

# A comparison of freshwater and marine/estuarine strains of *Pomphorhynchus laevis* occurring sympatrically in flounder, *Platichthys flesus*, in the tidal Thames

S. Guillen-Hernandez<sup>†</sup> and P.J. Whitfield\*

Environmental Sciences Research Group, Division of Life Sciences,  
King's College London, Franklin-Wilkins Building, 150 Stamford  
Street, London, SE1 8WA, UK

## Abstract

Collections of flounder, *Platichthys flesus*, at two sites on the tidal River Thames in 1994 and 1995 have, for the first time, revealed the sympatric occurrence of the freshwater and marine/estuarine strains of the acanthocephalan parasite *Pomphorhynchus laevis*. This natural co-occurrence of the strains has been employed to compare infection levels and a range of parasite attributes of the two strains under conditions of sympatry. At both Lots Road (Chelsea) and Tilbury the marine/estuarine strain was present at far higher infection levels than the freshwater form. In a detailed comparison of worms from Tilbury flounder, a range of differences was revealed between the two strains. In single strain infections in individual fish, freshwater and marine/estuarine worms had distinct but overlapping gut microhabitat use patterns, with the former having a central intestinal bias and the latter a bias for the posterior region of the gut. In mixed strain infections, niche contraction occurred so that no overlap occurred. Freshwater worms were larger and had more eggs, more ovarian balls, and a higher percentage of fully developed eggs than the marine/estuarine worms. These differences are thought to reflect intrinsic, presumably genetically determined, differences between the two strains as they occurred in the same fish host species collected at the same place and time. Apparent differences in strain reproductive potential in flounder in the tidal Thames are discussed in the context of previous studies and the intermediate host segment of the parasite life cycle.

## Introduction

While analysing and comparing distributions of the fish-inhabiting acanthocephalan *Pomphorhynchus laevis* in the British Isles, Kennedy *et al.* (1978) and Kennedy

(1986) concluded that this parasite comprised three different ecological strains; two in freshwater (found respectively, in the south of England and Ireland) and one in the Baltic and North Seas and the estuaries linked to them. Brown *et al.* (1986) described *P. laevis* as one of the six acanthocephalan species present in British freshwater fish. The literature on subspecific variation in *P. laevis* has used the term 'strain' for taxa within the species which typically use different intermediate and final hosts as well as different ecological zones. A strain in this sense is broadly equivalent to a sub-species with

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\*Author for correspondence

Fax: 020 7848 4195

E-mail: phil.whitfield@kcl.ac.uk

†Present address: Departamento de Ecología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán. C.P. 97000, A.P. 4–116, Itzimna, Yucatán, Mexico

the implication that in some circumstances interbreeding between sub-species will be possible.

The presence of this parasite in marine (MacKenzie & Gibson, 1970) and estuarine conditions (Munro *et al.*, 1989) and its great variability in morphological characters has caused some taxonomic confusion. The morphological variability within this species could be partly due to the use of different host species, differences in geographical location and seasonality in parasite growth and maturity (Brown, 1987). Given this complexity, no categorical morphological differences between the strains have been found (MacKenzie & Gibson, 1970; Kennedy *et al.*, 1978; Kennedy, 1984). It seems, however, that there are differences at a genetic level (Munro *et al.*, 1990; Munro, 1992) while the presence of an orange colour of adults in the English freshwater strain (Brown *et al.*, 1986) and a white/cream colour in the marine/estuarine strain have been used as morphological characters to differentiate them (Munro, 1992). Munro (1992) considered that parasite colour and the size (typically the freshwater strain is larger than the marine/estuarine one), along with knowledge of host utilization and habitat use of the parasite could together allow differentiation of the two parasite strains.

