

***Gammarus pulex* (L.) Feeding Bioassay—Effects of Parasitism**

D. Pascoe, T. J. Kedwards, S. J. Blockwell, E. J. Taylor

School of Pure and Applied Biology, University of Wales, College of Cardiff,
P.O. Box 915, Cardiff CF1 3TL, United Kingdom

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Taylor *et al.* (1993) described a pollutant bioassay based upon a change in the rate at which *Gammarus pulex* (L.) consumes a novel food source (eggs of the brine shrimp *Artemia salina*) when the animals are stressed by exposure to a pollutant. The bioassay is rapid and non-destructive, and produces results with less variation than reported using conventional leaf-feeding bioassays. However, it is important to determine the extent to which results may be influenced by the physiological status (*e.g.*, due to parasitism) of the animals used in the bioassay.

The acanthocephalan parasite *Pomphorhynchus laevis* Muller, 1776 uses the freshwater amphipod *G. pulex* as its sole intermediate host with the cystacanth, the final developmental stage in *G. pulex* being infective to fish, particularly the barbel *Barbus barbus* (L.) and chub *Leuciscus cephalus* (L.). Cystacanths are known to affect the respiration (Rumpus and Kennedy 1974), behavior (Brown and Thompson 1986), mating decisions (Poulton and Thompson 1987), reproduction (Poulton and Pascoe 1990), behavioral drift (McCahon *et al.* 1991) and hemolymph concentrations (Bentley and Hurd 1993) of their amphipod host.

The aim of this work was to determine whether the feeding activity of the shrimp *G. pulex* is also modified by the presence of the parasite, and, if so, the consequences for pollution evaluation using the feeding bioassay.

MATERIALS AND METHODS

Shrimps were collected by kick-sampling from the River Teme, Herefordshire (Brown and Pascoe 1989) where, at the time of collection, water quality conditions were: pH, 7.98; temperature, 12.5°C; dissolved oxygen, 9.5 mg/L; conductivity, 298 μ S/cm. Twenty large animals (approx. 7 mm in length) and 20 small (approx. 5 mm in length) were selected from the collection and transferred to individual perspex dishes (base area 18 cm²) containing 18 ml of river water, together with 10 shell-less eggs of *Artemia salina*. The feeding bioassay was carried out immediately, as described by Taylor *et al.* (1993), by recording, at regular time intervals, the number of eggs consumed by each animal.

On returning to the laboratory the length and weight of each shrimp were measured and the animals were then dissected to reveal the presence of any infestation with the parasite *P. laevis*. This allowed a classification of both large

Correspondence to: D. Pascoe

Table 1. Median Feeding Times (FT50, minutes) and slope functions (S) of probit response lines for parasitized and non-parasitized *G. pulex* divided into groups of large and small animals. 95% confidence limits are shown in parentheses.

		Parasitized		Non-parasitized	
Large <i>G. pulex</i> n = 20	FT50	378	(307-465)	180	(139-233)
	S	2.4	(2.1-2.8)	2.7	(2.3-3.1)
		n = 14		n = 6	
Small <i>G. pulex</i> n = 20	FT50	368	(315-430)	200	(172-231)
	S	2.0	(1.8-2.2)	1.9	(1.8-2.1)
		n = 12		n = 8	

and small gammarids into parasitized and non-parasitized groups. The feeding data were then examined by probit analysis of the cumulative percentage of eggs eaten by each batch of animals using a FORTRAN program written in this laboratory and based upon Litchfield's (1949) method of time-response analysis. Median feeding times (FT50, the time at which 50% of the eggs have been consumed) were thus determined and compared for the four groups of animals, i.e., large (parasitized and non-parasitized); small (parasitized and non-parasitized).

RESULTS AND DISCUSSION

Dissection indicated that 63% of the *G. pulex* were infected with up to 4 (mean = 1.6) cystacanths plus early larvae of *P. laevis*. The mean (plus standard error) values for length and weight of large shrimps were: 7.1 (0.2) mm & 32.2 (2.8) mg wet weight; and for small shrimps: 5.2 (0.1) mm & 13.0 (0.9) mg wet weight. The mean number and range of parasites (cystacanths and acanthellae) found in the large gammarids was 3.5 (1-7) and in the small gammarids, 2.9 (1-8). The FT50 values for each parasitized and non-parasitized (large and small) batch of animals are shown in Table 1, together with the slope function for each time-response probit line. It is evident for both large and small animals that the FT50 values are significantly ($p < 0.05$) longer for parasitized than for non-parasitized animals, whereas there was no significant difference between their slope functions. This indicates that parasitized animals took longer to consume the eggs provided than non-parasitized ones while the variation in feeding rate of individuals was similar for each group. It is also interesting to note that there were no significant differences ($p > 0.05$) between the corresponding large and small animals. It is not clear why parasitized animals fed at a slower rate than their non-parasitized conspecifics, but it has also been reported (McCahon *et al.* 1988) that in the laboratory they consumed less leaf material in a predetermined time than non-parasitized healthy animals. It is possible that this reflects the physiological/physical damage caused by the developing cystacanth parasites as they grow and compress the internal organs, including gut and hepatopancreatic caeca, of the host.

