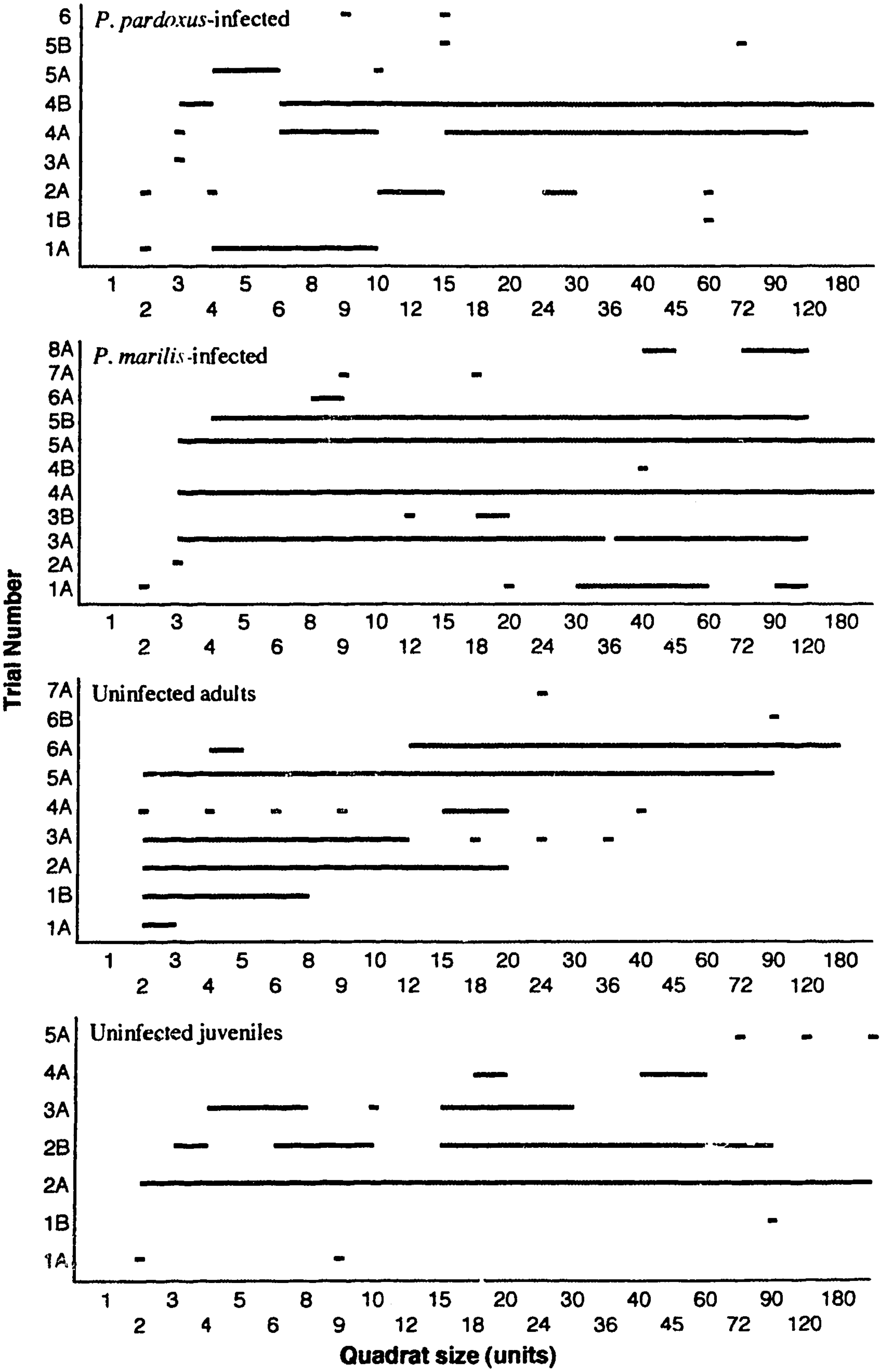
In the attraction/avoidance experiment, the distances of single *P. paradoxus*-, *P. marilis*-infected or uninfected gammarids from the ends of the track were similar for the three groups (ANOVA, $p = 0.29$). Thus, there were no differences in the positions of parasitized and uninfected gammarids in the test tracks. The distances

Table 2.5: The radial distribution of gammarids in a tray with six equal-area concentric zones; a comparison of the number of gammarids in the central five zones versus those in the outermost zone.

Zone	Number of gammarids per zone			
	P. paradoxus-infected	*P. marilis*-infected	Uninfected juveniles	Uninfected adults
Zones 1-5	104*	203*	40*	126*
Zone 6	157	228	109	131

* Significant difference between observed and expected numbers in the outermost zone; X^2 test, $p<0.01$

Figure 2.8: The distribution of gammarids at different spatial scales (Aggregation experiment). Bars denote the spatial scale at which gammarids were clumped (index of aggregation, Morisita's $I_\delta>1$). Each trial group was examined twice (A and B); in some trials, a clumped distribution was observed only once, and is represented by either A or B (the first and second photographs, respectively). For details on procedure and analysis see "Materials and Methods".



P. pardoxus-infected
6
5B
5A
4B
4A
3A
2A
1B
1A
P. marilis-infected
8A
7A
6A
5B
5A
4B
4A
3B
3A
2A
1A
Uninfected adults
7A
6B
6A
5A
4A
3A
2A
1B
1A
Uninfected juveniles
5A
4A
3A
2B
2A
1B
1A
1 3 5 8 10 15 20 30 40 60 90 180
2 4 6 9 12 18 24 36 45 72 120
Trial Number
Quadrat size (units)

between individuals were highly variable in paired trials. Therefore, instead of comparing absolute distances, the proportion of pairs found with less than a one-half-track distance between individuals were compared among the test groups. Over 50% of all pairs were found with less than half track distance between individuals (Table 2.6). There were no statistical differences among the five experimental groups ($X^2 = 4.27$, df = 4, $p = 0.37$); uninfected gammarids did not avoid infected gammarids.

All gammarids, whether parasitized adults, uninfected adults, or uninfected juveniles, displayed a significant preference for rocks over gravel or sand substrates (X^2 tests, $p < 0.01$; Table 2.7). There were no differences among the four experimental groups in the proportion of gammarids found on rocks versus gravel or sand substrates (X^2 tests, $p > 0.05$). None of the experimental gammarids showed a significant preference for sand over gravel substrates; however, the proportion of uninfected juveniles that chose the gravel substrate was significantly greater than those of *P. paradoxus*-infected and uninfected adults (X^2 tests, $p < 0.05$).

Gammarids from all four experimental groups settled, rather than swam, significantly more often over rock than sand or gravel substrates (Table 2.8). Gammarids found over sand or gravel substrates swam as often as they settled. There were no significant differences in the settlement versus swimming behaviour of parasitized adults, uninfected adults, or uninfected juveniles. The sole difference was found in the rock/sand choice experiment, where the proportion of *P. paradoxus*-infected gammarids found swimming over the rock substrate was significantly greater than that of gammarids from the other three groups (X^2 test, $p < 0.05$).

Polymorphus paradoxus-infected and uninfected gammarids, when given a choice of shallow water depths, were found almost exclusively on the bottom of the tank and in its deepest section (section D, 15-20 cm of water). The proportions of parasitized gammarids found in section D were 16/20 (80.0%), 11/19 (57.9%), and 12/20 (60%) during the first, second, and third hours of the experiment, respectively. Similarly, uninfected gammarids were found in zone D, 16/20 (80%), 14/19 (73.4%), and 16/20 (80.0%), during the first, second, and third hours of the experiment, respectively. There were no differences in the water depth choices of parasitized and uninfected adult gammarids.

Table 2.6: Attraction between infected and uninfected male *Gammarus lacustris* (*G.l.*) tested in a multi-track apparatus. Pairs of males were placed in each track and the distance between individuals measured. The proportion of pairs with less than one-half track distance between them was used to signify aggregation. No significant differences were found between pairs of infected or uninfected males (X^2 test), or among distances of single individuals from the track's end (ANOVA, $p>0.05$)

Gammarids	*G.l.* at <1/2 track distance between individuals Proportion of pairs (%)	Distance of single *G.l.* from track end (cm) $\bar{x} \pm sd$ (n)
P. paradoxus & *P. paradoxus*	15/22 (68.2)	
P. paradoxus & uninfected	20/25 (80.0)	
P. marilis & *P. marilis*	14/26 (53.8)	
P. marilis & uninfected	17/27 (63.0)	
Uninfected & uninfected	21/30 (70.0)	
P. paradoxus		7.4 ± 9.5 (11)
P. marilis		10.1 ± 7.5 (10)
Uninfected		8.0 ± 8.5 (13)

Table 2.7: Substrate preferences of infected and uninfected gammarids

Gammarids	Substrate choices					
	Rock N (%)	Gravel N (%)	Rock N (%)	Sand N (%)	Gravel N (%)	Sand N (%)
P. paradoxus-infected adults	46 (78)	13 (22)*	47 (78)	13 (22)*	29 (48)	31 (52)
P. marilis-infected adults	48 (69)	22 (31)*	47 (66)	24 (34)*	39 (56)	31 (44)
Uninfected juveniles	63 (71)	26 (29)*	69 (78)	20 (22)*	59 (66)	31 (34)+
Uninfected adults	76 (84)	14 (16)*	75 (84)	14 (16)*	33 (38)	55 (62)

* Significant differences between substrates (X^2 test, $p<0.05$)

\+ Significant differences between substrate choice of juvenile and those of *P. paradoxus*-infected and uninfected adult gammarids (X^2 test, $p<0.05$)

Table 2.8: Settlement behaviour of infected and uninfected gammarids on different substrates

Gammarids	Activity	Number of gammarids on substrate					
		Rock	Gravel	Rock	Sand	Gravel	Sand
P. paradoxus-infected adults	Swimming	7	7*	10+	6	11	10
	Settled	23	3	21	3	8	11
P. marilis-infected adults	Swimming	8	19*	6	17*	17	13
	Settled	40	13	41	7	22	18
Uninfected juveniles	Swimming	3	5	8	12*	10	13
	Settled	38	14	38	2	25	12
Uninfected adults	Swimming	5	5*	5	9*	13	13
	Settled	66	6	62	5	15	39

* Significant differences between substrates, Fisher's Exact Test, $p<0.01$

\+ Infected gammarids swam significantly more often over rock substrate than the other three groups (X^2 test, $p<0.05$)

Discussion

Gammarids infected with *P. paradoxus* were found in different habitats than *P. marilis*-infected and uninfected gammarids during the winter months. These are the first recorded findings of *P. paradoxus*-infected gammarids in winter. Under ice-cover, *P. paradoxus*-infected gammarids inhabited shallow water sites whereas *P. marilis*-infected gammarids were equally distributed between shallow and deep water sites. In winter, gammarids parasitized by *P. paradoxus* were often found in roots along the shoreline, particularly in areas with abundant emergent summertime vegetation. Similarly, *P. paradoxus*-infected gammarids prefer shallow vegetated regions, and those infected with *P. marilis* are evenly distributed in the lake in the summer (Tokeson, 1971; Bethel and Holmes, 1973). Thus, *P. paradoxus* alters the distribution and the habitat of infected gammarids not only during the summer months, but also during the winter period.

Shallow water sites were also the preferred habitat for small gammarids, ie. juveniles and small females. Females collected from deep sites were significantly larger than those from shallow water sites (Chapter 3). Uninfected males were more commonly associated with deep water sites; however, there were no length differences in males collected from shallow and deep waters (Chapter 4). Winter habitat of *G. lacustris* can be affected by both *P. paradoxus* infections and by amphipod size. There is little information on the winter habitat of gammarids in northern temperate lakes. Freshwater invertebrates can overwinter in boreal bodies of water by freezing into the ice or sediment (freeze-tolerant organisms), or by undergoing seasonal lateral movements to deeper waters (Olsson, 1982). The amphipods in Olsson's (1982) study underwent lateral migrations, and those that were trapped in ice did not survive thawing. Similarly, fish in winterkill lakes redistribute themselves by either moving towards the top of the water column (region with greatest dissolved oxygen (DO) concentration), or they move horizontally to plumes of oxygenated inlets or springs (Magnuson *et al.*, 1989). *Gammarus lacustris* can survive winter in anoxic lakes (Menon, 1966; de March, 1981), but gammarids die in the laboratory under anoxic conditions (Tokeson and Holmes, 1982). Thus, such lakes must have some regions, such as the ice-water interface or gas bubbles in the ice, which contain some DO. Menon (1966) suggested that *G. lacustris* may overwinter near the mud layer. However, I found that most gammarids welled up immediately after holes were cut in the ice, and Ekman samples yielded very few gammarids. It is more likely that *G. lacustris* overwinters at the ice-water interface than near the mud layer. This is a common overwintering behaviour of many aquatic poikilotherms in hypoxic and/or anoxic water bodies, since DO concentrations are highest at the ice-water interface (Clifford, 1969; Magnuson *et al.*, 1985; 1989).

Gammarids were not randomly distributed in the lake under ice-cover. Instead, both infected and uninfected gammarids were found to have patchy distributions during the winter, and exhibited aggregative distributions in laboratory tests. *Polymorphus paradoxus*-infected gammarids were aggregated on roots to which they were clinging. The distribution of *P. marilis*-infected and uninfected gammarids aggregates were not predictable, and patch size could not be determined by the sampling technique used.

Aggregated distributions are common to many aquatic invertebrates, including gammarids, and can be the outcome of animals congregating in preferred habitats (Miller and Buikema, 1977; Gee, 1982; Pringle, 1982). The preferred summer habitats of *G. lacustris* are shallow shoreline areas with emergent and submergent vegetation which provide gammarids with shelter (Menon, 1969). The numbers of gammarids found in open water in Big Island Lake (or other lakes with clear water) are negligible, and amphipods in open water in such lakes show a marked preference for the lake bottom (Menon, 1969). In contrast, because of the high turbidity at Cooking Lake, gammarids are distributed throughout the water column in all parts of the lake, but as in Menon's (1969) study, they show a preference for shallow near-shore regions (Bethel, 1972). Preferred habitats of stream gammarids are often dependent on the substrate particle size. There is a positive relationship between *G. pulex* length and substrate size, with larger gammarids associating with larger substrate particles (Birkhead and Clarkson, 1980; Gee, 1982; Pringle, 1982; Thompson and Moule, 1983). Substrate size is important as it provides shelter from predators and water currents, and because food availability (organic matter) differs with substrate.

The aggregated winter distribution of *G. lacustris* may be the outcome of their presence in preferred winter habitats. Habitat preferences may be dependent on one or several environmental factors such as substrate size and quality, or oxygen availability. The preferences for rock over sand or gravel substrates of infected and uninfected gammarids were identical and could not account for the differences in winter habitats of *P. paradoxus*-infected and uninfected gammarids. However, only three substrates were tested and the tests were conducted at summer temperatures. Other substrates such as mud or roots, and lower temperatures may result in different responses by infected and uninfected gammarids. Alternately, gammarids may be clumped in regions of DO availability, such as ice-water interface or gas bubbles. The available winter substrate in the lake is relatively uniform (mud and ooze) and the winter temperatures are constant (Fig. 2.1; Tokeson, 1971). Conversely, DO concentrations may vary with water depth, algal concentrations (Mitchell and Prepas,1990), or aquatic mammal activities (Magnuson *et al.*, 1985). It is therefore more likely that aggregation of uninfected and *P. marilis*-infected gammarids is the result of differences in oxygen availability rather than substrate preferences.

The differences in distribution of large (males and large females) and small (juveniles and small females) gammarids may be the outcome of differences in their responses to low DO concentrations. Costa (1967) found that young *G. pulex* were less sensitive than adults to reduced oxygen concentrations. Nevertheless, younger gammarids could detect and avoid hypoxic water but their reactions were very much delayed (up to five times slower than older gammarids). Smaller *G. lacustris* may also be slower to react to declining DO concentrations and therefore remain in shallower waters longer than the migratory adults. *Polymorphus paradoxus*-infected gammarids may also respond to low DO concentrations more slowly than uninfected adults, and therefore overwinter in the same regions as juveniles. Smaller gammarids may also have lower metabolic demands than larger amphipods and be better able to overwinter in shallower and more hypoxic waters.

Polymorphus paradoxus-infected gammarids may be aggregated in response to cues other than DO availability. Large numbers of infected gammarids were found only in regions that had roots and/or sticks in the mud layer. In summer, *P. paradoxus*-infected gammarids prefer habitats with emergent vegetation or other materials to which they can cling (Holmes and Bethel, 1972; Bethel and Holmes, 1973). These habitats also appear to be preferred by infected gammarids in the winter. Since regions with roots are limited and restricted to the shoreline, *P. paradoxus*-infected gammarids may be found aggregated in such substrates and in shallow waters.

Aggregative behaviour of gammarids may not be solely the outcome of preferred habitat. Gammarids were clumped along the periphery of trays with a uniform substrate (aggregation experiment) indicating their attraction to each other. Additionally, in the attraction/avoidance experiment, over 50% of *G. lacustris* pairs were less than one-half-track distance from each other, and no evidence of avoidance of infected gammarids was found. The cues that may result in attraction between male gammarids are unknown, but aggregation may be an amphipod adaptation allowing gammarids to locate regions of favorable habitat. That is, if an individual gammarid randomly encounters a desirable habitat other amphipods will be attracted to that individual and also profit from that habitat.

Polymorphus paradoxus may also alter the behaviour of infected gammarids in winter. Infected gammarids did exhibit photophilia, skimming and clinging behaviours in the laboratory at low temperatures (2°C; my unpublished observations), but altered photic responses probably do not affect winter distribution because the shoreline at Lakeview had more ice and snow cover than the rest of the lake and was therefore the region with least light penetration. Since *P. paradoxus*-infected gammarids are attracted to the brightest regions (Bethel and Holmes, 1973), it is unlikely for them to overwinter in the darkest region of the lake. Infected gammarids may be responding to under-ice disturbances, such as muskrats and beavers, underground springs, or human activities. However, the skimming and clinging responses (Bethel and Holmes, 1973) elicited by disturbances are not long lasting (maximum of 16 hours; Helluy, 1988) and may be insufficient to dramatically alter the winter distribution of infected gammarids.

The overwintering habitat of gammarids may be related to their survival and reproductive success. Overwintering *P. paradoxus*-infected gammarids were in shallow regions that froze earlier in the winter and may be frozen to the bottom of the lake. It is not clear whether parasitized gammarids remain in the frozen vegetated substrate or if they move to somewhat deeper waters. There is no evidence that gammarids can survive freezing; it is more likely that gammarids would die if frozen into the substrate. For example, tens of *P. paradoxus*-infected gammarids found along the frozen shoreline in November, 1991 were dead. Parasitized females are very scarce in the spring and are thought to be more susceptible to winter mortality than *P. marilis*-infected and uninfected females (Chapter 3). Therefore, near-shore sites may be more stressful overwintering environments than sites further from shore, predisposing gammarids that overwinter in the former sites (parasitized gammarids) to greater winter mortality than individuals in deeper waters.

Although *P. paradoxus*-infected gammarids in shallow water may be subjected to greater mortality than gammarids in deeper water sites, many do survive until spring. Shoreline regions become ice-free earlier than the rest of the lake and migratory birds, muskrats and beavers feed in these open-water regions. Thus, *P. paradoxus*-infected gammarids are available to predators as soon as the ice breaks. This parasite also overwinters as a cystacanth and is thus infective to its aquatic mammal definitive hosts during the winter, and immediately following ice break. Potentially, *P. paradoxus* may be transmitted in the winter to mammalian hosts (muskrats and beavers), thus the presence of infective cystacanths in winter may increase the transmission of this parasite. Indeed, adult *P. paradoxus* were found in the intestines of winter-collected muskrats and beavers from Cooking Lake (personal observations); however, it was not possible to determine whether the infections were acquired during the winter. The overwintering strategy of *P. paradoxus* may be two-fold; to alter winter distribution of infected gammarids to predispose them to predation in the spring by both avian and mammalian hosts, and to make infective larvae available for winter transmission to the resident definitive hosts.

In contrast to *P. paradoxus*-infected gammarids, those parasitized by *P. marilis* were equally distributed in deep and shallow water sites during the winter. These differences in distribution of infected gammarids are likely the outcome of differences in the degree of behavioural alterations caused by the two parasites. Whereas *P. paradoxus* causes grossly altered photic and evasive responses in infected gammarids, *P. marilis* only affects photic responses (Bethel and Holmes,1973). *Polymorphus marilis* overwinters primarily as an acanthor and its peak prevalence and transmission occur in June (Tokeson, 1971), not in the spring. Overwintering as an acanthor may minimize the energetic demands of the parasite on the host and ensures a source of infective larvae in the summer (Tokeson and Holmes, 1982). However, I found no evidence for selective winter mortality of gammarids bearing acanthella or cystacanth of *P. marilis* (Chapters 3 and 4) suggesting that this parasite does not negatively affect the overwintering survival of amphipods.