The freshwater strain of *P. laevis* has been found infecting a wide range of definitive hosts, but in each one has shown different levels of fecundity (Harris, 1972; Hine & Kennedy, 1974; Kennedy *et al.*, 1989; Molloy *et al.*, 1995; Kennedy, 1996; Munro *et al.*, 1999). Munro *et al.* (1989) and Munro (1992) considered the flounder, *Platichthys flesus*, to be the main definitive host of the marine/estuarine *Pomphorhynchus laevis* strain in the River Thames. From 32 fish species examined from the River Thames, 16 were found infected by *P. laevis*, but only in seven, including flounder, did the parasite reach full sexual maturity (Munro, 1992).

In the sampling programme of the present study in the River Thames, several instances arose where the freshwater and marine/estuarine strains of *P. laevis* were apparently existing in conditions of sympatry. This sympatric state existed at two levels: (i) two strains being found separately in different individual flounder collected in a single sample at one place and time; and (ii) two strains being found together in the alimentary tract of individual flounders.

These findings have provided a unique opportunity to carry out a comparative analysis of a variety of reproductive and other attributes such as parasite size and microhabitat use of the two strains in a natural context where confounding variables of differential host use, physicochemical and ecological conditions were completely eliminated.

## Materials and methods

From July 1994 to August 1995, twelve flounder samples were collected in the River Thames. Seven flounder samples were collected at Tilbury Power Station (NGR 755668) ( $n = 213$ ) and five were obtained from Lots Road Power Station (NGR PQ 246770) ( $n = 143$ ). Analyses of parasite infection levels were carried out on all the samples.

Further comparative analysis of characteristics of

individual parasites of the two strains (identified by trunk colour) in sympatric conditions was restricted to infections from Tilbury Power Station samples. Fish were dissected and the position along the intestine of each individual parasite was recorded. The total length of the intestine from the oesophagus to the anus was measured. The position of each individual parasite was transformed to a percentage distance along this total gut length. Parasites were preserved in 70% ethanol after removal from the gut.

Worms from the two *P. laevis* strains were measured and dissected. In order to compare the instantaneous reproductive capacity of the worms, the number of ovarian balls, the number of eggs and the proportions of developing and fully developed eggs were obtained (Keymer *et al.*, 1983). Twelve gravid females of the marine/estuarine strain and 12 of the freshwater strain were dissected. In each case, the parasites were part of an infrapopulation consisting of only a single *P. laevis* strain. Each female was placed in a Petri dish with 70% ethanol and the contents of the worm's pseudocoelome dispersed thoroughly. The bottom of the dish was divided into 0.5 cm squares to aid counting. The total numbers of ovarian balls and eggs in each dissected female were counted directly. The maximum length and width of random 100 ovarian balls from females of each of the strains were measured. Subsamples of 100 eggs were taken from each female and the proportion in each of three egg developmental stages was established. These stages were stage I (eggs in which the external egg envelope closely invests the developing embryo), stage II (where a distinct gap is present between the external egg envelope and the embryo, but no intermediate shell layer is present) and stage III (the mature shelled acanthor, possessing a shell layer with polar extensions and a fibrillar coat). Random samples of eggs in different developmental stages were taken from four females from each strain. The length and width of 150 eggs of the freshwater strain and 197 from the marine/estuarine strain were measured.

All comparative measurements of reproductive structures have been presented as means with standard deviations: means were compared using Student's *t* test, while chi squared Monte Carlo (randomization tests with 1000 repetitions) were employed to compare parasite attribute distributions (Romesburg & Marshall, 1985).

## Results

### *Parasite infection levels*

*Pomphorhynchus laevis* freshwater strain was present in five flounder samples from the River Thames. Three of these samples were collected at Tilbury Power Station and two at Lots Road Power Station. Tables 1 and 2 show that the marine/estuarine strain was more frequently present at both sites than the freshwater strain. In fact, in every collection at both sites, the marine/estuarine strain was present at prevalences varying between 20% and 100%. At Lots Road the marine/estuarine mean intensities were considerably higher than those for freshwater strain worms (July 1995, 1.1 vs. 0.1; August 1995, 22.0 vs.

Table 1. Infection characteristics of the *Pomphorhynchus laevis* freshwater strain in flounder from the River Thames.