The amphipod *Gammarus pulex* is a common and sensitive component of freshwater ecosystems and is used increasingly in laboratory (McCahon and Pascoe 1988; Naylor *et al.* 1989; Taylor *et al.* 1991) and field (Maltby *et al.* 1990; Pascoe *et al.* 1994) bioassays as an indicator of pollutants. The results of this study demonstrated that the *G. pulex* feeding bioassay can be used not only in the laboratory for evaluation of toxic chemicals (Taylor *et al.* 1993), but also in the field where it can provide a very rapid assessment of the status of collected animals and, therefore, of the quality of water from which they originate. Future work will examine the value of this bioassay in detecting toxic effects at points downstream of pollutant discharge sites. However, as with all toxicity tests, it is important to be aware of any biological and/or chemical factors which can modify the response of test animals. This study clearly demonstrates that gammarids which are infected with cystacanths of the acanthocephalan parasite *Pomphorhynchus laevis* have median feeding times which are significantly longer than those of non-parasitised animals. It is, therefore, recommended that animals which are either collected or cultured for use in toxicity tests should be assessed for parasite status, by dissection of a sample of the population, in order to avoid the possibility of erroneous results arising from the use of parasitized animals.

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REFERENCES

- Bentley CR, Hurd H (1993) *Pomphorhynchus laevis* (Acanthocephala): elevation of haemolymph protein concentrations in the intermediate host, *Gammarus pulex* (Crustacea: Amphipoda). *Parasitology* 107:193-198
- Brown AF, Pascoe D (1989) Parasitism and host sensitivity to cadmium: an acanthocephalan infection of the freshwater amphipod *Gammarus pulex* (L.). *J App Ecol* 26:473-488
- Brown AF, Thompson DBA (1986) Parasite manipulation of host behaviour: acanthocephalans and shrimps in the laboratory. *J Biol Educat* 20:121-127
- Litchfield JT (1949) A method for rapid graphic solution of time-per cent effect curves. *J Pharm Exp Ther* 97:399-408
- Maltby L, Naylor C, Calow P (1990) Field deployment of a scope for growth assay involving *Gammarus pulex*, a freshwater benthic invertebrate. *Ecotox Environ Safety* 19:292-300
- McCahon CP, Pascoe D (1988) Use of *Gammarus pulex* (L.) in safety evaluation tests: Culture and selection of a sensitive life stage. *Ecotox Environ Safety* 15:245-252
- McCahon CP, Brown AF, Pascoe, D (1988) The effect of the acanthocephalan *Pomphorhynchus laevis* (Müller 1776) on the acute toxicity of cadmium to its intermediate host, the amphipod *Gammarus pulex* (L.). *Arch Environ Contam Toxicol* 17:239-243
- McCahon CP, Maund SJ, Poulton MJ (1991) The effect of the acanthocephalan parasite *Pomphorhynchus laevis* on the drift of its intermediate host *Gammarus pulex*. *Freshwat Biol* 25:507-513
- Naylor C, Maltby L, Calow P (1989) Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. *Hydrobiologia* 188/189:517-523
- Pascoe D, Kedwards TJ, Maund SJ, Muthi E, Taylor EJ (1994) Laboratory and field evaluation of a behavioural bioassay - the *Gammarus pulex* (L.)

- precopula separation (GaPPS) test. Wat Res 28:369-372
- Poulton M, Pascoe D (1990) Disruption of precopula in *Gammarus pulex* (L.) - Development of a behavioural bioassay for evaluating pollutant and parasite induced stress. Chemosphere 20:403-415
- Poulton MJ, Thompson DJ (1987) The effects of the acanthocephalan parasite *Pomphorhynchus laevis* on mate choice in *Gammarus pulex*. Anim Behav 35:1577-1579
- Rumpus AE, Kennedy CR (1974) The effect of the acanthocephalan *Pomphorhynchus laevis* upon the respiration of its intermediate host *Gammarus pulex*. Parasitology 68:271-284
- Taylor EJ, Maund SJ, Pascoe D (1991) Toxicity of four common pollutants to the freshwater macroinvertebrates *Chironomus riparius* Meigen (Insecta:Diptera) and *Gammarus pulex* (L.) (Crustacea:Amphipoda). Arch Environ Contam Toxicol 21:371-376
- Taylor EJ, Jones DP, Maund SJ, Pascoe D (1993) A new method for measuring the feeding activity of *Gammarus pulex* (L.). Chemosphere 26:1375-1381