Overwintering in shallow waters did not appear to affect the susceptibility of uninfected small gammarids to winter mortality. Although winter mortality was significantly greater in juveniles and females than in the larger male gammarids, these differences in mortality occurred in both shallow and deep water sites (Chapters 3 and 4). However, without specific population estimates, it is difficult to draw conclusions regarding differential survivorship between sites.

The potential reproductive success of gammarids overwintering in shallow waters may be lower than that of those in deeper waters. Although females were more abundant in shallow than in deep waters, the available females in shallow sites were smaller than those in deep waters. In shallow water sites, males did not appear to select females based on size and therefore, may have paired at random (no correlation between male and female lengths); however, pairing in deep water was not random (significant correlations between male and female lengths). Male gammarids can assess female quality and prefer to pair with larger and more fecund females (Dick and Elwood, 1989). Precopulatory pairing may be random in shallow waters because in the absence of larger, more fecund females the males may not invest time in assessing females and will pair with any sexually mature female they encounter. The overwintering of *P. paradoxus*-infected males in regions abundant with juveniles and small females may impair their ability to search for and pair with fecund females.

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Chapter 3 - Reproductive consequences of parasitism with *Polymorphus paradoxus* and *P. marilis* (Acanthocephala) for their female intermediate host, *Gammarus lacustris* (Crustacea)

Introduction

That parasites can reduce the reproductive success of their hosts was recognized over a century ago (see review by Hurd, 1990). Barnard (1990) listed five ways in which a parasite may reduce the reproductive output of its host: parasitic castration (the alteration or destruction of the host's gonadal tissue; Baudoin, 1975), brood loss, reduced attractiveness or inclination of a partner to mate with an infected animal, reduced longevity, and the costs of counter-adaptation to the parasite (eg., immunity). The costs of counter-adaptation is not a direct measure of reduced fecundity, and the consequences of energy or resources being diverted from reproduction to defense against the parasite may affect any of the other four reproductive aspects. A wide variety of parasites, including protozoa, helminths and arthropods, can affect reproduction of their hosts. A detailed discussion of parasite induced reduction of host reproduction is provided in Chapter 1 (Introduction). In this study I examined the effects of two acanthocephalan parasites on the reproduction of their female amphipod intermediate host.

Many parasites interfere with their hosts' reproduction by diverting energy used in host reproduction into parasite production (Baudoin, 1975). Therefore, it is expected that parasites will affect those reproductive features in which the host invests the most energy. Females expend much energy in producing gametes; therefore, ovarian development is often affected by parasitism (Baudoin, 1975) Females also have long reproductive periods and often provide parental care; therefore, there is often a reduction in the secondary sexual characteristics and behaviours involved in parental care.

Acanthocephalans exert a variety of effects on the reproductive biology of their crustacean intermediate hosts, ranging from no pathology to complete sterilization (Kennedy, 1985). Much of the literature documents effects of acanthocephalans on the reproductive capacity of female amphipods and isopods (Superorder Peracarida). Parasitism may reduce body size of peracaridians (but see Oetinger and Nickol, 1981), and reduce or inhibit ovarian development, often resulting in sexually immature females, with altered development of secondary sexual characteristics, such as small, bristleless oostegites (eg. Hynes, 1955). Infected females may bear smaller (Poulton and Thompson, 1987) or no egg broods (Muzzall and Rabalais, 1975; Moore, 1983), or may be less attractive than uninfected females to potential mates (Ward, 1986). The effects of infection on the reproductive capacity of their hosts appear to be independent of cystacanth size (Kennedy, 1985). Most of the studies of acanthocephalan infections show some reduction in the reproductive success of infected female hosts (Table 3.1). Acanthocephalans appear to influence reproduction in female hosts by directly affecting gonadal development rather than through altering internal secretions such as hormones (see Chapter 1).

The fecundity of female amphipods is dependent on normal ovarian development, that is the ability to produce gametes and to develop secondary sexual characteristics necessary to bear a brood. In gammarid species in which females are receptive for only short periods of time after moulting, and where fertilization is possible only until the female's cuticle hardens (Conlan, 1991; Sutcliffe, 1992), males guard the females to ensure a mating opportunity following the females' moult (Elwood *et al.*, 1987; Elwood and Dick, 1990). Male gammarids guard their mates by inserting their gnathopods between the female's thoracic segments and carrying her until she moults and can oviposit (Conlan, 1991; Sutcliffe, 1992).

There has been considerable research on aspects of female quality that determine the mate choices of male gammarids (eg. Ridley and Thompson, 1985; Ward, 1992). Male peracaridians incur several costs while mate guarding: energetic costs, due to the transport of passive females and their defence from other males (Manning, 1980; Elwood *et al.*, 1987), and temporal costs since they cannot pair with other females while guarding (Elwood *et al.*, 1987). Therefore, males assess the female's utility (the number of eggs fertilized per reproductive unit) prior to pairing (Manning, 1975; Dick and Elwood, 1988). Males prefer to pair with larger females since they bear more eggs, have larger broods, and oviposit earlier than smaller females (Manning, 1975; Ridley and Thompson, 1985; Ward, 1988). Female gammarids do not actively participate in pair formation; however, larger females are more active and can resist pairing attempts of unsuitable (small) males (Ward, 1984).

The present study is a comparative examination of the effects two acanthocephalan parasites have on reproduction of female gammarids. *Polymorphus paradoxus* and *P. marilis* use *Gammarus lacustris* as an intermediate host (Denny, 1969). The life cycle of these two acanthocephalans and the behavioural alterations they induce in their *G. lacustris* host have been previously documented (Chapter 1). *Gammarus lacustris* has a univoltine life cycle in Alberta lakes (Canada). The

Table 3.1: The effects of acanthocephalan infections on the growth and reproduction of female peracaridians

Parasite & Host	Growth	Ovary	Secondary Sexual Characteristics	Fecundity+	Pairing Success	References
Acanthocephalus dirus						
Asellus intermedius *Lirceus garmani*	L	R	?	Sterile	R	Oetinger & Nickol 1981
A. jacksoni						
L. lineatus	L	R	?	Sterile	R	Muzzall & Rabalais, 1975 Oetinger & Nickol, 1981
Leptorhynchoides thecatus						
Hyalella azteca	N	R	N	N	?	[illegible] 1951 Uznanski & Nickol, 1980
Plagiorhynchus cylindraceus						
Armadillidium vulgare	S	R	?	Sterile	?	Moore, 1983
Polymorphus minutus						
Gammarus duebeni	?	R	N	Sterile	R	LeRoux, 1933
G. lacustris	S	R	Bristleless oostegites	Sterile	R	Hynes, 1955
G. pulex	S	R	Bristleless oostegites	Sterile	R	Hynes & Nicholas, 1963 Ward, 1986
Pomphorhynchus laevis						
Gammarus pulex	?	?	?	R	N	Poulton & Thompson, 1987

S - smaller; L - larger; R - reduced; N - no effect; ? - information unavailable
+ Females may be partially or completely sterile

reproductive behaviour and ecology of *G. lacustris* in Alberta lakes was described previously (Menon, 1966; Chapter 1). Briefly, pairing activity of *G. lacustris* peaks in early spring following ice thaw, coincident with the period of the final moult which results in ovulation. Ovigerous and brooding females are found in the spring and early summer and juveniles are released in June. Most of the adult population dies following mating and brooding, although some post-reproductive adults survive. Juvenile gammarids attain secondary sexual characteristics in the fall and form the breeding population during the following winter and spring (Menon, 1966).

I examined four of the five ways listed by Barnard (1990) in which parasites may reduce their hosts' reproductive output: 1. parasitic castration, based on the presence of ovaries and bristled oostegites; 2. brood loss, based on decreases in brood sizes of infected *G. lacustris*; 3. attractiveness to mates, as pairing success of infected and uninfected female *G. lacustris* in the lake and in laboratory tests; and 4. longevity, as the overwintering survival of infected female gammarids.

Materials and Methods

Gammarids were collected from Cooking Lake, Alberta, following the methods outlined in Chapter 2. Monthly samples were taken from December 1989 to September 1992, and from April-May, 1993. The gammarids were measured and necropsied following the procedure outlined in Chapter 2. Females were identified based upon the presence of ovaries, brood pouches bearing eggs or immatures, and the presence of bristled oostegites (Menon, 1966).

The number of eggs produced per female is dependent on her size (Hynes and Harper, 1972; de March, 1981). I compared the female reproductive output (the number of eggs or immatures borne) with female length. Eggs were removed from brood pouches of gravid females and counted. Regression of numbers of eggs or immatures on female lengths were used to compare brood sizes of *P. marilis*-infected and uninfected females.

Two laboratory experiments were conducted to compare the relative attractiveness of infected and uninfected females to uninfected males. Healthy, previously paired (to ensure their ability and inclination to pair) males were provided with one *P. paradoxus* or *P. marilis*-infected and one uninfected female, of similar sizes. Paired gammarids were separated by gently dislodging the females from the males' grasp. The choice of the male was assessed based on the female with which it paired. In 'alone' experiments, single males were provided with a single uninfected or parasitized female. The first experiment was conducted in April, 1990 and lasted 15 days. The second experiment was done in May, 1992 and lasted 120 days. All the gammarids used were field collected and naturally infected. The gammarids were acclimated to the environmental chambers for three to 14 days. The experiments were conducted in plastic cups, provided with 200 ml of water, a sand substrate and unlimited flake food (Tetramin). The animals were kept in environmental chambers at 4°C and 8L:16D light cycle. The cups were examined daily to establish when a pair formed and the male's choice of a female. Gammarids that died before the end of the experiment were excluded from analysis.

The proportion of infected and uninfected female gammarids found in precopula in the lake was used to assess pairing success, as a measure of attractiveness to males. The peak pairing periods differed among study years. The pairing success data were obtained from April in 1990, April-May in 1991, March-April in 1992, and April, 1993.

Length distributions of *P. marilis*-infected and uninfected female gammarids were compared during the pairing periods: January-May in 1990, March-May in 1991, March-April in 1992 and April-May in 1993. Sampling was done at shallow water sites near shore and deeper water sites further from shore. Length distributions for shallow and deep water sites were examined separately.

Brooding success, an additional estimate of attractiveness to mates, was expressed as the proportion of infected and uninfected females bearing broods of eggs or juveniles. It was determined during the peak brooding periods of May-June 1990-1993. Additional data were available from *G. lacustris* collections made during June to July of 1971-1974 (J. C. Holmes, unpublished). These data were used to increase the available data base and to compare brooding success between the 1990's and 1970's data. In 1971-1974 gammarids were collected from Cooking Lake and Hastings Lake, Alberta. Hastings Lake (53°25'N 113°55'W) is smaller (surface area 8.71 km^2) and deeper (mean depth 2.4 m) than Cooking Lake (Mitchell and Prepas, 1990). Hastings Lake is hyper-eutrophic and its aquatic macrophytes and benthic invertebrate compositions are similar to those of Cooking Lake (Kerekes, 1965). The average amphipod biomass is greater in Hastings Lake (Kerekes, 1965), and unlike Cooking Lake, fish (yellow perch) do survive winterkill conditions (anoxia) in this lake (Mitchell and Prepas, 1990). Hastings Lake is frequented by ducks such as lesser scaup and mallards, which provide a source of transmission for acanthocephalan infections.

The 1990's and 1970's collection techniques differed. The 1970s collections were from open water (at least 1 m deep) sites, obtained by vigorous stirring of a dip-net to ensure the inclusion of all gammarids from the bottom layers and throughout the water column. The proportion of breeding females from these data sets was based on records of broods (egg or immatures), and females bearing spines (bristles) on their oostegites (an indication that a brood was borne). These data were obtained during the period of brood release (Menon, 1966), therefore, the presence of bristled oostegites was used as indirect evidence of brooding.

The availability of adult females for breeding was determined by calculating the F:M ratio during the brooding periods of 1971-1974 and 1990-1992. The F:M ratio was not determined in 1993 because data were available for only a single collection taken early in the brooding period (early May).

Quantitative sampling that would enable calculation of gammarid densities was not done; thus overwinter survival (reduced longevity) could not be measured directly. The proportions of females that were uninfected or infected with *P. paradoxus* and *P. marilis* were compared between fall and spring periods to obtain an indirect measure of the ability of infected animals to overwinter. Monthly data were pooled for the two periods: fall (open water) based on September-October collections, and spring (ice free) based on April collections. The data for the ice free period were restricted to those collected in late April because increasing water temperatures in the spring (beginning in May) trigger an increase in the prevalence of *P. marilis* infection due to the development of overwintering acanthors (Tokeson and Holmes, 1982). *Polymorphus marilis* prevalences were based on presence of acanthellae and cystacanths; overwintering acanthor stages are too small to detect reliably using a dissecting microscope.

Statistical analyses were conducted using the following computer software packages:

- X^2 tests, G-tests and linear regressions using Statview (Macintosh version)
- Analysis of covariance (ANCOVA) and least square means using SuperAnova (Macintosh version)
- Fisher's exact tests and analysis of variance (ANOVA) using Systat for Windows (version 5).

Probabilities of 0.05 or less were considered significant.

Results

Parasitic Castration

All females found in the 1990s and 1970s, whether infected with *P. paradoxus* or *P. marilis*, possessed ovaries, and ovigerous *P. marilis*-infected females had bristled oostegites. Thus, there was no evidence that infection with either parasite affected the development of gonads or secondary sexual characters in female gammarids.

Reduced Fecundity

There was a positive relationship between egg number and female length for both *P. marilis*-infected and uninfected females collected in 1990, 1992 and 1993 (Fig. 3.1). The regressions accounted for 30-50% of the variance. In each year, the regression slopes of the infected and uninfected animals were not significantly different but the adjusted means did differ (ANCOVA, $p<0.05$). Females infected with *P. marilis* consistently bore three to five fewer eggs than uninfected conspecifics of the same length (Table 3.2).

In 1971-1974, there were significant positive correlations between brood size and female length for the uninfected females ($p<0.01$; Fig. 3.2). Unlike the regression values obtained in 1990-1993, the 1970s regressions accounted for only 6-12% of the variance. No significant correlations were found between brood size and female length for P. *marilis*-infected females. Analysis of pooled data from 1971-1974 also did not reveal a significant correlation between egg number and length of *P. marilis*-infected females. Based on the adjusted means of brood size, *P. marilis*-infected females consistently bore fewer eggs than uninfected ones (12.7±9.3 vs. 20.5±9.3, in 1971; 15.6±11.2 vs. 23.4±10.6, in 1972; 5.0±8.1 vs. 14.0±8.0, in 1973; and 12.8±9.8 vs. 22.5±9.8, in 1974. eggs or immatures per *P. marilis*-infected and uninfected females, respectively). Thus, based on both the 1990s and 1970s data, brood sizes of *P. marilis*-infected females are reduced by parasitism. However, the reduction in brood sizes of *P. marilis*-infected females could not be calculated based on the adjusted means from the 1970s data, because the regression slopes for the infected and uninfected groups were not the same.

Reduced Attractiveness to Mates

Males, given a choice between a *P. marilis*-infected and an uninfected female, preferred to pair with the uninfected female (G-test, $p<0.005$; Table 3.3). Males never paired with *P. paradoxus*-infected females when given a choice of an

Figure 3.1: B[illegible] (number of eggs) versus length of *Polymorphus marilis*-infected and uninfected female *Gammarus lacustris* (in 1990, 1992 and 1993)

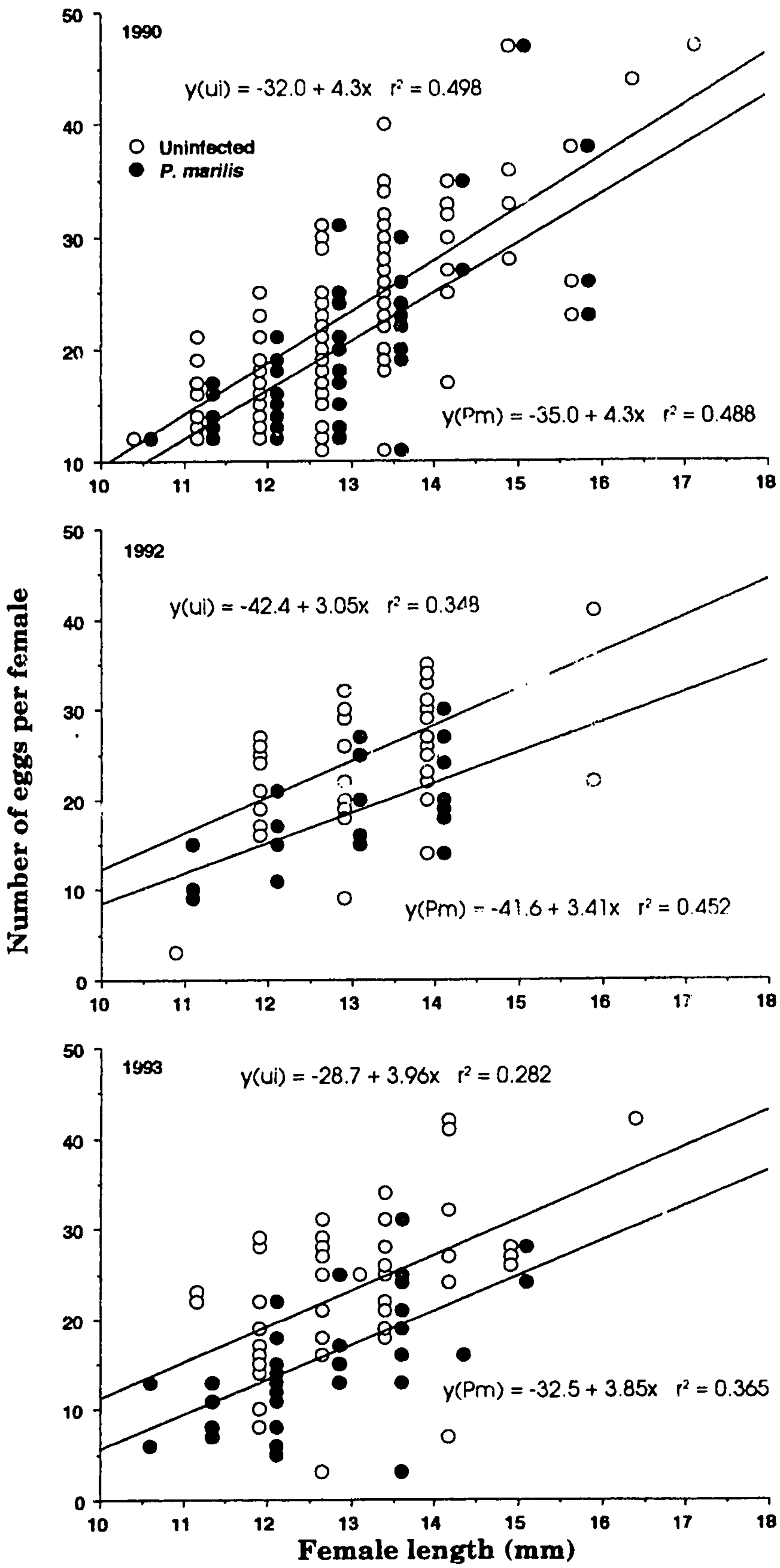

1990
y(ui) = -32.0 + 4.3x r² = 0.498
Uninfected
P. marilis
y(Pm) = -35.0 + 4.3x r² = 0.488
1992
y(ui) = -42.4 + 3.05x r² = 0.348
y(Pm) = -41.6 + 3.41x r² = 0.452
1993
y(ui) = -28.7 + 3.96x r² = 0.282
y(Pm) = -32.5 + 3.85x r² = 0.365
Number of eggs per female
Female length (mm)
0
10
20
30
40
50
10
11
12
13
14
15
16
17
18

Table 3.2: Analysis of covariance comparing brood sizes of *P. marilis*-infected and uninfected female *G. lacustris*

Year	Adjusted Means		Ancova	
	Count	$\bar{x}$ ± sd	F	p
1990				
Uninfected	56	24.6 ± 5.2	12.40	0.0006
Infected	52	21.1 ± 5.2		
1992				
Uninfected	50	25.1 ± 5.6	10.24	0.002
Infected	20	20.3 ± 6.1		
1993				
Uninfected	50	22.2 ± 6.6	13.59	0.0004
Infected	38	17.0 ± 6.7		

Figure 3.2: Brood size (number of eggs or immature gammarids) versus length of *Polymorphus marilis*-infected and uninfected female *Gammarus lacustris* (in 1971-1974)