Month	n	Tilbury Power Station			n	Lots Road Power Station			
		P%	I	$\bar{x}$		P%	I	$\bar{x}$	
July 1994	30	0	0	0	30	0	0	0	
October	30	0	0	0	–	–	–	–	
November	45	6.6	11.3	0.75	26	0	0	0	
February 1995	–	–	–	–	30	0	0	0	
May	30	6.6	4.0	0.26	–	–	–	–	
June	27	18.5	6.8	1.25	–	–	–	–	
July	30	0	0	0	30	3.3	1.5	0.1	
August	21	0	0	0	27	3.7	1.5	0.1	

P%, prevalence; I, intensity;  $\bar{x}$ , mean intensity; 0, fish collected, no parasites found; –, no collection was made.

0.1). On one occasion at Tilbury the freshwater strain mean intensity was higher than that of the other strain (June 1995; 1.03 vs. 1.25) while on the other two occasions the situation was reversed (November 1994; 4.1 vs. 0.75; May 1995, 0.6 vs. 0.26). The highest prevalence and mean intensity of the freshwater strain were observed in the June 1995 sample from Tilbury.

#### Comparative analysis of strain attributes

The analyses described below refer to adult worms from collections made at Tilbury Power Station in November 1994 and May and June 1995, when both strains were present. Unless otherwise defined, all the analyses below refer to measurements made on worms from single strain infections.

#### Microhabitat use

The distribution of the two *P. laevis* strains along the alimentary canal of flounder individuals, containing both parasite strains and single strain infections was analysed. At a population level (i.e. fish containing either strain or both), the freshwater *P. laevis* strain has a pattern of microhabitat use which is centred on a modal frequency in a central gut section (41–50% distance, see fig. 1). The overall distribution is one with a central bias. The distribution of marine/estuarine worms is quite different (chi squared = 8.6;  $P = 0.0001$ ;  $df = 10$ ) (fig. 1) with the 91–100% section being the modal one and the pattern being highly biased towards the posterior end of the gut. When both strains were present in the same individual

host, the marine/estuarine strain distribution along the intestine was restricted to the rectum and posterior part of the intestine (81–100%) (fig. 2). The freshwater strain was present mainly in the middle intestine regions, but was not observed in the rectum when the marine/estuarine strain was present in the same individual host. Under single strain infection conditions, the marine/estuarine strain was present from the 21–30% section of the gut to the rectal region (91–100%) (fig. 3). The freshwater strain in single strain infections was present mainly in the middle parts of the intestine (21–60%), but was also observed in the rectum (fig. 3).

#### Adult worm length

Considering each strain as a mixed-sex population sample, parasites of the freshwater strain are longer ( $10.89 \pm 1.75$  mm vs.  $7.3 \pm 1.6$  mm;  $t = 10.9$ ;  $P = 0.00001$ ;  $df = 118$ ) than those of the marine/estuarine strain. The freshwater strain females are longer than the marine/estuarine females ( $11.75 \pm 1.7$  mm vs.  $7.78 \pm 2.35$  mm;  $t = 5.14$ ;  $P = 0.00001$ .  $df = 25$ ).

#### Egg number

The number of eggs found in the female body cavity in both strains was very variable. The mean number of eggs per female worm was higher in freshwater worms than in the marine/estuarine worms ( $33567 \pm 16287$  vs.  $5894 \pm 7416$ ;  $t = 5.36$ ;  $P = 0.0001$ ;  $df = 15$ ). A positive relationship was demonstrated between gravid female worm length and the number of eggs found in such worms ( $r = 0.72$ ,  $P = 0.05$ ;  $df = 11$ ) for the freshwater

Table 2. Infection characteristics of the *Pomphorhynchus laevis* marine/estuarine strain in flounder from the River Thames.