* Statistically significant regression, $p<0.01$

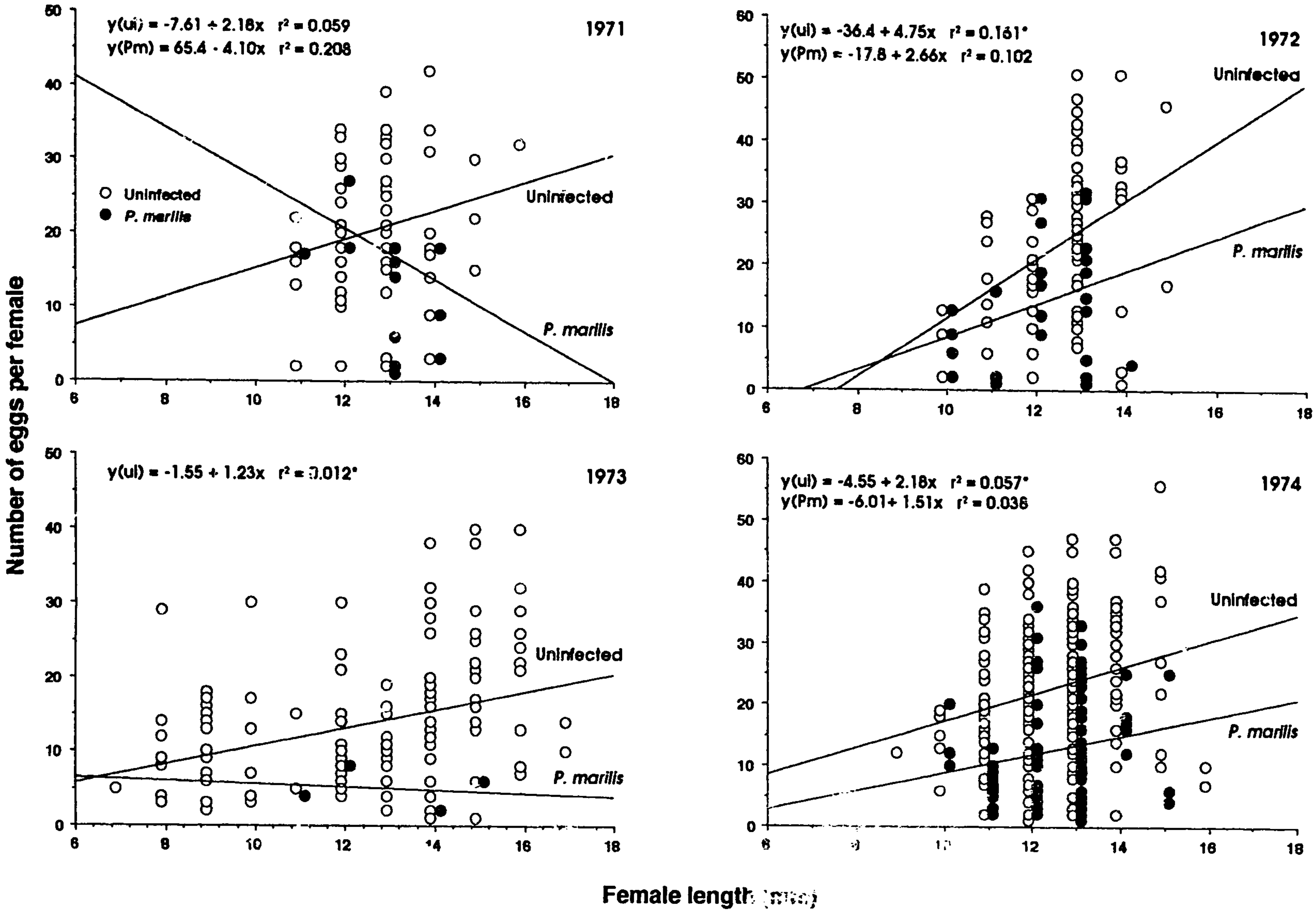

Number of eggs per female
Female length (mm)
1971
y(ui) = -7.61 + 2.18x r² = 0.059
y(Pm) = 65.4 - 4.10x r² = 0.208
○ Uninfected
● P. marilis
Uninfected
P. marilis
1972
y(ui) = -36.4 + 4.75x r² = 0.161*
y(Pm) = -17.8 + 2.66x r² = 0.102
Uninfected
P. marilis
1973
y(ui) = -1.55 + 1.23x r² = 0.012*
Uninfected
P. marilis
1974
y(ui) = -4.55 + 2.18x r² = 0.057*
y(Pm) = -6.01 + 1.51x r² = 0.036
Uninfected
P. marilis
0
10
20
30
40
50
60
6
8
10
12
14
16
18

Table 3.3: Pairing success of infected and uninfected female gammarids in choice experiments (a single male given the choice between an infected and an uninfected female) and alone (a single male presented with either an infected or uninfected female)

Experiment	Number of infected paired / total number or pairs formed	Total number of pairs formed / total number of set-ups (%)
Choice		
P.marilis		
Experiment 1	4/10 (40.0)	10/14 (71.4)
Experiment 2	2/14 (14.3)*	14/18 (77.8)
Combined 1+2	6/24 (25.0)*	24/32 (75.0)
P. paradoxus		
Experiment 2	0/9 (0.0)	9/14 (64.3)
Alone		
P.marilis		4/15 (26.7)
P. paradoxus		1/5 (20.0)
Uninfected		4/15 (26.7)

* Significant differences between infected and uninfected females (G-test, $p<0.005$)

unparasitized one. In choice experiments, over 65% of the males paired; however, few pairs formed in the 'alone' experiment (males provided with a single infected or an uninfected female). In the 'alone' experiment, which tested the attractiveness of infected females to males in the absence of uninfected females, *P. marilis*- and *P. paradoxus*-infected females had the same pairing success as the uninfected ones. The low pairing success and the small sample sizes make it difficult to interpret the 'alone' experiments results. However, males do choose to pair with uninfected females over parasitized ones.

The pairing success (proportion of females found in precopula) of uninfected females in the lake varied among years, ranging from 34 to 86% of the females (Table 3.4). Overall, *P. marilis*-infected females had similar pairing success to uninfected ones. The sole exception was in 1993 when the pairing success of *P. marilis*-infected females was greater than that of unparasitized animals (X^2 test, $p<0.05$). Overall, the pairing success of *P. paradoxus*-infected females was significantly lower than that of either uninfected or *P. marilis*-infected females (Fisher's exact test, $p<0.0$[illegible] In three of four years no parasitized females were found in precopula, but as previously mentioned, very few overwintering *P. paradoxus*-infected females were recovered in the spring. Parasitism with *P. marilis* did not affect the pairing success of infected females; however, the pairing success of *P. paradoxus*-infected females was greatly reduced.

Because male gammarids prefer to pair with larger females (Dick and Elwood, 19[illegible]d the length distributions of the available *P. marilis*-infected and unin[illegible]es. The female length distributions were significantly affected by collecting site (shallow and deep) and collection year, but not by the presence of infection (ANOVA, $p<0.05$; Table 3.5). Females from shallow sites were significantly smaller than those from deeper water sites. Most of the females were in the 1.05 and 1.2 cm length classes (Fig. 3.3) and few individuals < 1 cm were sexually mature (bearing ovaries and oostegites). Few females attained lengths > 1.5 cm; most of these were uninfected (Fig. 3.3). There was no significant difference in any year between the lengths of parasitized and unparasitized females. Thus, there is no evidence that *P. marilis*-infected gammarids were less attractive to potential mates because they were smaller.

However, when I compared the proportion paired between *P. marilis*-infected and uninfected females in each length class (based on data pooled from 1990-1993), the pairing success of infected females was lower in 5 out of 8 length classes in which paired *P. marilis*-infected females were found (Fig. 3.4). The reduced pairing

Table 3.4: The pairing success of *P. paradoxus*-, *P. marilis*-infected, and uninfected female gammarids at Cooking Lake

	Pairing Success Number of paired females / total number of female gammarids (%)				
	1990 (Apr)	**1991 (Apr-May)**	**1992 (Mar-Apr)**	**1993 (Apr)**	**Total**
P.paradoxus-infected	0/5 (0.0)	0/2 (0.0)	0/1 (0.0)	1/7+ (14.3)	1/15+ (6.7)
P. marilis-infected	6/6 (100.0)	4/14 (28.6)	6/17 (35.3)	68/74* (91.9)	84/111 (75.6)
Uninfected	91/105 (85.7)	27/80 (33.8)	47/105 (44.8)	908/1113 (81.6)	1073/1403 (76.5)

* Significant differences between the pairing success of *P. marilis*-infected and uninfected females (X^2 test, $p<0.005$)

\+ Significant differences between the pairing success of *P. paradoxus*-infected and uninfected females (Fisher's Exact test, $p<0.001$)

Table 3.5: Analysis of variance comparing the lengths of *Polymorphus marilis*-infected and uninfected female gammarids among years (1990-1992), and between shallow and deep water sites

Source	Sum of squares	df	Mean square	F-ratio	p
Year	0.321	2	0.161	11.644	0.000
Site	0.090	1	0.090	6.538	0.011
Infection	0.001	1	0.001	0.052	0.819
Year*site	0.011	2	0.005	0.382	0.683
Year*infection	0.036	2	0.018	1.294	0.275
Site*infection	0.000	1	0.000	0.031	0.861
Year*site*infection	0.009	2	0.005	0.339	0.713
Error	19.446	1410	0.014		

Figure 3.3: Length distribution of *Polymorphus marilis*-infected and uninfected female *Gammarus lacustris* from shallow and deep water sites during pairing periods (no deep water sample was available from 1993). Continued on next page.

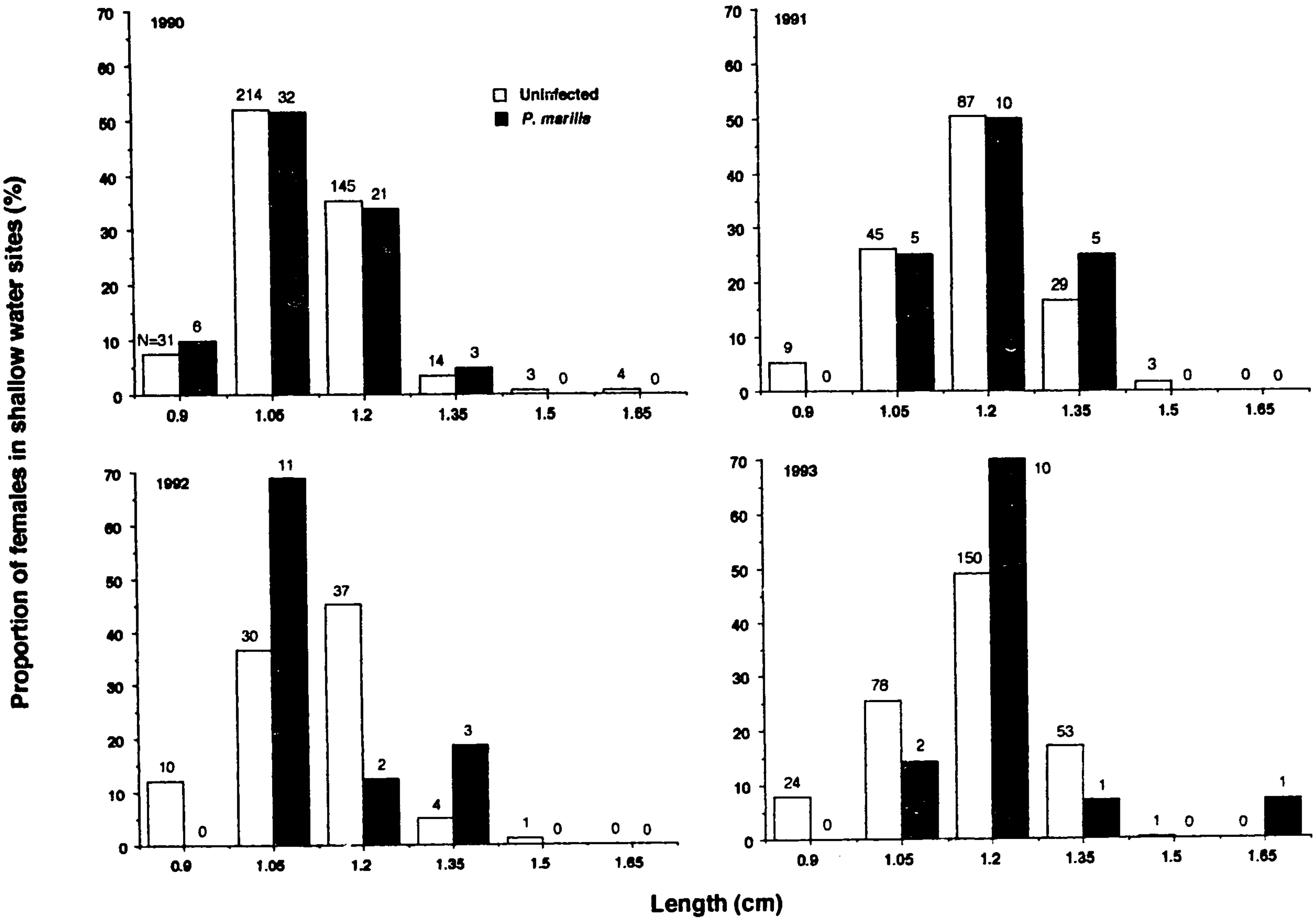

Proportion of females in shallow water sites (%)
Length (cm)
☐ Uninfected
■ P. marilis
1990
1991
1992
1993
70
60
50
40
30
20
10
0
0.9
1.05
1.2
1.35
1.5
1.65
N=31
6
214
32
145
21
14
3
3
0
4
0
9
0
45
5
87
10
29
5
3
0
0
0
10
0
30
11
37
2
4
3
1
0
0
0
24
0
78
2
150
10
53
1
1
0
0
1

Figure 3.3: Continued

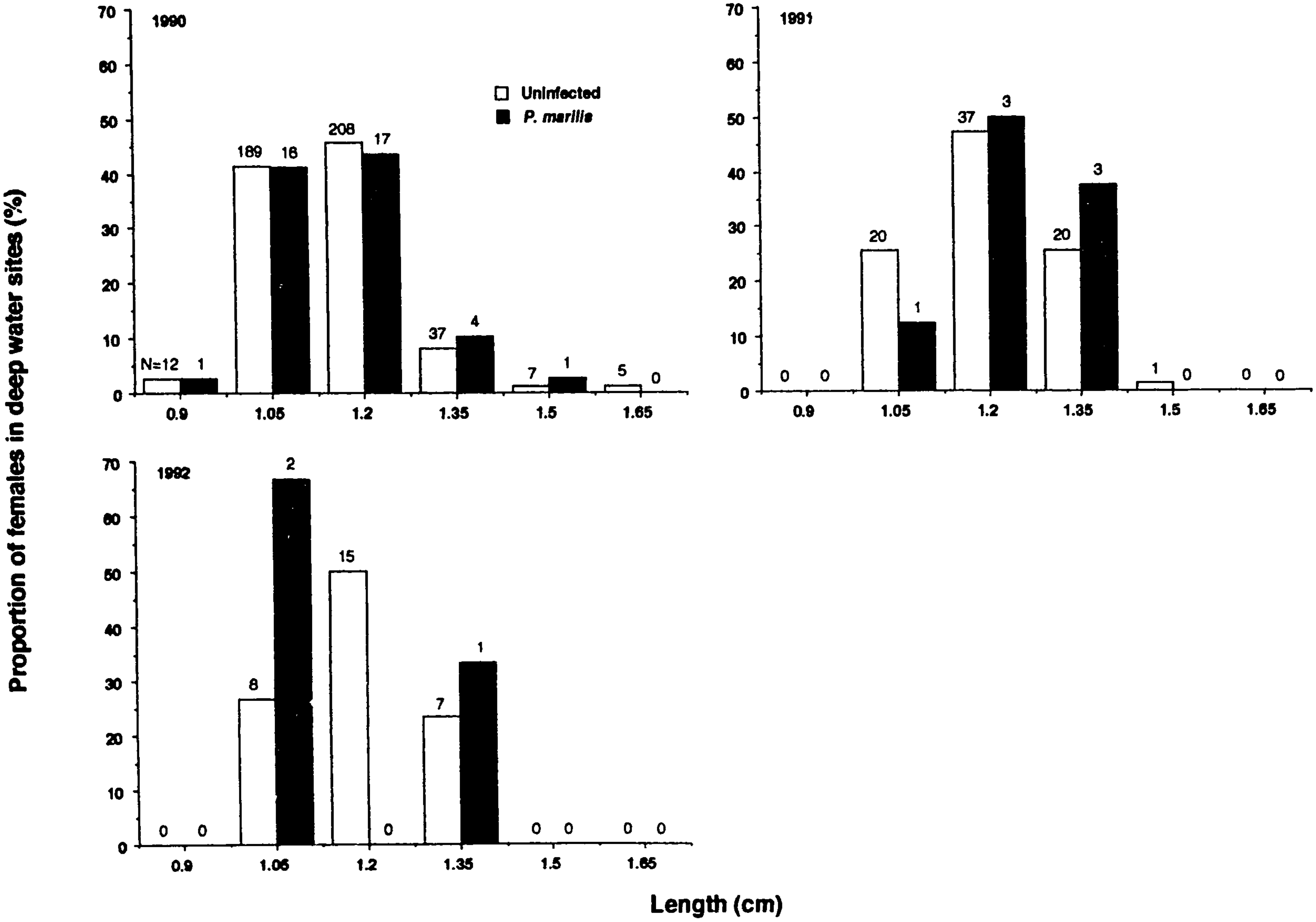
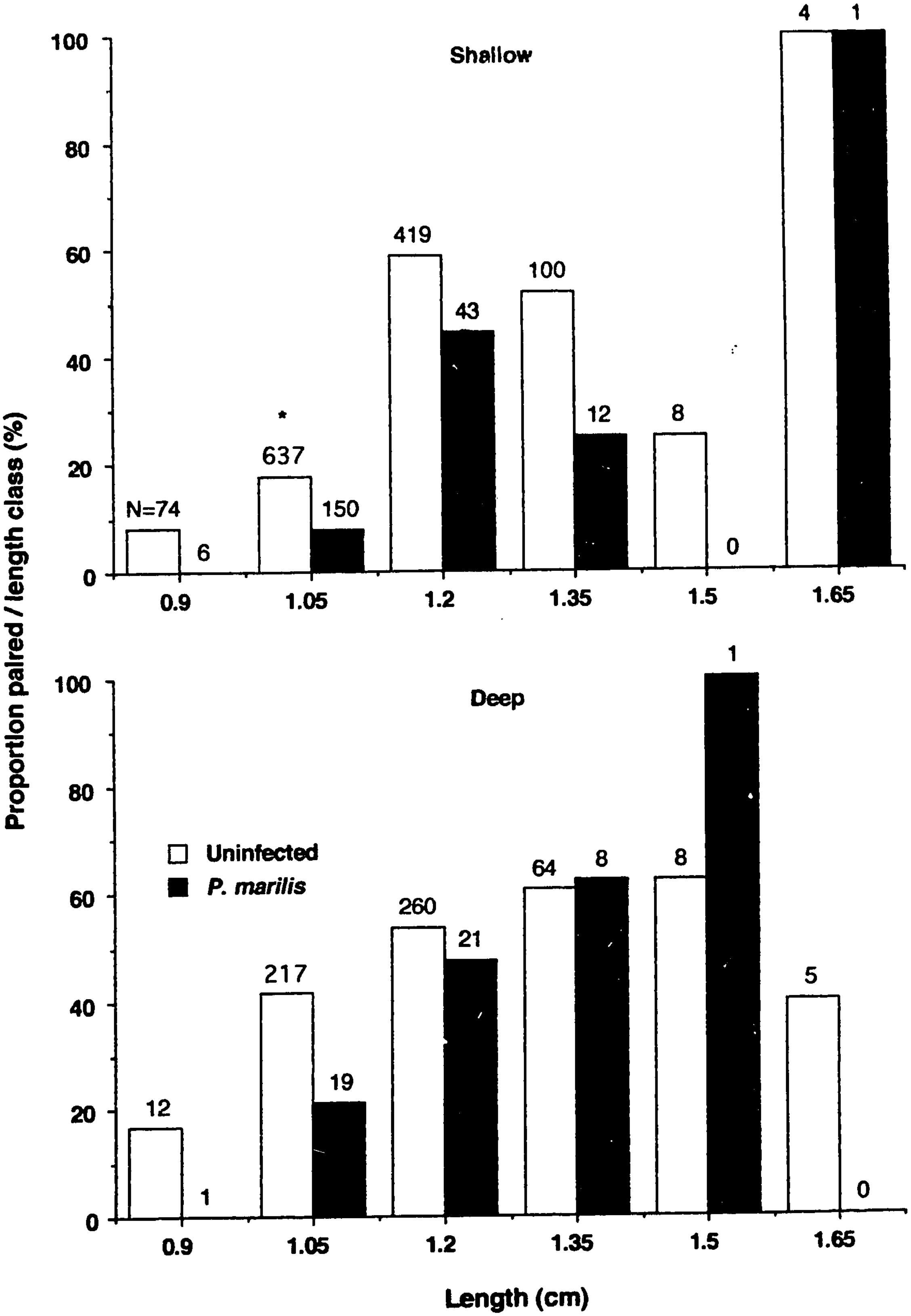

Proportion of females in deep water sites (%)
Length (cm)
1990
1991
1992
Uninfected
P. marilis
0
10
20
30
40
50
60
70
0.9
1.05
1.2
1.35
1.5
1.65
N=12
1
189
16
208
17
37
4
7
1
5
0
20
1
37
3
20
3
1
0
8
2
15
0
7
1

Figure 3.4: The proportion of *Polymorphus marilis*-infected and uninfected female *Gammarus lacustris* in precopula in each length class (data pooled across years)

* Significant differences between infected and uninfected females (X^2 test, $p<0.001$)


Shallow
Deep
Proportion paired / length class (%)
Length (cm)
0
20
40
60
80
100
0.9
1.05
1.2
1.35
1.5
1.65
N=74
6
*
637
150
419
43
100
12
8
0
4
1
12
1
217
19
260
21
64
8
8
1
5
0
□ Uninfected
■ P. marilis

success was most noticeable in the smaller length classes. Infected females in the 0.9 cm class were never paired. However, pairing success of *P. marilis*-infected females was significantly lower than that of uninfected ones in only a single case, the 1.05 cm class from shallow collections. Males may discriminate against small *P. marilis*-infected females, but the attractiveness of larger infected females was not affected.

In all study years (1990's) uninfected females had a high brooding success (bearing egg or immature broods), with over 80% of females bearing broods (Fig. 3.5). In three of the four years, females infected with *P. marilis* had levels of brooding success similar to those of uninfected females. However, in 1992, a significantly greater proportion of *P. marilis*-infected gammarids bore broods (X^2 test, F=4.77, p =0.03). The proportion of uninfected and *P. marilis*-infected females with broods and/or bristled oostegite in 1973 and 1974 (Fig. 3.6), was the same as in 1990-1993 (over 80% of the females; Fig 3.5). There were no differences in the brooding success of parasitized and uninfected females. Conversely, in 1971 and 1972, the pairing success of uninfected females was < 80% and *P. marilis*-infected females bore significantly fewer broods than uninfected ones. It appears that in years when most females bear broods, *P. marilis*-infected females are mated, but in years when fewer females are fertilized, infected females are discriminated against.

In the 1990's the brooding success of *P. paradoxus*-infected females was lower than that of uninfected females (Fig. 3.5). Only one of the six *P. paradoxus*-infected females collected in 1990-1993 bore a brood. No infected females were found during the brooding periods of 1991 and 1992. In contrast to the 1990-1993 data, in 1971-1974 eight of nine *P. paradoxus*-infected females bore broods or spines (Fig. 3.6). The contradictory patterns, and the very few parasitized females found during the brooding periods, make it impossible to draw any conclusions about the brooding success of *P. paradoxus*-infected females.

The sex ratio of mate-guarding peracaridians determines the males' "choosiness", guarding times and guarding tenacity (Ridley and Thompson, 1985; Manning, 1980). Females were more abundant than males during the late brooding periods (June-July) of 1971-1974, with F:M ratios of 1.5-2.2 (Fig. 3.7). However, males outnumbered females during the earlier brooding periods (May-June) of 1990-1992, with F:M ratios of 0.3-0.5.

Figure 3.5: The proportion of uninfected, *Polymorphus paradoxus*-, and *P. marilis*-infected female *Gammarus lacustris* bearing broods (May-June)

* Significant differences between *P. marilis*-infected and uninfected females (X^2 test, $p<0.05$)

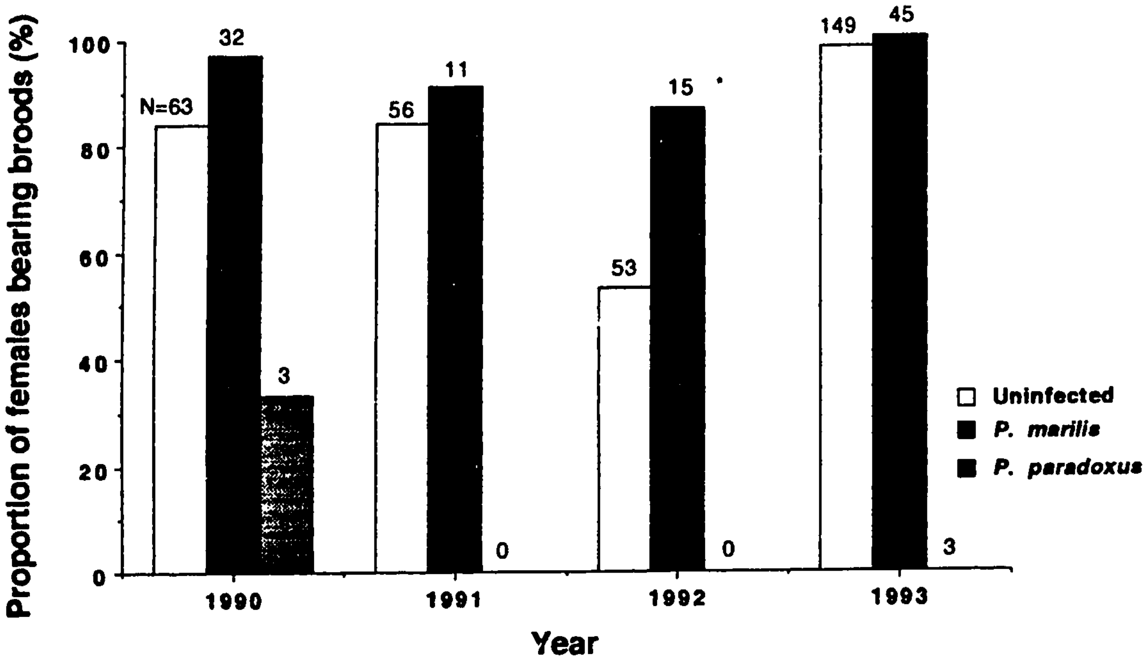

Proportion of females bearing broods (%)
0
20
40
60
80
100
N=63
32
3
56
11
0
53
15
0
149
45
3
1990
1991
1992
1993
Year
☐ Uninfected
■ P. marilis
■ P. paradoxus

Figure 3.6: The proportion of uninfected, *Polymorphus paradoxus*-, and *P. marilis*-infected females *Gammarus lacustris* bearing broods or bristles on their oostegites (indicating a brood has been released; June-July)

* Significant differences between *P. marilis*-infected and uninfected females (X^2 test, $p<0.05$)

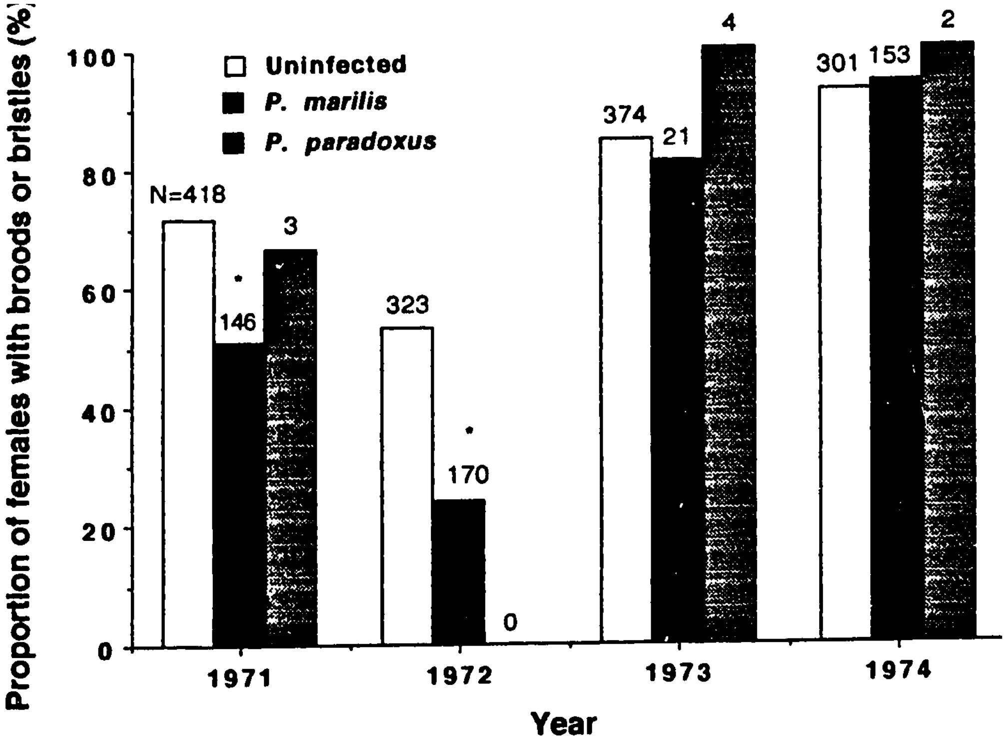
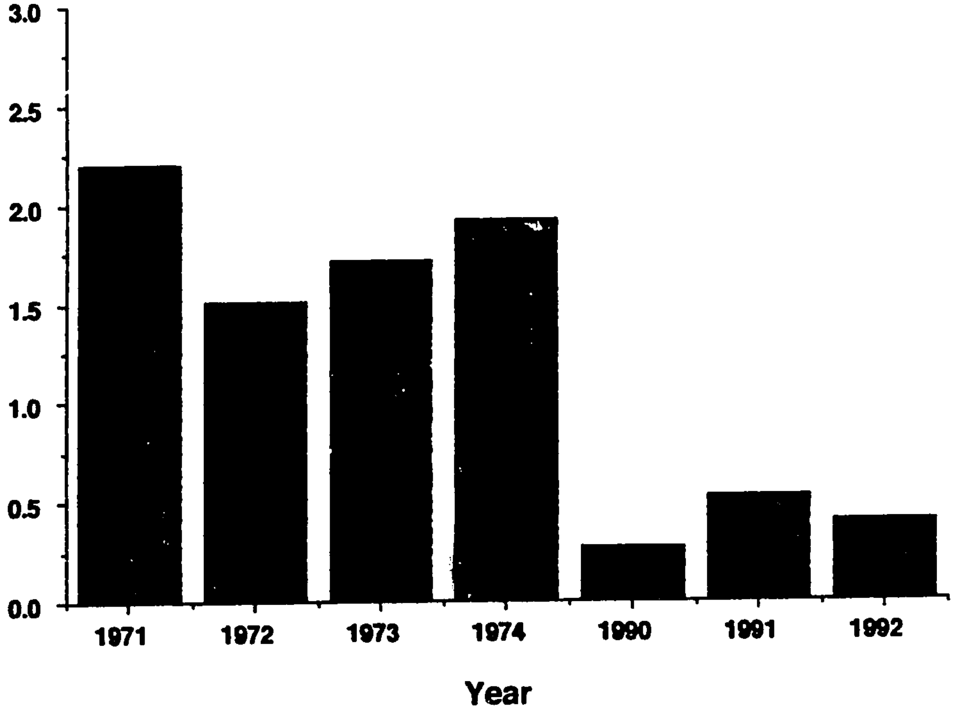

Proportion of females with broods or bristles (%)
100
80
60
40
20
0
Uninfected
P. marilis
P. paradoxus
N=418
*
146
3
323
*
170
0
374
21
4
301
153
2
1971
1972
1973
1974
Year

Figure 3.7: Female to male ratio of *Gammarus lacustris* during brooding seasons (June-July, 1971-1974 and May-June, 1990-1992)



3.0
2.5
2.0
1.5
1.0
0.5
0.0
1971
1972
1973
1974
1990
1991
1992
Year

Reduced Longevity

Longevity of female gammarids was indirectly assessed by comparing changes in sex ratios (as proportion of females) and prevalence of infection between fall and spring periods. Declines in the proportions of parasitized females, or of infection prevalences, may indicate that infected gammarids are susceptible to selective winter mortality. In the spring of all study years, 40% or less of the uninfected gammarids were female (Fig. 3.8). In two of three years, there was a significant decline in the proportions of females among uninfected gammarids between the fall, prior to ice-formation, and spring, following ice break-up (G-tests, $p<0.005$). The proportions of females among *P. marilis*-infected gammarids were also lower in the spring than in fall samples. However, this decline was significant in only one of the four study years. In three of the years, there were no significant differences between the proportions of females among uninfected and *P. marilis*-infected gammarids (G tests, $p>0.05$). The prevalence of *P. marilis* infections did not decline between fall and spring in any of the study years (Fig. 3.9). The data show that both uninfected and *P. marilis*-infected females experienced selective winter mortality; however, there was no evidence that *P. marilis*-infected females were more susceptible to mortality than uninfected females.

The proportions of females among *P. paradoxus*-infected gammarids were significantly lower in the spring than in fall collections in all years, except in 1990 when no infected females were recovered in the fall samples (Fig. 3.8). Females infected with *P. paradoxus* were very scarce in the spring and made up less than 20% of the overwintering infected population. The prevalence of *P. paradoxus* in females was not calculated due to the difficulties of random sampling infected gammarids (because of their altered behaviour). The overwinter decline in the proportions of *P. paradoxus*-infected females and the recovery of very few females following ice-break (21 females in four years), suggest that parasitized females may be susceptible to greater winter mortality than uninfected or *P. marilis*-infected females.

Discussion

Both *P. paradoxus* and *P. marilis* exerted some negative effect on the reproductive success of infected female gammarids, although neither castrated the females. The two acanthocephalans affected reproduction in their female hosts in a variety of ways (Table 3.6). *Gammarus lacustris* females infected with *P. paradoxus*

Figure 3.8: The proportion of females among *Polymorphus marilis*- and *P. paradoxus*-infected *Gammarus lacustris* during fall (open water, September-October) and spring (open water, April)

* Significant difference in the proportion of females between spring and fall (G-test, *p*<0.05)

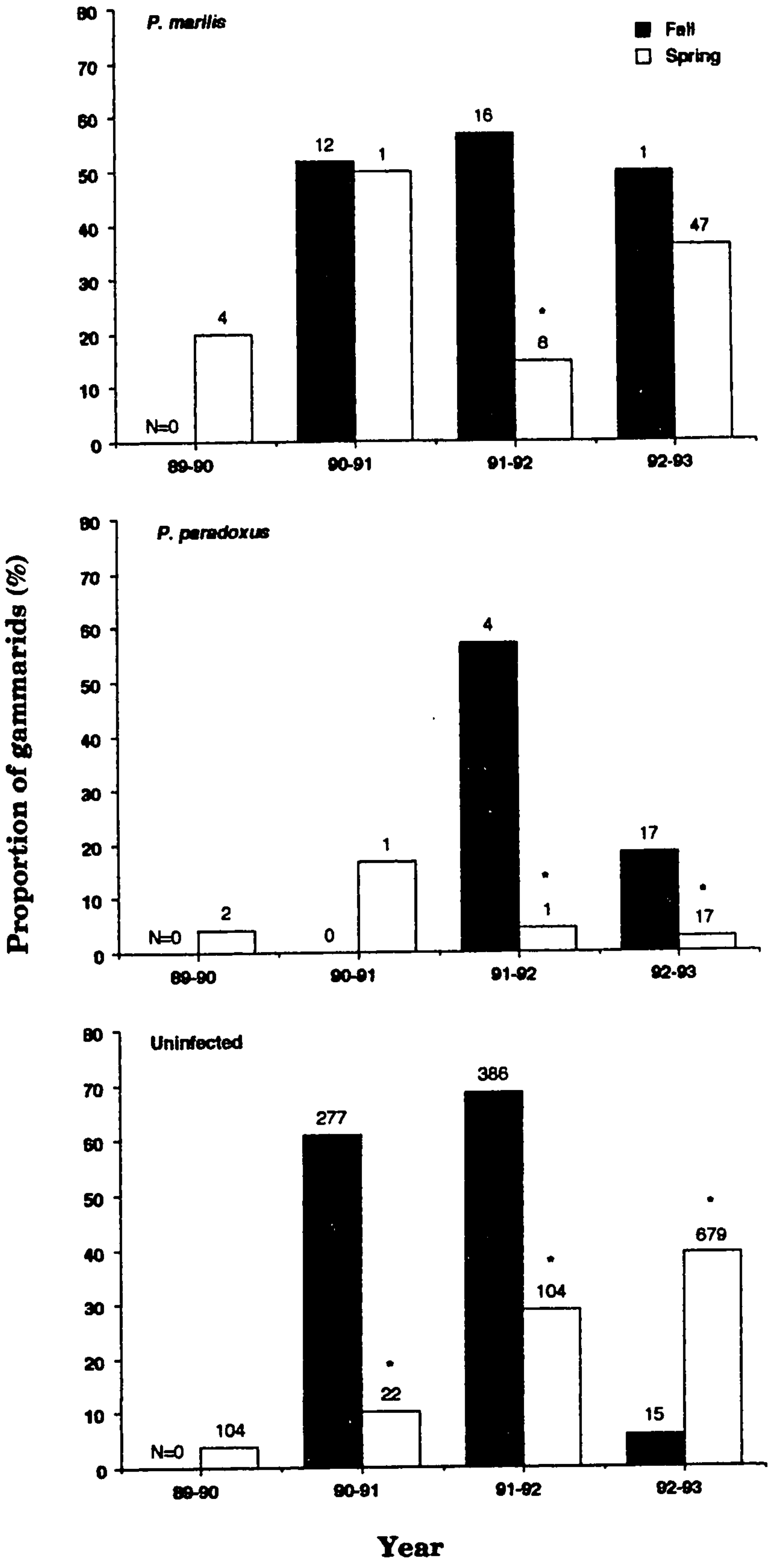
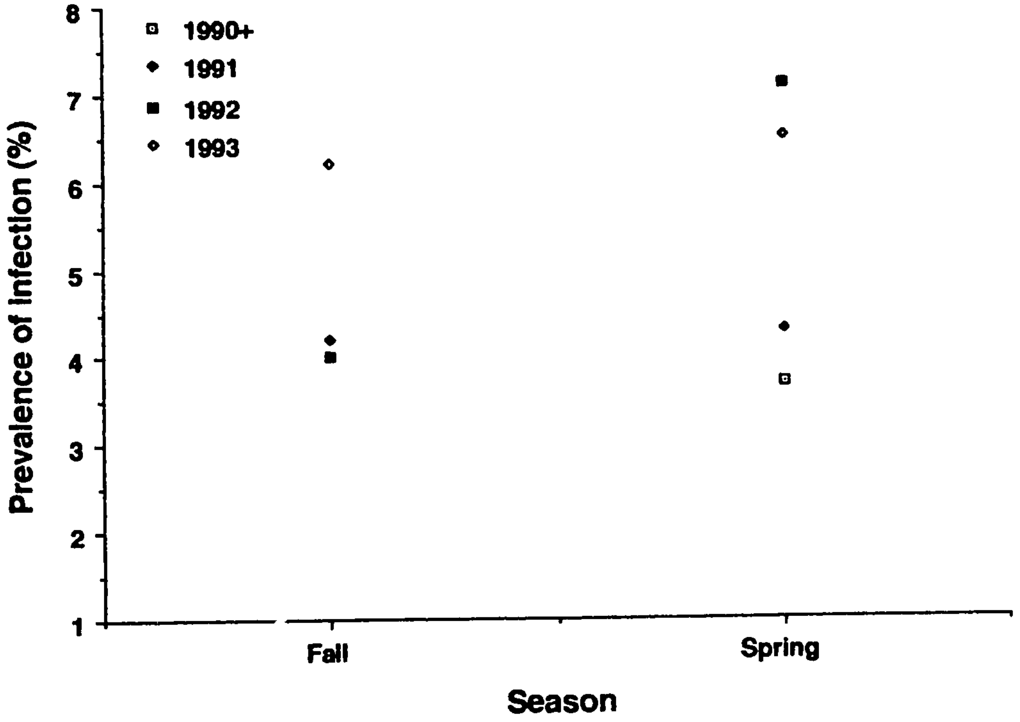

Proportion of gammarids (%)
P. marilis
Fall
Spring
N=0
4
12
1
16
8
*
1
47
89-90
90-91
91-92
92-93
P. paradoxus
N=0
2
0
1
4
1
*
17
17
*
89-90
90-91
91-92
92-93
Uninfected
N=0
104
277
22
*
386
104
*
15
679
*
89-90
90-91
91-92
92-93
0
10
20
30
40
50
60
70
80
Year

Figure 3.9: The prevalence of *Polymorphus marilis* in female *Gammarus lacustris* during fall (open water, September-October) and spring (open water, April)

\+ No sample available for fall, 1990



8
7
6
5
4
3
2
1
Prevalence of Infection (%)
□ 1990+
◆ 1991
■ 1992
◇ 1993
Fall
Spring
Season

Table 3.6: Summary of the effects of *Polymorphus paradoxus* and *P. marilis* infections on reproduction of female *Gammarus lacustris*

Parasite	Ovary	2° Sex Characters	Size of Brood	Pairing Success		% with Brood	Longevity
				Field	Lab		
P. marilis	N	N	R	N, R*	R	N(90s), R(70s)	N
P. paradoxus	N	N	?	R	R	R	R

N = normal, R = reducted, ? = not studied

* N when sex ratio is male biased, R when female biased; R in small females

suffered from greatly reduced pairing and brooding success. However, the greatest detriment to the reproductive capacity of *P. paradoxus*-infected females was their winter mortality. Female gammarids infected with *P. marilis* were affected less severely. Their absolute egg output was lower than that of uninfected females and they were less attractive to males during periods of female biased sex ratios.