Month	n	Tilbury Power Station			n	Lots Road Power Station		
		P%	I	$\bar{x}$		P%	I	$\bar{x}$
July 1994	30	20	1.6	0.43	30	86.6	4.8	4.2
October	30	26.6	22.1	5.9	–	–	–	–
November	45	42.2	9.8	4.1	26	77.0	18.8	14.5
February 1995	–	–	–	–	30	83.3	16.8	14.0
May	30	26.6	2.5	0.6	–	–	–	–
June	27	40.7	2.5	1.03	–	–	–	–
July	30	33.3	2.8	0.9	30	56.6	1.9	1.1
August	21	47.6	11.3	5.3	27	100	22.0	22.0

P%, prevalence; I, intensity;  $\bar{x}$ , mean intensity; –, no collection was made.

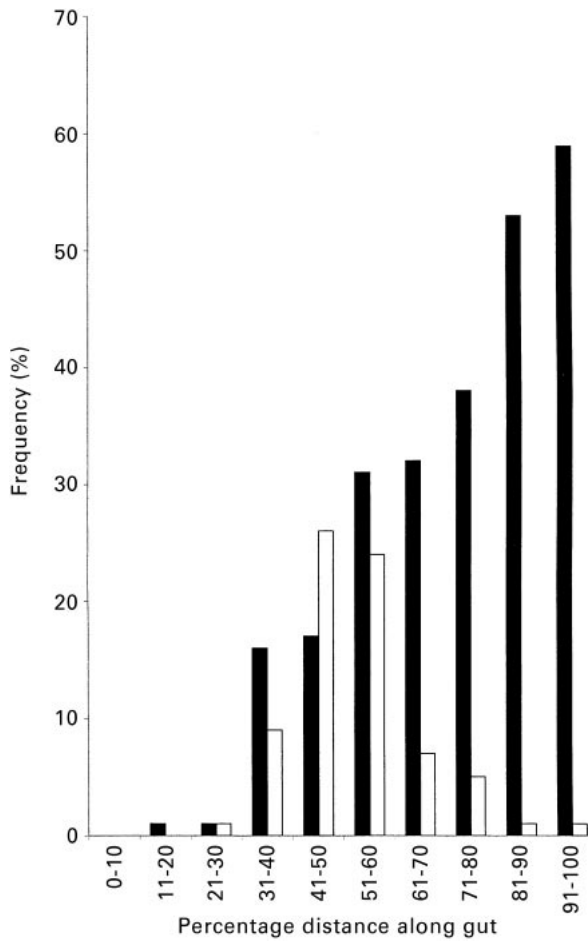


Fig. 1. Distribution of freshwater (□) and marine/estuarine (■) strains (mixed and single strain infections) of *Pomphorhynchus laevis* along the flounder gut.

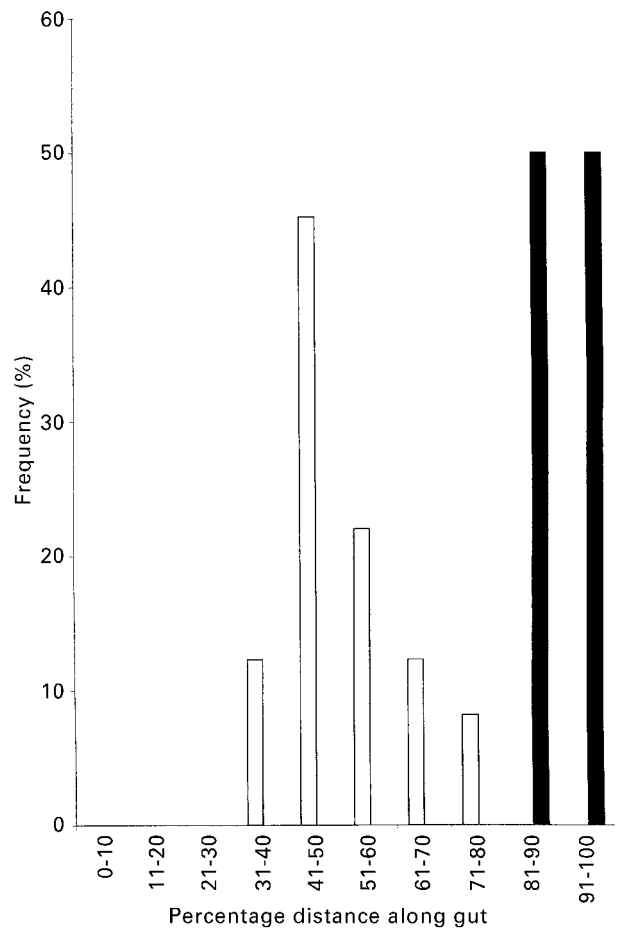


Fig. 2. Distribution of freshwater (□) and marine/estuarine (■) strains of *Pomphorhynchus laevis* along the flounder gut in mixed infections.