Parasitic Castration

The was no evidence of parasitic castration of either *P. paradoxus* or *P. marilis*-infected females; all females had ovaries, and there was no obvious reduction in secondary sexual characteristics (bristled oostegites). Ovarian development controls the development of secondary sexual characteristics (Hartnoll and Smith, 1978); therefore, *P. marilis*-infected females should have bristled oostegites. This was true for *P. marilis*, however, due to the small number of ovigerous, *P. paradoxus*-infected females, it was difficult to determine the effects of this parasite on female gammarids. Based on the presence of ovaries in all infected females, it is unlikely that either acanthocephalan affects host reproduction through hormonal modulation.

Although many other acanthocephalans can sterilize their female intermediate hosts (Table 3.1), the sterilization is not always complete. Various studies have found infected females bearing broods even when infected with castrating parasites. For example, *Polymorphus minutus* interferes with ovarian development of *G. pulex* females, and most infected individuals possess empty brood pouches and bristleless oostegites (ie. non-breeding condition; Hynes and Nicholas, 1963); however, males do pair with those females that can bear broods (Ward, 1986). In addition, Rumpus (1973, in Kennedy, 1985) calculated that in a locality where *Pomphorhynchus laevis* prevalence in *G. pulex* population was 26%, only 2.3% of the females were actually sterile. Such systems, in which some, but not all, the females are castrated may be good systems to investigate the effects of timing of infection on ovarian development.

Reduced Fecundity

Brood size in amphipods is well known to be a function of body size (Ward, 1984). Although in all study years there was a positive relationship between brood size and body length for uninfected female gammarids, regression values for the summer samples of 1971-1974 were lower than those from the spring samples of 1990-1993. The broods of the former females were more mature, and included immature gammarids as well as eggs. The 1990's broods were composed of eggs only. Menon (1966) reported that all eggs in brood pouches hatch into immatures, with no measurable egg loss. However, immatures are more likely to escape from brood pouches, during both collection and laboratory examination, resulting in counts that are less accurate than egg counts. Possibly, the weaker correlations from 1971-1974 were the outcome of less accurate brood counts due to the loss of immature gammarids.

The relationship between egg number and length for *P. marilis*-infected females was also significant for 1990's collections. However, there were no significant correlations between brood size and length of infected females in 1971-1974. The lack of relationship between brood and body size of *P. marilis*-infected gammarids may be due to losses of immatures from the brood pouch. Alternately, the hatching or incubation success of infected females may be lower than that of uninfected amphipods.

Females infected with *P. marilis* suffered from some reduction in the absolute number of eggs borne. Parasitized females bore 3-5 fewer eggs than uninfected females of the same size. Such egg losses constitute a greater proportional loss for small infected females which bear samller broods than larger females. Therefore, reduction in brood sizes will have a greater negative influence on samll than on large female. It was difficult to assess the effects of *P. paradoxus* on brood size since few brood-bearing parasitized females were found during the study years. Many other acanthocephalans significantly decrease the brood sizes of infected females and may even cause complete sterility (Table 3.1). Although I was able to demonstrate a brood reduction only for *P. marilis*-infected females, the data were not adequate to rule out brood reductions for *P. paradoxus*-infected females.

Reductions in brood sizes of acanthocephalan-infected amphipods may be attributed to one or more of three main factors: mechanical interference, hormonal modulation, or alteration of energy budgets. It is unlikely that mechanical interference obstructs ovarian development. The ovaries of *Hyalella* infected with *Leptorhynchoides thecatus* are compressed and anteriourly displaced (Spaeth, 1951), but egg numbers and egg development of parasitized females are unaffected (Spaeth, 1951; Uznanski and Nickol, 1980). In addition, other large cystacanths, such as *Metechinorhynchus truttae*, do not affect egg production in amphipods (Hynes and Nicholas, 1958). Cystacanths may also interfere with female reproduction through direct or indirect interference with hormonal processes (for a review of parasite modulation of host reproduction see Holmes and Zohar, 1990). However, as previously described, neither *P. paradoxus* nor *P. marilis* castrated female gammarids, suggesting that these parasites do not modulate reproductive hormonal processes.

The most likely mechanism behind the reductions in brood size is parasite modulation of the host's energy budget. The metabolic requirements of the cystacanths may be energetically demanding to females that must expend energy for reproductive development. Energetic drains imposed by acanthocephalans may result in declining egg production or complete female sterility (Frenec, 1959; Hynes and Nicholas, 1963).

Attractiveness to Potential Mates

In laboratory tests, male gammarids select females based on size (the larger the female the more fecund) and moult stage or the proximity to the short period of female receptivity to fertilization (Dick, 1992). Males can make complex decisions about the egg utility of females, and choose to pair with females with the greatest number of eggs that can be fertilized per reproductive effort (Elwood *et al.*, 1987; Poulton and Thompson, 1987). Mate selection in peracaridians is also dependent on female densities, and males become less discriminating of female quality as the sex ratio becomes male biased (Manning, 1980). Three aspects of female attractiveness to males were examined in the present study. The attractiveness of infected versus uninfected females was tested in the laboratory where the length and sex ratio of gammarids could be controlled. The pairing success of female gammarids was also assessed based on the proportions that were paired in the lake. Based on the lake samples, the influence of sex ratio on the attractiveness of parasitized and uninfected females could be assessed. The last measure was the proportion of females bearing broods as an indirect measure of the attractiveness of female to mates.

Under experimental conditions significantly more males chose to pair with uninfected female *G. lacustris* than with *P. paradoxus*- or *P. marilis*-infected individuals of equal length. Apparently, males could discriminate between infected and uninfected females and rejected the former, presumably based on their lower egg output. However, males did pair with some parasitized females. In *G. pulex* infected with *Pomphorhynchus laevis*, there is a five egg reduction in broods of infected as compared with uninfected females of the same size (Poulton and Thompson, 1987), but in mate choice experiments, males did not prefer to pair with uninfected females. Male *G. pulex* can assess the quality of females based on both size and proximity to moult, and choose to guard females with higher egg gain rate (number of eggs fertilized divided by the length of the precopula period; Poulton and Thompson, 1987). I controlled for female length, but not proximity to moult. Perhaps, *G. lacustris* males may also be able to assess egg gain rate and paired with females with a higher gain rate regardless of infection with *P. marilis*.

In field collections made during peak pairing periods, 35 to 80% of the uninfected females were in precopula. Overall, the pairing success of *P. marilis*-infected females did not differ from that of uninfected females. Nevertheless, the pairing success of small females infected with *P. marilis* was significantly lower than that of uninfected females of equal length. In this study, as in other studies on *Gammarus* (eg. Ward, 1984; Dick and Elwood, 1988), males preferentially paired with larger females presumably because smaller females produce fewer eggs and moult later than larger ones (Elwood *et al.*, 1987). Small, parasitized females may be particularly unattractive because, due to their size and infection they will bear the least number of eggs.

The high pairing success of females infected with *P. marilis* may also have been the outcome of the scarcity of females in the overwintering *Gammarus* population. Mate guarding periods are affected not only by female quality (Ward, 1984; Ridley and Thompson, 1985), but also by female density (Manning, 1980; Ward, 1983; Dunham and Hirshman, 1990). Manning (1980) examined the relationship between sex ratio and mate guarding in isopods. As the sex ratio became more male biased, males entered into precopula earlier, accepted females with lower egg gain rates, and the strength of association between timing of pairing and oviposition was reduced. Gammarids also tend to guard for longer periods as the male bias increases, ie. with increasing number of competitors (Ward, 1983). All the overwintering populations (spring populations) I studied were male biased, with males making up about 69% of the population. The competition for females at Cooking Lake may be relatively high, resulting in males being less selective and more willing to guard infected females, thus ensuring some degree of reproductive success.

Almost no *P. paradoxus*-infected females were found paired in field collections. In part, this may be the result of the very low numbers of females that survive until spring. The habitat differences of parasitized females (in shallower waters) and uninfected males (in deeper waters; see chapter 2), may reduce their chances of encountering males. All infected females were > one cm in length and possessed ovaries; thus, they should have been attractive to males. The low pairing success of *P. paradoxus*-infected females may be attributed to their scarcity, habitat differences, and grossly altered behaviour. They may also have very low egg utility, rendering them unattractive to males; however, I have no evidence for this possibility.

The attractiveness of females was further examined on the basis of the proportions of infected and uninfected females which bore broods. In May and June of the 1990's, over 80% of the uninfected females in the *Gammarus* populations bore broods. In most years, the proportion of females with broods (in summer) was greater than the proportion of paired females (in spring), indicating that females were guarded at different times, and that most females were mated. The brooding success (ie. the proportion mated) of females with *P. marilis* was the same as that of uninfected females. Several factors may account for the high brooding success of parasitized females. In most experimental situations, male gammarids prefer to pair with larger females (eg. Elwood *et al.*, 1987); however, Ward (1983) found no difference in the sizes of field-collected paired and unpaired females. Crespi (1989) suggested that all females that near moult are guarded. As described earlier, males choose females not only on the basis of their size, but also on proximity to moult, and the availability of females in the population. The male biased sex ratio during the reproductive period (spring and early summer) at Cooking Lake (at least in the 1990's) probably resulted in the mating of most adult females, regardless of infection.

In 1971 and 1972, however, the brooding success of uninfected females was < 80%, and *P. marilis*-infected females bore significantly fewer broods than uninfecteds. These differences cannot be attributed to a male skewed sex ratio, as in all four years (June-July, 1970-1974), the *Gammarus* population was dominated by females. An even or female-biased sex ratio in the overwintering populations in 1971 and 1972 would decrease the pressure on the males to mate with all available females. However, I do not have winter sex-ratio data for 1971-1974, and the reasons for the differences between 1971-1972 and 1973-1974 are not clear. The trends indicate that when the brooding success of gammarids was high (> 80% of the females bore broods), the brooding success of infected females was unaffected. However, when brooding success of the gammarids was low, males may have been more selective and preferentially paired with uninfected females.

Reduced Longevity

Although I could not assess overwinter mortality directly (because no quantitative measurements of population sizes were made) selective overwinter mortality could be assessed for *G. lacustris* by changes in sex ratios or prevalences of parasites from fall to spring samples. The proportions of female *G. lacustris* declined over winter, indicating greater overwinter mortality than in males. The ice cover during the winter months, in Cooking and Hastings Lakes, is associated with increasing hypoxia leading to anoxia, often resulting in winterkill of fish (Kerekes, 1965). Menon (1966) attributed winter mortality of *G. lacustris* to low water temperatures and oxygen depletion. The greatest winter mortality occurred in March when the water under the ice became anoxic. Menon (1966) reported that winter mortality was greatest among the smaller animals, especially pre-reproductive stages and female gammarids.

Parasitism with *P. marilis* and *P. paradoxus* had different effects on the winter survival of infected females. There was no evidence that *P. marilis*-infected females were susceptible to greater winter mortality than uninfected females. The prevalence of *P. marilis* infections did not decline over winter and there were no differences in the proportions of overwintering infected and uninfected females. Conversely, very few *P. paradoxus*-infected females were found in the spring relative to uninfected females. Therefore, *P. paradoxus*-infected females may be more susceptible to winter mortality than uninfected or *P. marilis*-infected females.

McMahon *et al.* (1991) found *Pomphorhynchus laevis*-infected *G. pulex* to be more sensitive to reduced dissolved oxygen concentrations than uninfected gammarids. They suggest that the stress of the parasite results in increased mortality of infected gammarids (they did not distinguish between the sexes). Cystacanths metabolize glucose and oxygen; their energetic demands on intermediate hosts are seldom noticed in experimental conditions, but may weaken the hosts under natural conditions (Crompton, 1970). Acanthocephalans can reduce the respiratory rates of infected gammarids (Ferenc, 1959; Rumpus and Kennedy, 1974) and reduce their feeding rates (McMahon *et al.*, 1991). *Polymorphus paradoxus* cystacanths are larger than those of *P. marilis*; therefore, infection with the former may be a greater energetic burden. Gammarids infected with *P. paradoxus* were predominantly found in shallower and more anoxic water than uninfected females (Chapter 2). Potentially, the stress of the more anoxic environment (shallow sites) and the larger cystacanth, contribute to the severe winter mortality of *P. paradoxus*-infected females.

The reproductive output of *P. paradoxus*- and *P. marilis*-infected female *G. lacustris* was reduced by a variety of means (Table 3.6). Female reproduction was least affected by *P. marilis*; infected females suffered from reductions in brood sizes, probably as a result of the energetic demands of the parasite. Potentially, the reduction in brood size resulted in their diminished attractiveness to males when sex ratios were not male biased. *Polymorphus paradoxus* infections severely affected the reproductive output of infected female gammarids. Reduced longevity, as a result of overwintering mortality, eliminating the reproductive potential of most *P. paradoxus*-infected females. Parasitized females were also unattractive to males, either due to reduced brooding success (not assessed in this study), or because of the grossly altered behaviour and winter distribution of infected females. This is the first reported case of altered habitat resulting in the reduced reproductive success of parasitized hosts.

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Chapter 4 - Reproductive consequences of parasitism with *Polymorphus paradoxus* and *P. marilis* (Acanthocephala) for their male intermediate host, *Gammarus lacustris* (Crustacea)

Introduction

Parasites can cause many different modifications in their invertebrate host's reproductive behaviour and output (Baudoin, 1975; Hurd, 1990). Barnard (1990) listed five means by which a parasite may reduce the reproductive output of its host: parasitic castration (the alteration or destruction of the host's gonadal tissue; Baudoin, 1975), brood loss, reduced attractiveness or inclination of a partner to mate with an infected animal, reduced longevity, and the costs of counter-adaptation to the parasite (eg., immunity). The cost of counter-adaptation is not a direct measure of reduced fecundity, and the consequences of energy or resources being diverted from reproduction to defense against the parasite may affect any of the other four reproductive aspects. A wide variety of parasites, including protozoa, helminths and arthropods, can affect reproduction of their hosts. A detailed discussion of parasite-induced reduction of host reproduction is provided in Chapter 1. In the previous chapter, I examined the effects of two acanthocephalans, *Polymorphus paradoxus* and *P. marilis*, on the reproduction of their female intermediate hosts, *Gammarus lacustris*. In this chapter, I examine their effects on male hosts.

Baudoin (1975) postulated that parasites have different effects on reproduction in male and female hosts due to the differences in the sexual behaviour and parental contributions of the two sexes. He suggested that because energy expenditures to gametes are lower in males than in females, testes are less likely to be affected by infections than ovaries. In contrast, males invest more energy in secondary sexual characteristics involved in defense and fighting (eg. chelae of crustaceans), which tend to be reduced in castrated males. Similarly, males generally invest more energy in courtship and defense than in brood rearing, so the first two would be expected to be affected by parasites.

There is sparse information about the effects of acanthocephalans on the reproductive output of male peracaridians. Most studies report no obvious effects on growth, secondary sexual characteristics, pairing success, or spermatogenesis of infected males (eg. Hynes and Nicholas,1963; Schmidt and Olsen, 1964; Brattey, 1980; Moore, 1983). LeRoux (1933) found that two secondary sex characteristics, the first gnathopod and third uropod, grew more slowly in *G. duebeni* infected with

P. minutus than in uninfected males. Interestingly, she found that there was an inverse relationship between parasite burden and growth rate; however, these differences were not statistically significant and reduction in growth rate is unlikely to be dependent on parasite burden. The isopods *Asellus intermedius* and *Lirceus garmani* infected with *Acanthocephalus dirus* were significantly smaller than uninfected conspecifics in one out of six field collections (Oetinger and Nickol, 1981). However, there were no differences in the sizes of the reproductive systems of infected and uninfected isopods, and spermatozoa were found in the vas deferens of infected males. Similarly, the testes and spermatozoa of infected *G. duebeni* developed normally (LeRoux, 1933). However, only 0.6% of paired *G. pulex* males collected in the field were infected by *P. minutus*, whereas 5.1% of the unpaired males were infected (Ward, 1986). The pairing success of the infected gammarids was significantly lower than that of the uninfecteds. No experimental studies on the pairing success of acanthocephalan-infected male peracaridians exist in the published literature.

The reproductive output of male gammarids depends on their ability to produce gametes, develop secondary sexual characteristics, procure a female and successfully fertilize her. The reproductive behaviour of many species of gammarids, in which the female can be fertilized only immediately after moulting, involves a period of precopulatory mate guarding (Birkhead and Clarkson, 1980; Conlan, 1991). To ensure a mating opportunity, male gammarids attach to the female with their gnathopods and guard her until she moults and can be fertilized (Hartnoll and Smith, 1978; Elwood *et al.*, 1987; Elwood and Dick, 1990; Conlan, 1991; Sutcliffe, 1992).

There has been considerable research on the behavioural ecology of mate choice, size assortative mating and mate guarding behaviour (Ward and Porter, 1993). Such studies attempt to determine which factors influence the formation of precopulatory pairs, and the costs and benefits associated with mate guarding (Ward, 1983; Elwood *et al.*, 1987; Elwood and Dick, 1990). Males may incur energetic costs during the guarding period because they carry the female when swimming (Adams and Greenwood, 1983). Paired amphipods are also more susceptible to predation than single animals (Strong, 1973; Ward, 1986). Another cost of mate guarding is the loss of mating opportunities with other females (Elwood *et al.*, 1987); however, Dick (1992) found that males in precopula can assess the quality of unpaired females and change partners. A male gammarid needs to be able to assess the optimal time and duration of mate guarding to ensure that he will mate following her moult while minimizing the costs of guarding. Elwood *et al.* (1987) suggest that the optimal time for which a male should guard is influenced by factors including the operational sex-ratio, search time, costs of searching, cost of guarding, number of eggs likely to be fertilized, and the possibility of take-overs.

In most studies of field-collected *G. pulex* there is a positive size-assortative pairing whereby larger males are paired with larger females and smaller males pair with smaller females (Hynes, 1955; Dick and Elwood, 1990). Ward and Porter (1993) reviewed five hypotheses about the causes of assortative pairing of *G. pulex*:

1. Mechanical hypothesis proposing small males are incapable of pairing with large females (Crozier and Snyder, 1923).
2. Habitat segregation hypothesis suggesting that large and small gammarids occupy different substrates and locations (Birkhead and Clarkson, 1980).
3. Loading constraints hypothesis suggesting that pairs with high male/female size ratios can move faster in a current (Adams and Greenwood, 1983).
4. Sexual selection hypothesis assuming that large males have an advantage in competition for females (Ridley and Thompson, 1985). Larger males are better able to take-over females in precopula with smaller males (Ridley and Thompson, 1985; Dick and Elwood, 1990) and are better able to resist take-overs when paired (Ward, 1983).
5. Guarding time hypothesis based on differences between large and small males. Larger males enter into precopula earlier in the females' cycle and larger females are paired earlier than smaller females (Elwood *et al.*, 1987). This results in indirect male competition for females (Ward and Porter, 1993).

Ward and Porter (1993), rejected the mechanical and loading constraints hypotheses as the causes for size-assortative pairing. Using a simulation study, they compared the roles of habitat segregation and male-male competition in the formation of size assortative mating and concluded that the habitat structure is more important in determining this mating system. Dick and Elwood (1993) disagreed with Ward and Porter's (1993) conclusions and suggested that the role of habitat in size-assortative pairing is negligible. Most likely, as Ward (1986) suggested, habitat segregation and male-male competition may both contribute to size-assortative pairing. Therefore, in my study I considered the role of both habitat (Chapter 2) and male-male competition on pairing in *G. lacustris* (present chapter).

In this study I examined the role of infection with *Polymorphus paradoxus* and *P. marilis* on the reproductive biology and behaviour of male *G. lacustris*. The life-cycles of the two parasites were summarized in chapter 1. Both parasites induce some degree of behavioural pathology in their *G. lacustris* hosts, ranging from positive photic responses to altered evasive behaviour (Bethel and Holmes, 1973; 1977; and see chapter 1). I studied three of Barnard's mechanisms which may affect reproduction in male hosts: parasitic castration, as the presence of gonads, spermatogenesis and secondary sexual characteristics; the ability to compete for females, and the inclination to pair (based on the likelihood of single males to pair with females under non-competitive situations) of infected versus uninfected males in both the field and laboratory; and the longevity of infected and uninfected males based on their overwintering survivorship.

Materials and Methods

Gammarus lacustris were collected from Cooking Lake, Alberta according to the methods described in Chapter 2. Monthly samples were taken from September 1989 to September 1992, and in April and May 1993. The gammarids were measured and necropsied following the procedure outlined in Chapter 2. Males were identified based upon the presence of testes and secondary sexual characteristics, the presence of calceoli on the flagellum of the second antenna, and the modified propodus of the second gnathopod (Menon, 1966).

Presence of testes and the development of secondary sexual characteristics were examined in 25 *P. marilis*-infected, 25 *P. paradoxus*-infected and 25 uninfected males. The body length of the gammarid was measured, testes were removed, squashed on a slide using a cover slip, and examined at 40X magnification for the presence of mature sperm. The propodus of the second gnathopod, a secondary sexual characteristic, was measured using a calibrated ocular micrometer. The presence and the number of calceoli on the flagellum of the second antennae were determined.

Relative pairing success was measured by the proportion of infected and uninfected males found in pre-copula. Data for the calculation of pairing success came from the period of peak pairing activity, based on the data available: April, 1990; April-May, 1991; March-April, 1992; and April, 1993. The distribution of lengths of *P. marilis*-infected and uninfected males during the pairing periods were compared for 1990-1993. The length distribution of *P. paradoxus*-infected male gammarids was compared with those of *P. marilis*-infected and uninfected males in 1993. The data used in this analysis included males collected from the onset to the peak of the pairing periods: January-May, 1990; March-May, 1991; March-April, 1992; and April, 1993. There were no significant differences between the length distributions of males found in sites closer to shore and those from sites further off shore (ANOVA, $p > 0.05$); therefore, the data were pooled.

A series of eight laboratory experiments was conducted to study the ability of *P. marilis*- and *P. paradoxus*-infected males to pair with females, either alone or in the presence of uninfected males. A number of experiments, differing slightly in design, starting date and duration, were carried out in 1990-1993 (Table 4.1). In all experiments the gammarids were field collected and naturally infected. The gammarids were acclimated to the environmental chambers for three to 14 days. Gammarids were placed in plastic cups (200 ml in volume), provided with a substrate of sand (experiments 1-4) or aquarium gravel (Hagen, 1-2 mm in diameter; experiments 5-8) and unlimited flake food (Tetramin). The animals were examined daily to determine whether they had entered into precopula. Gammarids that died before the end of the experiment were excluded from analysis. Each experimental cup contained either a competitive situation of two males (one infected, one uninfected) and a previously-paired uninfected female, or a single infected or uninfected male 'alone' with a previously-paired, uninfected female. Previously paired uninfected females were used to ensure their pairing suitability. All males were size matched to eliminate the bias of size in competitive situations. All the females were 1.20-1.35 cm in length. Experiments 1-4 were conducted at 4°C and maintained at a 8L:16D cycle. Experiments 5-8 were conducted at 8°C and 12L:12D to mimic field conditions more closely but below the temperature threshold at which overwintering *P. marilis* acanthors will develop (Tokeson and Holmes, 1982). The details of experiments 1 -8 are summarized in Table 4.1.

No quantitative collections that allowed calculation of density data were taken, so overwinter survival could not be measured directly. Instead, overwinter survival was determined indirectly by comparing the prevalence of *P. marilis* in male gammarids between fall (open water) and spring (open water) periods. A decline in parasite prevalence may provide an indirect measure of selective winter mortality of infected gammarids. Monthly data were pooled for the two periods: September-October for fall observations and April for spring data. The spring open-water period was restricted to those collected in April because increasing water temperatures in the spring trigger an increase in the prevalence of *P. marilis* infection due to the development of overwintering acanthors (Tokeson and Holmes,

Table 4.1: Summary of experiments on the pairing success of *Polymorphus paradoxus*-(P. p.) and *P. marilis*-infected (P. m.) male *Gammarus lacustris* . In 'competition' tests, one infected and one uninfected (Un.) male were provided with an uninfected female. Single males provided with an uninfected female tested the inclination of infected and uninfected males to pair, and served as controls in the 'alone' tests.

Experiment	Start Date	Duration (d)	'Alone' Single males (#)			'Competition' Pairs of males (#)	
			Un.	P. m.	P. p.	P.m.+	P.p.+
$1^{a,c}$	Apr. 26, 90	15	28			18	20
$2^{a,c}$	Apr. 28, 90	10	14	14	14	18	19
$3^{a,d}$	May 17, 91	10				15	15
$4^{a,d}$	May 25, 92	120	15	15	14	19	18
$5^{b,d}$	Apr. 15, 93	7	25		25		25
$6^{b,d}$	Apr. 26, 93	7	20	25	20	25	20
$7^{b,d,e}$	May 5, 93	7	20	20			
$8^{b,d}$	May 10, 93	7	20				

a = 4°C, 8L:16D photoperiod, substrate sand
b = 8°C, 12L:12D photoperiod, substrate aquarium gravel
c = placed in cups at same time as females
d = males placed in cups 3 hours before females
e = also included 20 cups with 2 uninfected males + 2 females, 19 cups with uninfected male + 2 females
# = number of experimental setups in each experiment

1982). Prevalences are based on acanthellae and cystacanths only, and do not include overwintering acanthor stages which are too small to detect under a dissecting microscope. The prevalence of *P. paradoxus* was not measured because this parasite alters the distribution and evasive responses of gammarids (Bethel and Holmes, 1973); thus, it is difficult, if not impossible, to obtain random samples of infected gammarids.

Statistical analyses were done using the following computer packages:

- χ^2 tests, G-tests and linear regressions using Statview (Macintosh version)
- Analyses of covariance (ANCOVA) and least square means were obtained from SuperAnova (Macintosh version)
- Fisher's exact tests and analyses of variance (ANOVA) were run using SYSTAT for DOS (version 5).

Probabilities of 0.05 or less were considered significant.

Results

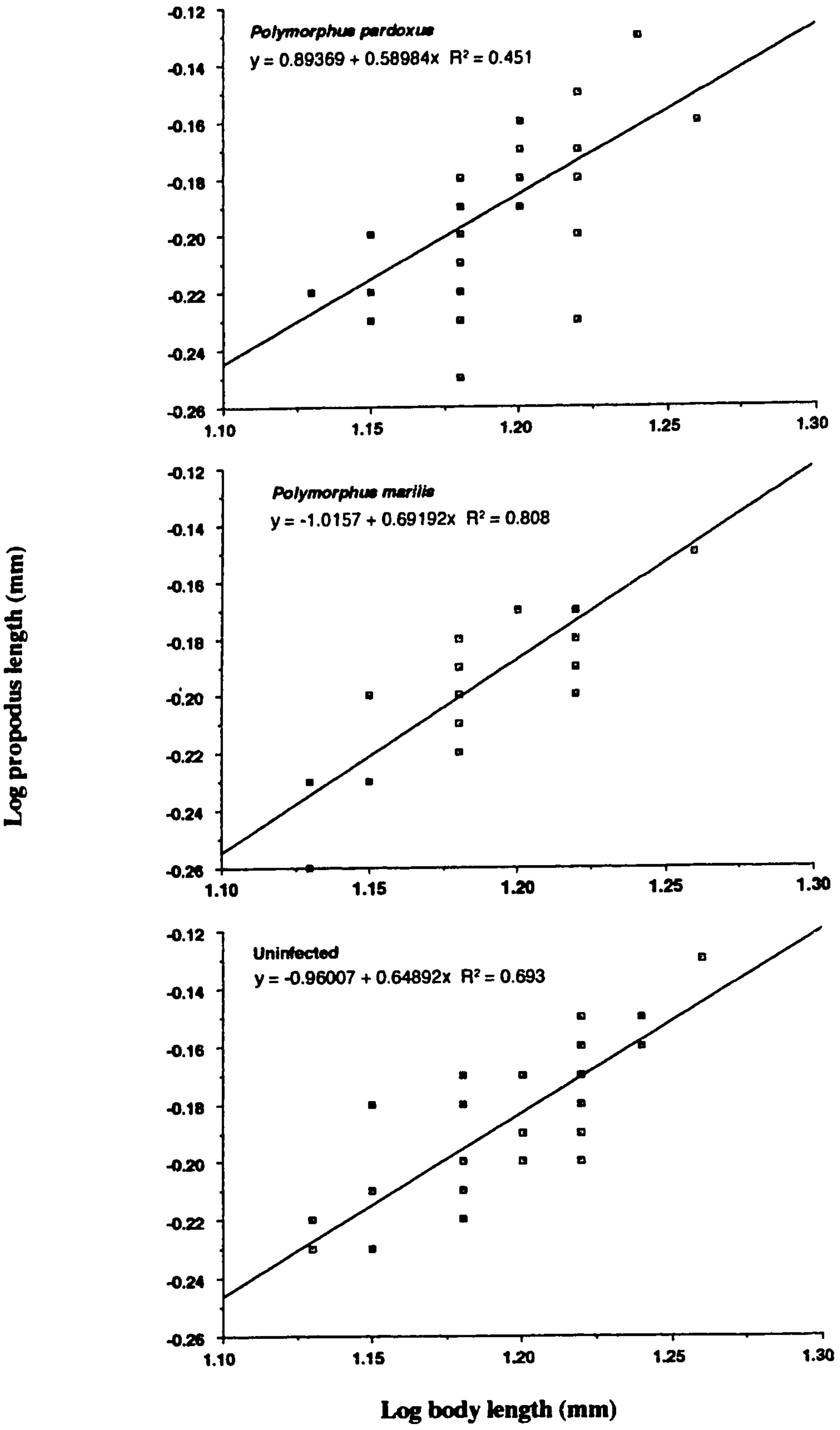
Parasitic Castration

All males, whether *P. paradoxus-*, *P. marilis*-infected or uninfected, had testes which contained mature sperm. There were positive correlations between the length of the propodus of the first gnathopod and the body length of *Polymorphus paradoxus-*, *P. marilis*-infected and uninfected males (Fig. 4.1). The regressions accounted for 45, 75 and 69% of the variance for *P. paradoxus-*, *P. marilis*-infected and uninfected gammarids, respectively. There were no significant differences between the propodus lengths of infected and uninfected gammarids when treating body length as a covariate (ANCOVA, df=2, F=0.48, p=0.62). Additionally, all of the above male *G. lacustris* possessed nine calceoli on the flagellum of their second antennae. Infection with either parasite did not adversely affect spermatogenesis (at least, not qualitatively) or either of the secondary sexual characteristics examined.

Pairing Success in the Lake

Males infected with *P. paradoxus* had a significantly lower pairing success (the proportion of males found in precopula in the field) than uninfected males in three of the four years. Overall, only three out of 302 *P. paradoxus*-infected males were found paired, a significantly lower pairing success than the 46% of uninfected males

Figure 4.1: The length of propodus of the second gnathopod versus body length of *Polymorphus paradoxus*-, *P. marilis*-infected and uninfected male gammarids (n = 25 / group)


Polymorphus pardoxus
y = 0.89369 + 0.58984x R² = 0.451
Polymorphus marilis
y = -1.0157 + 0.69192x R² = 0.808
Uninfected
y = -0.96007 + 0.64892x R² = 0.693
-0.12
-0.14
-0.16
-0.18
-0.20
-0.22
-0.24
-0.26
1.10
1.15
1.20
1.25
1.30
Log propodus length (mm)
Log body length (mm)

(Table 4.2). An additional *P. paradoxus*-infected male was found 'paired' with an uninfected, adult male; the gnathopods of the infected male were inserted into the carapace of the other male as if it was carrying a female. This was the only case of homosexual pairing observed during this study.

The pairing success of *P. marilis*-infected gammarids was greater than that of those infected with *P. paradoxus* in two of the study years, but significantly lower than that of uninfected males in all study years (Table 4.2). Although both parasites reduced the pairing success of infected males in the field, the reduction due to *P. paradoxus* infection was significantly greater ($X^2=72.4$, df=1, $p=0.001$).

Because the pairing success of male gammarids may be affected by their size (Dick and Elwood, 1990), I compared the length distributions of infected and uninfected males. The most abundant size class of both infected and uninfected male gammarids was 1.5 cm during all four years (Fig. 4.2). There were no significant differences between the length distributions of *P. marilis*-infected and uninfected males in the four years (Table 4.3). *Polymorphus marilis*-infected males were found in all size classes. In contrast, *P. paradoxus*-infected were significantly smaller than uninfected males (Tukey's test, $p<0.05$). However, these length differences were small, the least square mean length of *P. paradoxus*-infected males was 1.50 cm, compared to 1.59 cm for uninfected males and 1.55 cm for *P. marilis*-infected males.

There were no significant differences in the length distributions of paired *P. marilis*-infected and uninfected males (ANOVA, F=0.57, df=1, $p=0.45$). The majority of the *P. marilis*-infected males in precopula were in the 1.35 cm, 1.50 cm and 1.65 cm length classes (Fig. 4.3), representing the classes with the highest proportions of male gammarids. Small gammarids measuring less than 1.20 cm were never found in precopula. Very large gammarids, in the 1.80 and 1.95 cm classes, had a lower pairing success than those in the median length classes; these may have been mainly post-reproductive males. The length distributions of *P. marilis*-infected males were the same as those of uninfected males; therefore, male size was not likely the cause of the parasitized males' reduced pairing success.

Pairing success and decisions of male gammarids are often governed by the male: female ratio (Ward, 1983). Therefore, the proportion of males in the *G. lacustris* population was calculated for fall and spring. In all years males made up a greater proportion of the spring population than females (Fig. 4.4). Over 60% of spring-collected *P. marilis*-infected or uninfected individuals were males. Additionally, almost all *P. paradoxus*-infected individuals (over 80%) collected in

Table 4.2: Pairing success of *Polymorphus paradoxus*-, *P. marilis*-infected, and uninfected male gammarids at Cooking Lake

	Number of males paired / Total number of male gammarids (%)				
	1990 (Apr)	1991 (Apr-May)	1992 (Mar-Apr)	1993 (Apr)	Total (1990-1993)
P. paradoxus-infected	0/29 (0.0)	0/7 (0.0)	2/8 (25.0)	1(+1♦)/258~ (0.4)	3/302~ (1.0)
P. marilis-infected	5/22* (22.7)	0/26 (0.0)	3/57~ (5.3)	49/129* (38.0)	57/234* (24.4)
Uninfected	91/203 (44.8)	31/139 (22.3)	50/292 (17.1)	927/1740 (53.3)	1099/2374 (46.3)

/~ Significant differences between the pairing success of infected and uninfected males (X^2 test, $p < 0.05$; ~ Fisher's exact test, $P<0.05$)

♦ *P. paradoxus*-infected male in precopula with a male gammarid

Figure 4.2: Length distributions of *Polymorphus paradoxus*-, *P. marilis*-infected, and uninfected male gammarids

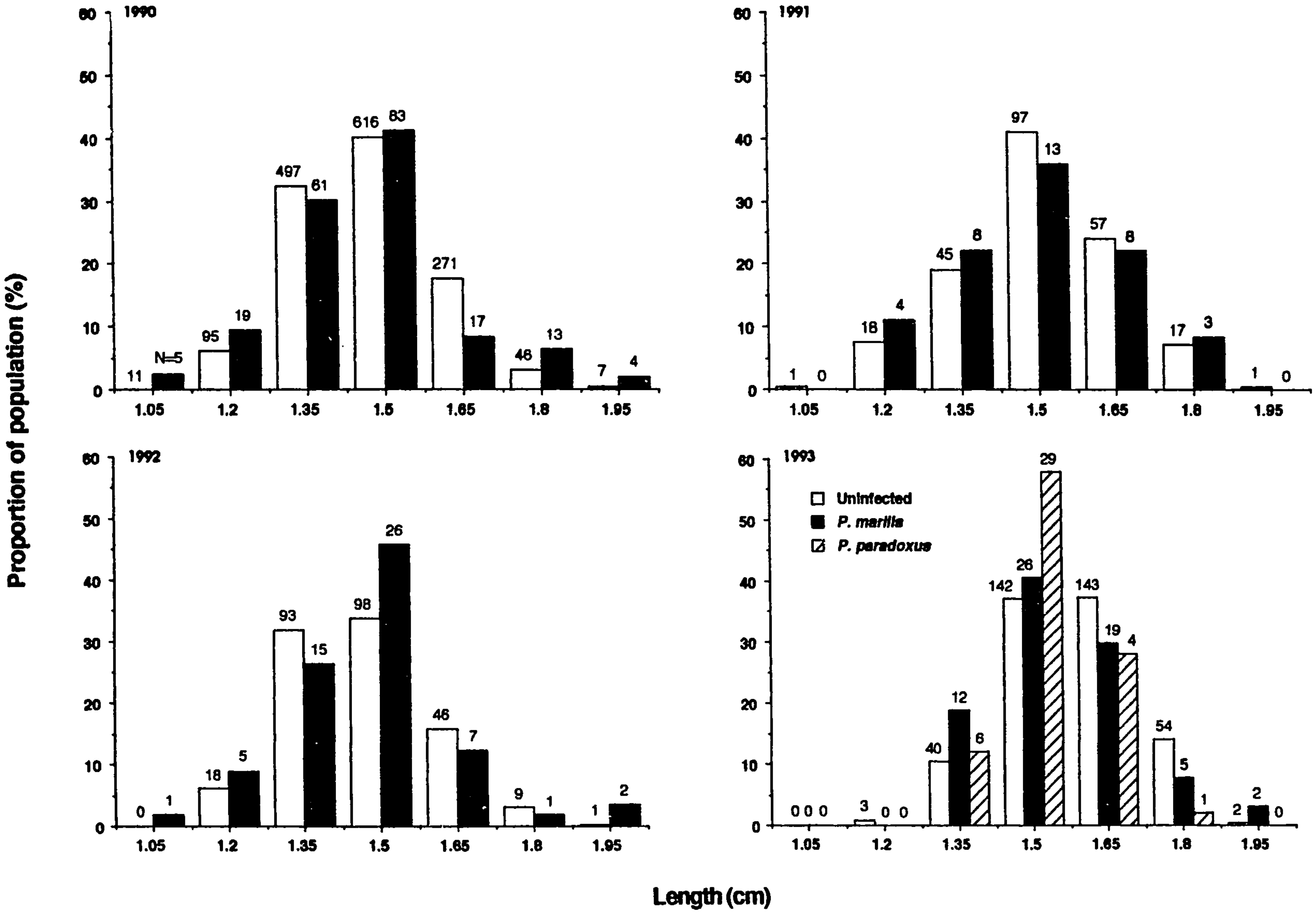
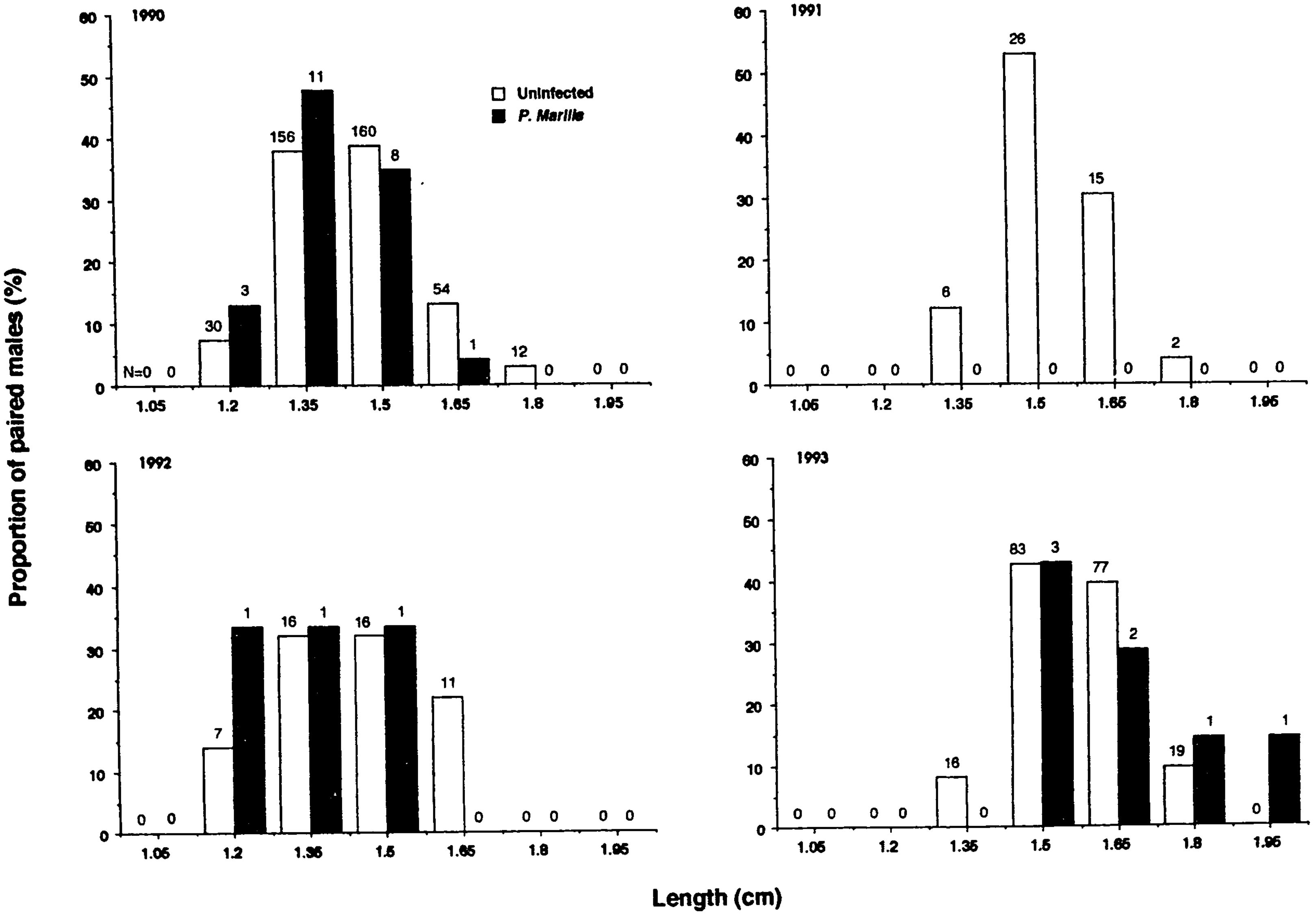