strain and  $r = 0.54$  ( $P = 0.05$ ;  $df = 11$ ) for the marine/estuarine strain.

*Ovarian balls*

More ovarian balls were observed per female worm in the freshwater strain than in the marine/estuarine one ( $432 \pm 97$  vs.  $278 \pm 106$ ;  $t = 3.71$ ;  $P = 0.013$ ;  $df = 21$ ). Equally, they were longer ( $129.8 \pm 32.8 \mu\text{m}$  vs.  $92.8 \pm 22.2 \mu\text{m}$ ;  $t = 9.27$ ;  $df = 169$ ) and wider ( $97.2 \pm 13.1 \mu\text{m}$  vs.  $63.4 \pm 14 \mu\text{m}$ ;  $t = 12.42$ ;  $df = 159$ ) than the ovarian balls from the marine/estuarine strain.

*Egg development stages*

The percentages of eggs in different development

Table 3. Percentage of different egg developmental stages of the two *Pomphorhynchus laevis* strains.

Strain	Stage I	Stage II	Stage III
Marine/estuarine	41.7	50.9	17.4
Freshwater	14.4	30.6	65.0

stages in the body cavity of gravid females from the two *P. laevis* strains were different (see table 3). In the freshwater strain, stage III, i.e. mature eggs, are the most abundant (65.0%), and in the marine/estuarine strain, this stage is the least frequent (17.3%). Eggs in different developmental stages from the two strains showed differences in their sizes. The stage I eggs ( $57.35 \pm 3.2 \mu\text{m}$  vs.  $57.9 \pm 3.9 \mu\text{m}$ ) of the two strains are similar in length, but stage II eggs ( $75.9 \pm 9.0 \mu\text{m}$  vs.  $67.2 \pm 5.8 \mu\text{m}$ ;  $t = 6.49$ ;  $P = 0.00001$ ;  $df = 100$ ) and stage III eggs ( $98.0 \pm 5.9 \mu\text{m}$  vs.  $85.7 \pm 6.6 \mu\text{m}$ ;  $t = 9.12$ ;  $P = 0.00001$ ;  $df = 53$ ) of the freshwater strain are longer than those of the marine/estuarine strain.

**Discussion**

All previous studies on *P. laevis* in fish from the River Thames have assumed that the parasites studied were all individuals of the marine/estuarine strain (Munro, *et al.*, 1989, 1990, 1999; Munro, 1992; Lee, 1993). Extensive investigations by these workers on this acanthocephalan in flounders from West Thurrock and Lots Road Power