Proportion of population (%)
Length (cm)
1990
1991
1992
1993
0
10
20
30
40
50
60
1.05
1.2
1.35
1.5
1.65
1.8
1.95
11
N=5
95
19
497
61
616
83
271
17
46
13
7
4
1
0
18
4
45
8
97
13
57
8
17
3
1
0
0
1
18
5
93
15
98
26
46
7
9
1
1
2
Uninfected
P. marilis
P. paradoxus
0 0 0
3 0 0
40
12
6
142
26
29
143
19
4
54
5
1
2
2
0

Table 4.3: Analysis of variance comparing the lengths of *Polymorphus marilis*-infected and uninfected male gammarids among years (1990-1992), and between shallow and deep water sites

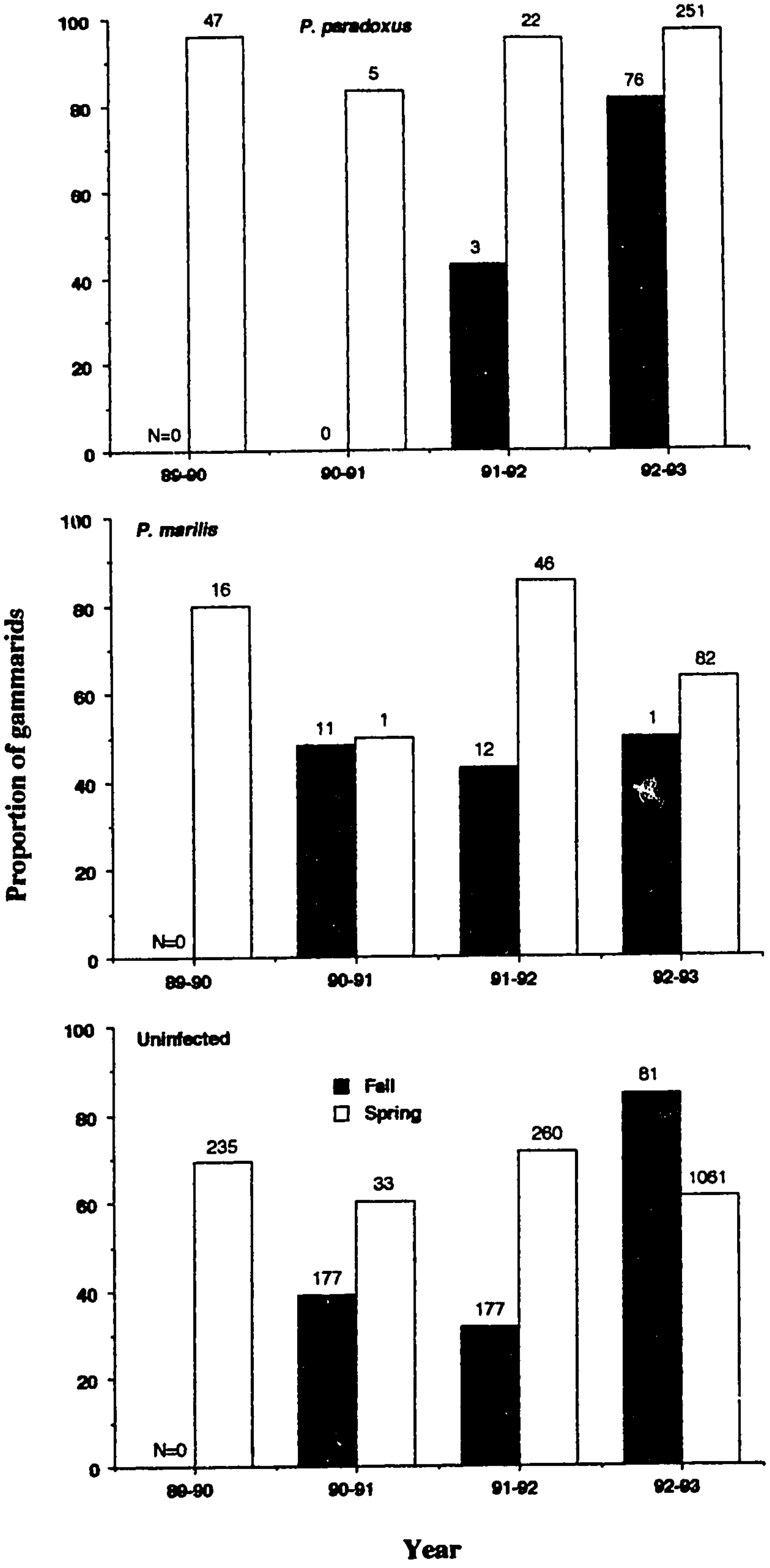
Source	Sum of squares	df	Mean square	F-ratio	p
Year	0.118	2	0.059	2.764	0.063
Site	0.066	1	0.065	3.053	0.080
Infection	0.004	1	0.004	0.210	0.646
Year*site	0.053	2	0.026	1.229	0.292
Year*infection	0.015	2	0.008	0.358	0.698
Site*infection	0.004	1	0.004	0.201	0.653
Year*site*infection	0.022	2	0.011	0.520	0.594
Error	49.850	2317	0.021		

Figure 4.3: Length distribution of *P. marilis*-infected and uninfected paired male gammarids



Proportion of paired males (%)
Length (cm)
1990
Uninfected
P. Marilis
N=0 0
30 3
156 11
160 8
54 1
12 0
0 0
1.05 1.2 1.35 1.5 1.65 1.8 1.95
1991
0 0 0 0
6 0
26 0
15 0
2 0
0 0
1.06 1.2 1.35 1.5 1.65 1.8 1.96
1992
0 0
7 1
16 1
16 1
11 0
0 0 0 0
1.06 1.2 1.36 1.5 1.65 1.8 1.95
1993
0 0 0 0
16 0
83 3
77 2
19 1
0 1
1.06 1.2 1.35 1.5 1.65 1.8 1.96
0 10 20 30 40 50 60

Figure 4.4: Proportion of *Polymorphus paradoxus*-, *P. marilis*-infected, and uninfected gammarids that were males during the fall (September–October) and spring (April)


P. paradoxus
100
80
60
40
20
0
N=0
47
0
5
3
22
76
251
89-90
90-91
91-92
92-93
P. marilis
16
11
1
12
46
1
82
N=0
Uninfected
■ Fall
□ Spring
235
177
33
177
260
81
1061
N=0
Proportion of gammarids
Year

spring were males. Thus, during peak breeding seasons (spring) the sex ratio of the *G. lacustris* population was male biased.

Pairing Success in Laboratory Tests

In a preliminary experiment I found that previously paired, uninfected males acquired a mate more frequently than single (previously unpaired), uninfected males (9/14 and 5/14 pairs formed, respectively). Although these differences were not statistically different (X^2 test, $p>0.05$), only single males were used in all subsequent experiments to eliminate potential differences between previously paired and single males. Additionally, most *P. paradoxus*-infected males were single, necessitating the use of single *P. marilis*-infected and uninfected males. The pairing success of *P. paradoxus*- and *P. marilis*-infected males in competition with uninfected conspecifics is summarized in Table 4.4. Gammarids infected with *P. paradoxus* paired significantly less often than the uninfected competitors in five of six experiments. Overall, only 15 infected males paired (out of 90 pairs). *Polymorphus marilis*-infected males paired significantly less often than uninfected males in only one of five experiments. However, overall, only 19 *P. marilis*-infected males paired (out of 73 pairs). There were no statistical differences between the pairing success of *P. paradoxus*- and *P. marilis*-infected males. Thus, males parasitized with either *P. paradoxus* or *P. marilis* may be poorer competitors than uninfected males.

The inclination of infected and uninfected males to mate was tested by providing single males with a single female ('alone'). In three of four tests, the pairing success of *P. paradoxus*-infected males was lower than that of uninfected ones. Overall, *P. paradoxus*-infected males paired significantly less often than both *P. marilis*-infected and uninfected males (Table 4.5). In two of four experiments, the pairing success of *P. marilis*-infected males was significantly lower than that of uninfected males. However, there was no significant difference between the overall pairing success of *P. marilis*-infected and uninfected males. There were no differences in the mean number of days to precopula between *P. paradoxus*- and *P. marilis*-infected and uninfected males (ANOVA, $p>0.05$). Thus, in non-competitive situations, males infected with *P. paradoxus* were less inclined to pair with a female than either *P. marilis*-infected and uninfected males.

In each of the experiments, the pairing activity of uninfected males was greater in 'competition' situation of two males than when 'alone' with a female.

Table 4.4: Pairing success of *Polymorphus paradoxus*-, *P. marilis*-infected, and uninfected male gammarids in competition for a single female

Experiment	No. of infected pairs / Total no. of pairs formed (%)	Total no. of pairs formed / Total no. of setup (%)
1		
P. paradoxus	3/17 (17.6)*	17/20 (85.0)
P. marilis	6/16 (37.5)	16/18 (88.9)
2		
P. paradoxus	5/16 (31.2)	16/19 (84.2)
P. marilis	4/12 (33.3)	12/18 (66.7)
3		
P. paradoxus	0/5 (0.0)	5/15 (33.3)
P. marilis	2/9 (22.2)	9/15 (60.0)
4		
P. paradoxus	3/16 (18.8)*	16/18 (88.9)
P. marilis	5/18 (27.8)	18/19 (94.7)
5		
P. paradoxus	2/19 (10.5)*	19/25 (76.0)
P. marilis	NA+	
6		
P. paradoxus	2/17 (11.8)*	17/20 (85.0)
P. marilis	2/17 (11.8)*	17/25 (68.0)
Overall		
P. paradoxus	15/90 (16.7)*	90/117 (77.0)
P. marilis	19/73 (26.0)*	72/95 (75.8)

* Significant differences between the pairing success of infected and uninfected male gammarids (G-test, $p<0.01$)

\+ Not examined

Table 4.5: Pairing success of single *Polymorphus paradoxus*-, *P. marilis*-infected, and uninfected male gammarids provided with a single female ('alone' experiments)

Experiment	Pairs formed / Total of setups (%)	Number days prior to pairing (x ± sd)
2		
P. paradoxus	5/14 (35.7)	1.0 ± 0.0
P. marilis	8/14 (57.1)	2.8 ± 2.5
Uninfected	5/14 (35.7)	1.2 ± 0.4
4		
P. paradoxus	2/14 (14.3)	11.5 ± 14.8
P. marilis	11/15 (73.3)	10.1 ± 8.4
Uninfected	7/15 (46.7)	9.7 ± 6.5
5		
P. paradoxus	0/25 (0.0)*	
Uninfected	12/25 (48.0)	2.1 ± 3.0
6		
P. paradoxus	1/20 (5.0)*	1.0 ± 0.0
P. marilis	10/25 (40.0)+	1.7 ± 1.3
Uninfected	15/20 (75.0)	2.1 ± 2.4
7		
P. marilis	7/20 (35.0)+	6.0 ± 4.8
Uninfected	18/20 (90.0)	1.0 ± 0.0
8		
Uninfected	17/20 (85.0)	1.0 ± 0.0
Overall		
P. paradoxus	8/73 (11.0)*♦	
P. marilis	36/64 (56.3)	
Uninfected	92/104 (69.2)	

/+ Significant differences between the pairing success of infected and uninfected male gammarids (Fisher's exact test, $p<0.001$; + G-test, $p<0.001$)

♦ Significant differences between pairing success of *P. paradoxus*- and *P. marilis*-infected male gammarids (G-test, $p<0.001$)

I therefore investigated pairing in other 'competition' situations. When two males were provided with two females (Experiment 7) pairs formed in 30 out of 40 potential pairs (75%). Single males presented with two females paired in 16/19 cups (84%). In both of these 'competition' tests the pairing success was not significantly different than that of uninfected males alone with a single female (90%). The pairing success of males in experiment 7 was high, indicating that regardless of the male to female ratio (2:1 or 1:1), males were highly inclined to pair during the test period.

The pairing success of uninfected males, whether in competition or alone, varied greatly among dates. Elwood *et al.* (1987) found that males prefer to guard larger females that are closer to moult. Because males become more inclined to enter into precopula as the female approaches moult, I compared the pairing frequency of uninfected males alone with an uninfected female collected on four occasions from April 15 to May 10, 1993 (experiments 5-8). The pairing frequency doubled between April 15 and May 5, reaching about 90% in May (Fig. 4.5). The mean number of days to precopula pairing was halved from April to May samples. Additionally, there were large variances in the April samples (experiments 5 and 6); however, all the May collected males (experiments 7 and 8) paired within 24 hours following the start of the experiment. Thus, peak pairing activity, which occurs approximately three to four weeks following the spring ice break, accounts for much of the variation in pairing activity in uninfected gammarids.

Longevity

The prevalence of *P. marilis* infection was similar (about 5%) during fall and spring of one of the three study years for which data were available (1991; Fig. 4.6). In contrast, during the other two years (1992 and 1993), the prevalence of infection in spring was significantly greater than that in fall (X^2 test, $p<0.05$). No sample was available for fall, 1990. There was no decline in infection prevalence over the winter; thus, *P. marilis*-infected males do not appear to be susceptible to selective winter mortality.

Discussion

Only one of the three aspects of reproductive output of male *G. lacustris* examined in this study was adversely affected by infection with *P. paradoxus* and *P. marilis*. The pairing success of males infected with either parasite was reduced both

Figure 4.5: The proportion of uninfected males paired, and the onset of pairing in 'alone' experiments (1 male and 1 female)

(SD=standard deviation)

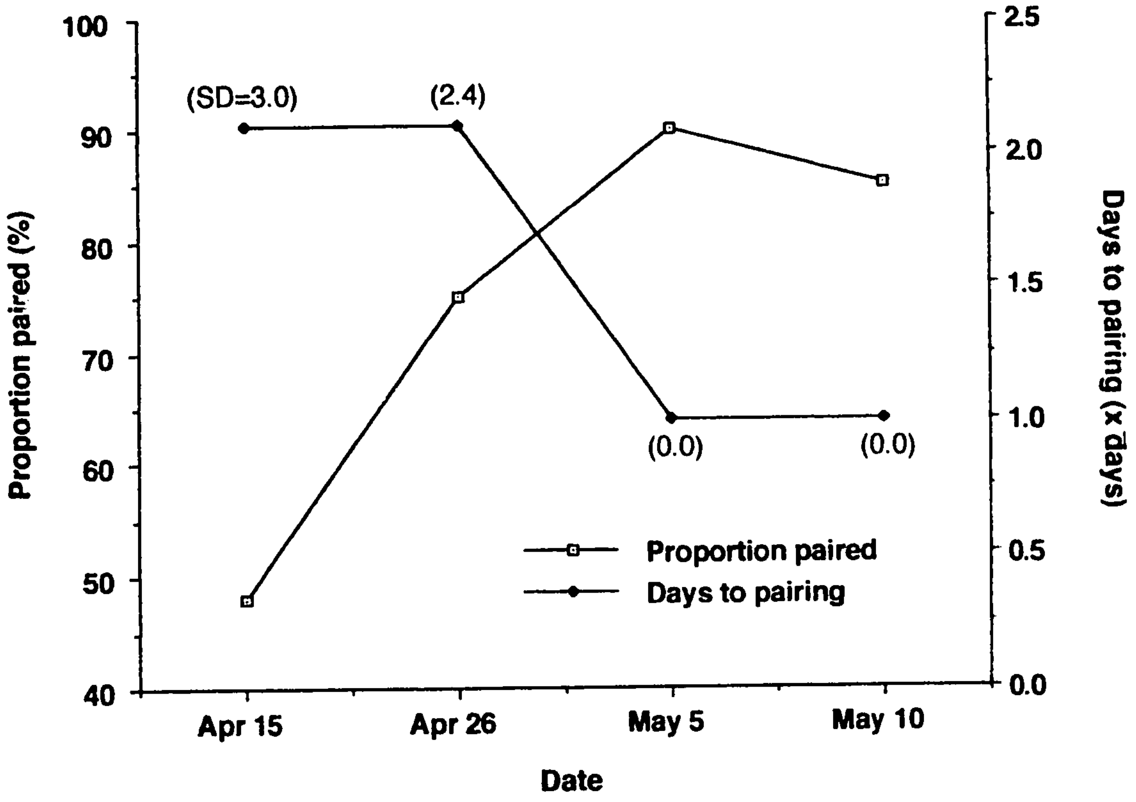
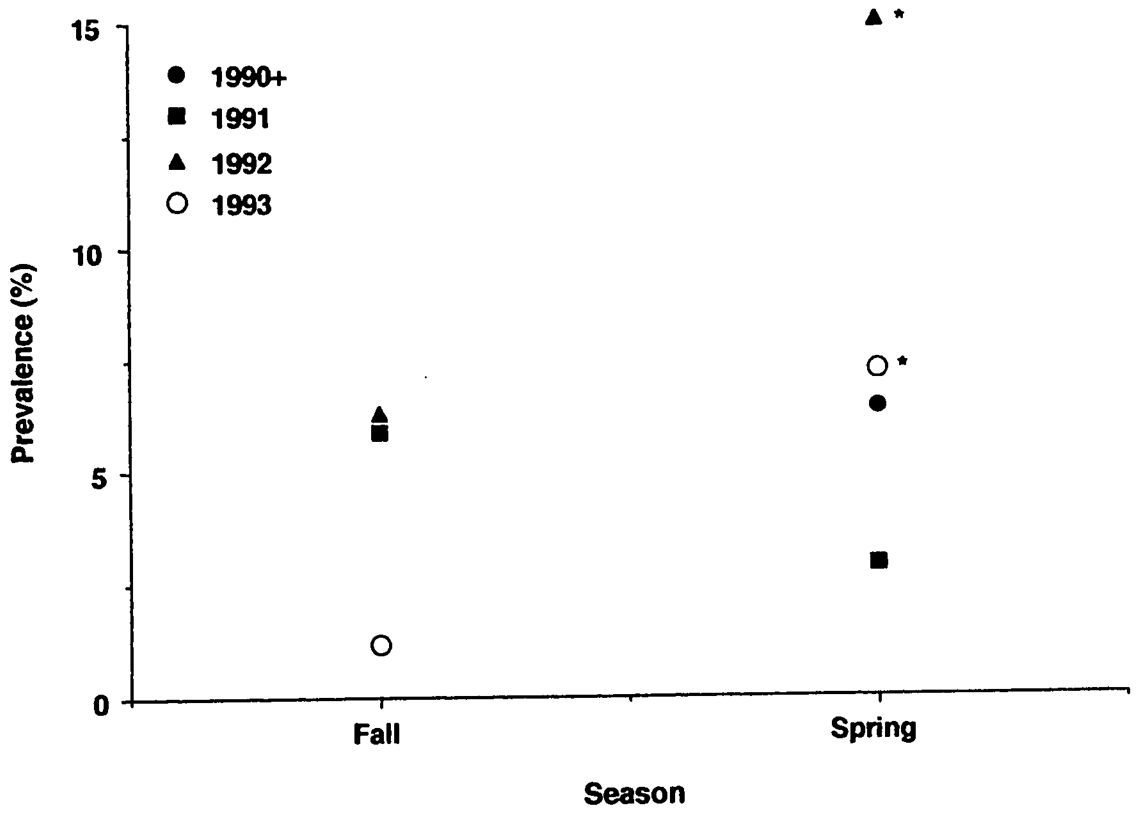

100
90
80
70
60
50
40
Proportion paired (%)
2.5
2.0
1.5
1.0
0.5
0.0
Days to pairing (x̄ days)
(SD=3.0)
(2.4)
(0.0)
(0.0)
Proportion paired
Days to pairing
Apr 15
Apr 26
May 5
May 10
Date

Figure 4.6: Prevalence of *Polymorphus marilis* in *Gammarus lacustris* males at Cooking Lake

* Significantly different from fall prevalences (X^2 test, $p<0.05$)
\+ No data available for fall, 1990


15
10
5
0
Prevalence (%)
● 1990+
■ 1991
▲ 1992
○ 1993
*
*
Fall
Spring
Season

in the field and in laboratory tests. Pairing success was more severely affected by infection with *P. paradoxus* than with P. *marilis*, and may be related to the degree of altered behaviour induced by each parasite. In contrast, there was no evidence that either parasite castrated their male host, or reduced their longevity (overwintering survival).