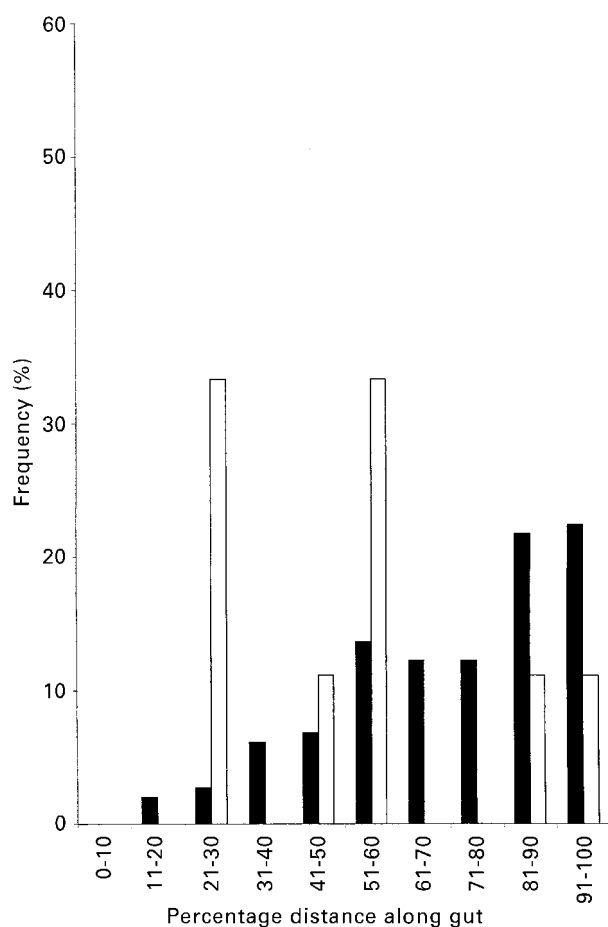


Fig. 3. Distribution of freshwater (□) and marine/estuarine (■) strains of *Pomphorhynchus laevis* along the flounder gut in single strain infections.

Stations have provided considerable evidence for this assumption and part of that evidence related to the white/cream colour of the trunk of all worms.

The present study using flounder collected from Lots Road and Tilbury Power Stations in 1994 and 1995 has, for the first time, found yellow/orange *P. laevis* infecting flounders in the Thames, albeit normally at much lower infection levels than that shown by the white/cream forms (see tables 1 and 2). The findings of white/cream and yellow/orange worms in sympatric circumstances and in a single host species, *Platichthys flesus*, means that differential attributes which are shown to be linked with the colour dimorphism are very likely to be a direct result of intrinsic, presumably genetically determined differences in the two parasite taxa. Co-occurrence in the same fish species at the same place and time means that such differences are most unlikely to be caused by external factors, such as geographical, seasonal, nutritional or host species utilization differences.

The results of initial separation of samples into two subsamples (putatively marine/estuarine and freshwater worms) have been that it has been possible to show clear differences in other attributes between the two groups.

Such differences have been demonstrated for adult body size (mixed sex samples), female body size, distribution along the flounder gut, number of eggs per gravid female worm, number of ovarian balls per gravid female worm, egg size and details of egg development. Given that these differences cannot be attributed to developmental status (gravid worms from each subsample were compared in analysis of reproductively related attributes), it would seem that the simplest hypothesis to explain this list of differences is that the two sub-samples actually do represent distinct strains or subspecies of *Pomphorhynchus laevis*. The ecological situation apparently now existing in two regions of the tidal Thames is of small but finite spatial and temporal overlap of the two parasite strains in the same fish host species.

Most adult specimens of acanthocephalan species are confined to the lumen of the small intestine, and often to a particular zone of it (Crompton, 1973). Kennedy *et al.* (1976), speculated that the major factor determining the distribution and site preference of *P. laevis* in the intestine of cyprinids and salmonids is the place of liberation and activation of the parasite.

The two *P. laevis* strains, as analysed in the present study, have partially overlapping but different distributions along the flounder's intestine. In previous surveys, this difference in microhabitat use has previously been described by MacKenzie & Gibson (1970) and Kennedy *et al.* (1989), but always in single strain infections. The present study is unique in providing an opportunity to study the microhabitat utilization of the two strains when present together. Figures 2 and 3 show that when present in single strain infections the two strains have overlapping but distinctive distributions, with the anterior bias of the freshwater strain and the posterior bias of the marine/estuarine strain already present. When occurring together in the same intestine (see fig. 2), there is clear evidence of niche width reduction in both strains, with zero overlap being observed in the fish examined. Niche width reduction in such circumstances can be regarded as a reproductive isolating mechanism which will reduce the chance of inter-strain mating occurring even when the two strains occur together in the same fish gut. Along with the other differences between the two strains, this phenomenon might suggest that the strains are incipient new species. At present, unfortunately, there are no experimental or field data on the existence or outcome of inter-strain mating, nor have cross-feeding experiments with eggs of the two strains and different *Gammarus* species been successfully achieved. Such work, though, would certainly illuminate the status of the strains/subspecies.

Previous studies on what are assumed to be marine/estuarine strain worms (Munro, 1992; Lee, 1993; Munro *et al.*, 1999) have examined differences in *P. laevis* reproductive activity in different fish hosts in the tidal Thames. Of the 16 species investigated, four, including eels (*Anguilla anguilla*) and flounder, demonstrated infections where over 20% of females worms were gravid, another three species including smelt (*Osmerus eperlanus*) possessed infections with between 1% and 20% of gravid females while in the other nine fish hosts examined, gravid females were never found. Detailed investigations compared marine/estuarine *P. laevis*



reproductive potential in flounder and smelt from the tidal Thames (Lee, 1993; Munro *et al.*, 1999). All components of overall parasite reproductive potential in the final hosts showed greater fitness in flounder compared with the smelt. Parasite prevalence (97% vs. 74%), parasite intensity (35 vs. 15), percentage of female worms gravid (50% vs. 10%) mean number of eggs per female (29,000 vs. 1200) and percentage of eggs at the shelled acanthor stage of development (35% vs. 0.5%), all showed this disparity. In the final host segment of the life cycle marine/estuarine *P. laevis* is achieving a far higher reproductive potential in flounder than in smelt.

The present study allows a different type of comparison of *P. laevis* reproduction for the first time: a direct comparison between freshwater strain and marine/estuarine strain reproductive potential in the same host (flounder) in sympatric conditions in the tidal Thames. The results of this investigation showed clear differences between the strains. In all the measures that are likely to indicate higher reproductive potential: egg number per female worm (c. 33,500 vs. 6000), ovarian ball number per female worm (c. 430 vs. 280) and the percentage of eggs at the shelled acanthor stage of development (65% vs. 17%), the freshwater strain parasites show enhanced values compared with those of the marine/estuarine strain. These differences are probably not due to parasite age/size differences since large females of the marine/estuarine strain had lower egg numbers than worms of the same size of the freshwater strain. Ignoring the possible contribution to overall reproductive success of the intermediate host segment of the life cycles, these results suggest that in flounders in the tidal Thames freshwater strain parasites have higher reproductive potential than marine/estuarine parasites.

This situation would, if actually the case, be an unstable one as in conditions of sympatry the freshwater strain would be expected to rapidly become the dominant strain. This was not the case in 1994 and 1995. Two hypotheses suggest themselves to explain this anomaly. First, it is possible that a process of strain replacement is under way now and that the present dominance of the marine/estuarine strain will be altered by an increasing proportion of freshwater worms. Second, it is possible that it is invalid to ignore the intermediate host segment of the life cycles in estimating overall reproductive potential.

The first hypothesis seems unlikely to be tenable because regular collections since 1994/95 (unpublished results) have showed no decline in the dominance of the marine/estuarine strain in the tidal Thames. In relation to the second hypothesis, there is evidence (Munro, 1992; Munro *et al.*, 1989, 1999) that there are distinct intermediate host use patterns for the freshwater and marine/estuarine strains, with the former being adapted for *Gammarus pulex* and the latter for *G. zaddachi*. To a first order of approximation, there are no *G. pulex* in the tidal Thames, with *G. zaddachi* being the dominant species in the study zone (Munro, 1992). This means that despite an enhanced potential output of shelled acanthors by freshwater worms in flounder, the chances of these eggs infecting appropriate gammarids is probably very low or zero in the tidal Thames. If this were true, it would explain the continuing dominance of the marine/

estuarine strain. It would further suggest that the freshwater strain worms that are seen in flounder in the tidal Thames are likely to have been acquired outside the tidal regions, in freshwater tributaries where *G. pulex* is present. This analysis suggest that the parasite sympatry observed in this study is generated by a low level of immigration of flounders infected in this way into the tidal Thames as part of the normal migrational movements of these catadromus fish.

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