Parasitic Castration

Although there are very few studies that have examined the effects of acanthocephalans on the sexual development of male intermediate hosts, acanthocephalans have not been found to affect spermatogenesis in peracaridians (LeRoux, 1933; Moore, 1984). In addition, acanthocephalans seldom alter the development of secondary sexual characteristics; LeRoux (1933) provided the only evidence of slower growth of these characteristics in infected gammarids. In my study, neither *P. marilis* nor *P. paradoxus* had any negative effect on normal spermatogenesis, or on the secondary sexual characteristics, the morphology and size of the second gnathopod or the number of calceoli. Most likely, acanthocephalans do not interfere with the functioning of the androgenic gland which regulates spermatogenesis and sexual differentiation (Charniaux-Cotton and Payen, 1982).

Pairing Success

The pairing success of uninfected males varied from 17 to 53% among the four study years. Pairing activities were greatest about three weeks following ice-break (Fig. 4.5 and personal observations). Because ice break occurred on different dates in 1990-1993 and sampling was conducted on different dates among the four years, I encountered varying proportions of paired males. Despite the variability, it is obvious that not all males in the population can or will pair and potentially mate. In all overwintering populations there was a preponderance of males in the population (male bias was also observed in Big Island Lake, Menon, 1966). Assuming that all adult females become paired, if females make up only 40% of the *G. lacustris* population and males can pair and mate with only one female, a maximum of 67% of the males will be paired. This estimate is greater than the pairing success observed in the field; therefore, my observations may underestimate the actual pairing success, because I included sampling times prior to the peak of pairing activities.

In all four study years only four of 302 field-collected, *P. paradoxus*-infected males were found in precopula, and one of the four was attached to another male.

The pairing success of *P. marilis*-infected males, while greater than *P. paradoxus*-infected males, was still about 50% lower than that of uninfected gammarids. Both parasites adversely affected the pairing success of infected males.

As described previously, many factors may contribute to the acquisition of a female to guard. Assortative pairing is commonly observed among mate guarding arthropods (Ridley and Thompson, 1985; Crespi, 1989) and has also been documented for *G. lacustris* at Cooking Lake (Chapter 2). In the present study, the uninfected male/female length ratio in this study was 1.2:1, similar to the ratio of 1.3:1 for *G. pulex* (Birkhead and Clarkson, 1980). The importance of size to the pairing success of gammarids has been demonstrated in numerous studies (Ward, 1983; Elwood *et al*, 1987; Elwood and Dick, 1990). There are several advantages for larger males because they are better able to take over a paired female and can better guard against take overs (Ward, 1983); they can also guard females for longer periods of time and enter into precopula earlier than smaller males (Ward, 1983; 1984). Therefore, larger males are more likely to pair with larger, more fecund females. The absolute length of male *G. lacustris* was unaffected by *P. marilis*, but *P. paradoxus* -infected gammarids were significantly smaller than uninfected males. However, the mean length of *P. paradoxus*-infected males was only one mm less than that of uninfected males, unlikely to be a biologically significant size difference. Thus, the reduced pairing success of infected male gammarids cannot be attributed to size differences between them and uninfected males.

Gammarus pulex exhibit a spatial heterogeneity in their distribution. Larger gammarids are associated with larger substrate particle sizes (Thompson and Moule, 1983). Birkhead and Clarkson (1980) hypothesized that assortative pairing is the outcome of these differences in habitat distributions of large and small *G. pulex* in a stream. The habitat segregation hypothesis as the cause of assortative mating has been criticized (Thompson and Moule, 1983; Dick and Elwood, 1993) since large gammarids are found paired with small females, assortative mating occurs in experimental situations providing uniform substrates, and males search for females from different sites and settle on particle size that suits the pair, not the individual (but see Ward, 1993). Juvenile *G. lacustris*, small females, and those infected with *P. paradoxus* predominated in samples taken from shallow water sites closer to shore, whereas *P. marilis*-infected gammarids were equally distributed between shallow near-shore and deeper off-shore water sites (Chapter 2). Assortative pairing was evident in deep off-shore samples; however, there was no significant correlation between male and female size of paired gammarids from shallow near-shore samples, suggesting pairing in the latter site was random (Chapter 2). The spatial segregation between *P. paradoxus*-infected males and most of the reproductive females may result in their reduced pairing success. Most of the females in near-shore sites were small and may not be attractive to the large infected males. The habitat segregation hypothesis may provide a partial explanation to the lowered pairing success of *P. paradoxus*-infected males, but not for *P. marilis*-infected ones, which have the same distribution as uninfected males.

Male *G. pulex* infected with *P. minutus* did not pair as often as uninfected gammarids in a stream (Ward, 1986). The author suggested that the lowered pairing success may be the outcome of behavioural alterations precipitated by the acanthocephalan. Gammarids suffering from altered behaviour may not be able to engage in normal courtship and pairing activities. In this study, pair formation was experimentally studied under two situations, competition between two males for a single female and the inclination of single males to pair when provided with a single female. Male gammarids infected with *P. paradoxus* were capable of pairing in the laboratory, but consistently lost to uninfected males in competitive situations and paired less often than uninfected in most of the 'alone' experiments. These infected males are not only poor competitors but are also not inclined to mate as often as uninfected conspecifics. Interestingly, in two cases where *P. paradoxus*-infected males paired in a competitive situation (experiment 5), the uninfected losers had only immature sperm cells and no mature sperm. However, the two uninfected losers from another experiment (6) did have mature sperm.

Polymorphus marilis did not impair the inclination of infected males to pair in 'alone' experiments, but overall, males infected with *P. marilis* paired significantly less often than uninfected males in the competition experiments. I therefore concluded that there was a reduction in the competitive ability of *P. marilis*-infected males, but not in their inclination to pair. These results also corroborate the field observations on the pairing success of infected males (Table 4.2).

Several factors may be responsible for lowering the pairing success of infected male gammarids. Pair guarding males incur some energetic costs since they must actively carry the female. Precopula has been found to reduce the feeding rates of paired *G. lawrencianus* males by 53%, with a consequent 45% reduction in their growth rates (Robinson and Doyle, 1985). Paired amphipods are also more susceptible to fish predation (Strong, 1973), are less able to swim in a current (Ward, 1983) and are more susceptible to anoxia (McMahon *et al.*, 1989; 1991). Feeding rates and survival at low oxygen concentrations are also adversely affected by infections with *Pomphorhynchus laevis* (McMahon *et al.*, 1991); therefore, the combination of parasitism and mate guarding may be too energetically costly to infected males.

It is not clear how altered photic responses may disturb normal pairing behaviour, but the clinging behaviour of *P. paradoxus*-infected males may interfere with using their gnathopods to hold a female. *Polymorphus paradoxus*-infected males may also be unable to search effectively for females as they may spend much of their time skimming and clinging in response to disturbances. Behaviourally altered males may be rejected by females. Female gammarids can kick and struggle with males, rejecting their pairing attempts. Females may be selected to choose to reject infected males if susceptibility to infection with acanthocephalans is heritable or if the quality of sperm of infected males is inferior; however, there is no evidence for either circumstance. Females paired with infected gammarids may predispose themselves to greater predation risks since the behavioural alterations induced by *P. paradoxus* infections increase their susceptibility to predation by mallards and muskrats (Bethel and Holmes, 1977).

Longevity

The prevalence of *P. marilis* infections did not decline over winter suggesting that the parasite had no obvious negative effects on winter survival of male gammarids. The failure to find evidence of greater overwinter mortality in infected male gammarids was surprising. *Gammarus pulex* infected with the acanthocephalan *Pomphorhynchus laevis* are so susceptible to reduced oxygen concentrations that they are used as bioassays in aquatic toxicology studies (Poulton and Pascoe, 1990; McMahon *et al.*, 1989; 1991). Given the winter anoxia at Cooking Lake (Mitchell and Prepas, 1990); it might be expected that infections with *P. marilis* or *P. paradoxus* predispose infected *G. lacustris* to greater winter mortality than uninfected males. There is evidence that infection with *P. paradoxus* may predispose infected females to selective winter mortality (Chapter 3). However, the effects of this parasite on male gammarids could not be assessed because of the difficulties associated with the estimation of *P. paradoxus* prevalences.

Conclusions

Parasitism with *P. paradoxus* precipitated a greater loss of reproductive success in males than did infection with *P. marilis*. This may be the outcome of greater energetic demands of *P. paradoxus*, the larger parasite, or the result of the more grossly altered behaviour induced by *P. paradoxus*. As seen in the present study, reduced reproductive success is not always physically or physiologically obvious and may only be detected with behavioural assays. The reproductive success of other acanthocephalan-infected male peracaridians may also be affected in subtle ways that have not yet been assessed.

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Chapter 5 - General Discussion and Conclusions

The acanthocephalans that I studied, *Polymorphus paradoxus* and *P. marilis*, significantly reduced reproduction of both male and female gammarid intermediate hosts. However, *P. paradoxus* and *P. marilis* differed in the way they interfered with reproduction of both male and female hosts. Here I compare my results with the literature, in terms of Barnard's (1990) list of means by which reproductive output of parasitized hosts may be reduced: parasitic castration, brood losses, reduced mating succ[illegible]uced longevity. In addition, I examine the effect of altered behaviour a[illegible]lection on reproduction.

There was no evidence [illegible]ic castration by either parasite, in either male or female hosts. There were [illegible]ications of complete or partial destruction of the gonads, nor were there any signs of negative effects on any secondary sexual characters. At least some females infected with each species of acanthocephalan produced viable broods, and males infected with either *P. paradoxus* or *P. marilis* produced mature, motile sperm.

However, very few *P. paradoxus*-infected *Gammarus lacustris* were found with broods. This could be regarded as evidence of some sterilization, particularly as castration of their hosts by some other acanthocephalans is not always complete. Female *G. lacustris* and *G. pulex* infected with *Polymorphus minutus* have immature ovaries and bristleless oostegites (Hynes, 1955; Hynes and Nicholas 1963). However, 10% of infected females do produce eggs (Hynes, 1955). Additionally, Rumpus (1973, in Kennedy, 1985) calculated that in a locality where the prevalence of *Pomphorhynchus laevis* in *G. pulex* was 26%, only 2.3% of the females were actually sterile. Such systems with partial castration would provide a good opportunity to investigate how, and under what conditions, acanthocephalans do castrate their hosts. Below, I suggest that in the systems I studied, the lack of broods was probably due to a lack of mating success, not castration.

Brood reduction was a common feature of *P. marilis* infections, in which infected females bore broods of 3-5 fewer eggs than similar-sized uninfected females. The most likely mechanism producing brood reduction is a reduced energy budget in infected amphipods. Infection with *P. paradoxus* may also produce brood reductions, but numbers of brood-bearing infected females were too low to establish their brood sizes.

The mating success of infected females was reduced by both *P. marilis* and *P. paradoxus*. The attractiveness to males of *P. marilis*-infected females in the field, as evidenced by the proportion of females paired or with broods, was reduced only for small females, or in years when there was a female skewed sex ratio. In the laboratory, males given a choice between an infected and an uninfected female, preferred the uninfected ones. Females parasitized with *P. paradoxus* were more severely affected; such females were seldom found in precopula or with a brood, and in laboratory experiments, were always rejected by males when given the choice between an infected and an uninfected female. Reduced mating success is common among female peracarid crustaceans infected with other acanthocephalans, such as *Acanthocephalus dirus*-infected isopods (Oetinger and Nickol, 1981) and *Polymorphus minutus*-infected gammarids (LeRoux 1933; Hynes, 1955; Hynes and Nicholas, 1963; Ward, 1986).

Two factors may influence the attractiveness of infected females: the extent to which the behavioural alterations produced by the parasite interferes with courtship activity (discussed below) and the extent to which the female's reproductive value is reduced by infection. Males can assess the brood size and time to ovulation of females (Dick and Elwood, 1989, and see Chapter 3), so they would be expected to reject infected females to the extent that the female's reproductive value is compromised, and the male has a choice. Given the common feature of a male-biased sex ratio in the spring (see Chapter 2), and the even higher effective sex ratio (if males can mate with more than one female), it is likely that most females that can bear a brood will usually be mated (Crespi, 1989).

The mating success of parasitized males was also reduced by infection. In the field, the pairing success of males infected by *P. marilis*, *P. paradoxus* (Chapter 4), or *P. minutus* (Ward, 1986) was significantly lower than that of uninfected male gammarids. In laboratory tests, males infected with *P. marilis* and *P. paradoxus* were inferior competitors, and appeared to be less inclined to pair than were uninfected males. The pairing success of those infected with *P. paradoxus* was more adversely affected than those infected with *P. marilis*. This aspect of male reproduction is seldom considered, yet it may be the most significant influence on the reproductive success of individual male hosts. The examination of attractiveness to mates or the inclination to pair of acanthocephalan-infected arthropods may provide an interesting and important subject for future research.

There was no evidence of reduced survival to the breeding season, based on overwintering survival, of *P. marilis*-infected females and males. Conversely, females infected with *P. paradoxus* were more susceptible to selective winter mortality than *P. marilis*-infected and uninfected females. *Polymorphus paradoxus*-

infected males survived the winter better than parasitized females; however, because of the difficulties of quantifying the prevalence of this parasite, it was not possible to compare their winter survivorship to that of uninfected gammarids.

I also found it impossible to evaluate a second aspect of longevity, survival through to release of the brood. The numbers of *P. paradoxus*-infected gammarids appeared to decrease through spring and early summer, but this apparent decrease is very difficult to quantify. The numbers of *P. marilis*-infected gammarids increases during the same period, due to the development of overwintered acanthors (Tokeson and Holmes, 1982), precluding any calculations of their survival rates. However, predation rates on infected gammarids appear to be high during this period, based on the rapid buildup of populations of adults in migratory definitive hosts (Hair, 1975; J. C. Holmes, personal communication). It is likely that this aspect of the longevity of infected gammarids, especially the females, is an important means of reduced reproductive output.

Change in habitat use due to parasite-induced behavioural modifications may also impinge on reproductive output. The altered behaviour of *P. paradoxus*-infected gammarids resulted in their overwintering in shallower water sites and closer to the shoreline than uninfected adult gammarids. The greatly reduced pairing success of *P. paradoxus*-infected female and male gammarids may be influenced by their overwintering distribution. Shallow water sites were dominated by juvenile and small females, thus reducing the chances of infected females to encounter adult males, and lowering the chances of infected males to encounter large (more fecund) adult females.

The degree of reduced reproduction in female and male gammarids was dependent on the parasite with which they were infected. Although the fecundity and pairing success of *P. marilis*-infected gammarids was lower than that of uninfected gammarids, the effects of this parasite were less severe than infection with *P. paradoxus*. Such differences may stem from differences in the energetic demands of the two cystacanths. Cystacanths of *P. paradoxus* (1.212 mm long by 0.825 mm wide) are about three times the volume of those of *P. marilis* (0.800 mm long and 0.533 wide) (Denny, 1969). Therefore, the metabolic demands of the former parasite on gammarids are probably greater than those of *P. marilis,* diverting more resources necessary for host reproduction into parasite production or maintenance. Females may be more susceptible than males to the parasites' energetic demands, as evidenced by the brood losses experienced by all parasitized female amphipods. However, male gammarids invest energy both in gonadal development and in precopulatory mate guarding. Reductions in pairing success of parasitized males may have been affected by the parasites' energetic demands.

The differential effects of the two acanthocephalans on reproduction may also be the outcome of the different behavioural modifications caused by these parasites. Bethel and Holmes (1973) found that *P. marilis*-infected gammarids are photophilic, but negatively phototactic when disturbed, whereas *P. paradoxus*-infected gammarids are strongly photophilic, positively phototactic, and in response to disturbances they skim the water surface and cling to any material they encounter (eg. vegetation). In this study, the winter distribution of *P. paradoxus*-infected gammarids, but not those of infected *P. marilis*, were modified (discussed above). In addition, *P. paradoxus*-infected gammarids of both sexes may spend more time clinging, further reducing the possibility of encountering potential mates. Clinging to substrate may also interfere with the parasitized males' ability to hold a female in precopula. Helluy (1988) suggested that the clinging response of *P. paradoxus*-infected gammarids is the same as precopulatory behaviour in uninfected males, but is elicited under the wrong circumstances and as a response to the wrong stimuli. Thus, *P. paradoxus*- infected males may not be able to perform normal precopulatory assessment of, or attachment to, females. However, a few parasitized males were found in precopula in both the field and laboratory (present study) suggesting that at least some infected males can perform normal precopulatory behaviour.

Parasite-induced modifications in intermediate host behaviour and reductions in host reproduction may have an effect not only on individuals, by increasing their susceptibility to predation and lowering their reproductive success, but may also affect host populations. Much of the attention given this topic has been directed to the possibility that the parasites regulate the populations of their intermediate hosts (Kennedy, 1985; Dobson, 1988). Kennedy (1985) considered several means by which acanthocephalans can regulate their intermediate hosts' populations. The stabilizing factors are overdispersion, observed in most parasite populations , and host mortality as the result of multiple infections or over-crowding of the early developmental stages (acanthors); such host mortalities are parasite density-dependent (Seidenberg, 1973; Camp and Huizinga, 1980; Amin *et al.*, 1980). The increased vulnerability to predation as a consequence of altered pigmentation (Hindsbo, 1972; Camp and Huizinga, 1979; Oetinger and Nickol, 1981) or behavioural alterations (Hindsbo, 1972; Holmes and Bethel, 1972) is not density dependent, and therefore not considered a regulatory process (Uznanski and Nickol, 1980). Parasite-induced reduction in their hosts' reproductive potential is considered the principal destabilizing factor for populations of intermediate hosts (Kennedy, 1985).

Both of the acanthocephalans examined in the present study were found to have negative effects on the reproduction of female amphipods. They affected the proportion of females bearing broods, the average brood size, or both. These results concur with observations from other studies reporting some degree of reduced reproduction in most acanthocephalan-infected female arthropods (Moore, 1984). Do these reproductive consequences affect intermediate host populations? A closer examination of the possible influence of *P. marilis* in *G. lacustris* in Cooking Lake would be instructive, in that prevalences are very high in the system. In the present study, *P. marilis*-infected females made up about 10% of overwintering female gammarids, but other studies at Cooking Lake have found prevalences of 30-40% during the gammarids' breeding period (Denny, 1967; Tokeson, 1971). Using the higher value for prevalence, and assuming no reduction in the proportion carrying broods (likely from my data), no reduction in longevity through to brood release (unlikely, due to predation, see above), and an average reduction of 5 eggs per female (from an average of 32 eggs; Menon, 1966), the overall loss of reproductive potential would be only 6.25% of the eggs. In high density gammarid populations, such as in Cooking Lake, other density-dependent processes are apt to have much greater effects.

None of the studies examining the influence of acanthocephalans on their male arthropod hosts provide any evidence of reduced reproduction (but see LeRoux, 1933). Although male fertility (gametogenesis, development of secondary sexual characteristics) appears to be unaffected by acanthocephalan infections, their pairing success may be markedly affected. However, this reduced pairing success is unlikely to affect the *G. lacustris* population. The sex ratio of the breeding population is male biased (approximately 60% males and 40% females); thus, a maximum of 67% of the male population can mate (see chapter 4). The prevalence of *P. marilis* in overwintering males is about 10%; therefore, even if none of the infected males mated, enough males exist in the uninfected population to fertilize every female in the population. Additionally, males infected with *P. marilis* do pair, and presumably mate, with females, so the lowered reproductive success of individuals probably has a negligible effect on the *G. lacustris* population. Indeed, Bethel (1972) suggested that because many parasites that reduce their host's reproduction are also susceptible to predation (due to altered host behaviour), predation on parasitized hosts actually removes excess and expendable individuals (non-reproducing individuals).

Kennedy (1985) concluded that although both stabilizing and destabilizing (principally, reduced host reproductive potential) processes occur in many acanthocephalan infrapopulations, there is insufficient evidence to indicate whether the populations are regulated. In Cooking Lake, the *G. lacustris* population incurred reproductive losses due to acanthocephalan infections. Based on this study and the available literature, it is likely that all acanthocephalans impair the reproductive output of female, and potentially male, arthropods. Reproductive losses in acanthocephalan-arthropod systems range from the loss of a few eggs per female (*P. marilis*-infected gammarids; this study) to complete sterility (*Acanthocephalus dirus*-infected isopods; Oetinger and Nickol, 1981), and from reducing the attractiveness of parasitized animals to mates (*P. paradoxus*-infected female gammarids; this study) to the reduction of their propensity to mate (*P. paradoxus*-infected male gammarids; this study). Such reproductive losses can be costly to individual hosts and potentially, to the intermediate hosts' population. However, they are unlikely to have much influence in the Cooking Lake system.

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