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Digenean metacercariae (*Timoniella* spp., *Labratrema minimus* and *Cryptocotyle concava*) from the flounder, *Platichthys flesus*, in the tidal Thames

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Abstract

A detailed examination of the abundant flatfish species *Platichthys flesus*, the flounder, in the tidal Thames has revealed the presence of four digenean metacercarial parasites, *Cryptocotyle concava* (Creplin, 1825), *Timoniella imbutiforme* (Molin, 1859), *T. praeterita* (Looss, 1901) and *Labratrema minimus* (Stossich, 1887). Flounders were recorded as a new second intermediate host for *T. praeterita* and *L. minimus*. They were also recorded as second intermediate hosts for the first time in British waters for *T. imbutiforme*. The temporal and spatial characteristics of these infections were examined and were believed to provide indirect parasitological evidence of the movement patterns of flounders during their utilization of the Thames Estuary as a nursery ground. From these data it was also surmised that the first intermediate host of *T. imbutiforme*, *T. praeterita* and *C. concava* was probably the molluscan species *Hydrobia ulvae* in the lower Thames Estuary, whereas *L. minimus* was most likely to occur in the molluscan host *Cerastoderma edule*, also present in the lower estuary.

Introduction

The flounder, *Platichthys flesus* (Pleuronectidae), is a euryhaline teleost that is widespread in the coastal waters of Britain, north west Europe, the Baltic, and the Mediterranean (Beaumont & Mann, 1984). This facultatively catadromous flatfish occupies estuaries and inshore waters during most of its juvenile life, returning to the sea, when mature, to spawn (Wheeler, 1969). Its wide distribution and high abundance makes it an important food fish, especially in northern Europe and the Baltic (Wheeler, 1969; Hardisty & Badsha, 1986). The flounder is the only European flatfish that enters and, during the early stages of its development, prefers reduced levels of salinity, enabling it to penetrate deep into the upper reaches of many estuaries and also into freshwater (Hutchinson & Hawkins, 1993).

Older flounders, between the age of 4+ or 5+, spend most of their time in the lower estuaries, unlike many of the youngest age groups which travel further upstream into brackish waters, sometimes as far upriver as the tributaries (Beaumont & Mann, 1984; Weatherley, 1989). Some accounts suggest that 0+ flounders enter freshwater rivers in spring as post-larvae, and remain there for up to 3 years before returning to the sea to spawn (Johnston, 1981 cited in Weatherley, 1989), while Summers (1979) and Hardisty & Badsha (1986) describe an autumn immigration of all flounder age groups, annually, to a lower position in the estuaries. Mature flounders continue out to the sea to spawn, and the majority of spent adults remain close to their spawning grounds outside the estuaries (Hardisty & Badsha, 1986).

This migratory pattern of juvenile and pre-spawning flounders through freshwater, tidal rivers and estuaries, inevitably exposes the fish to various, ecologically distinct environments (Scott, 1985; Gibson, 1972). Biotic, as well as abiotic factors within these environments will consequently have an effect on the migrating fish, but only if their period in such locations is sufficiently long (Dogiel, 1961; MacKenzie, 1983). A useful indicator of fish movements is their parasite load, which will often demonstrate

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the habitat or habitats to which a population of fish has been exposed, particularly if the habitats harbour a particular parasite or group of parasites specific to them (Gibson, 1972; MacKenzie, 1983, 1987).

Salinity differences exhibited by mixohaline and euryhaline habitats are an important abiotic factor in the distribution of many parasite species, not only through the parasites' own tolerance of such conditions, but also, and more importantly, through the salinity-related distribution of the parasites' intermediate hosts or final hosts (Dogiel, 1961; Williams & Jones, 1994). The metacercariae of the digenean *Stephanostomum baccatum*, for example, which is restricted to marine coastal waters where its first intermediate host *Buccinum undatum* exists, can only infect flatfishes that come into the vicinity of the first host. Young flounders are thus exempted from infection because they do not encounter this host due to their brackish and freshwater distribution in their early developmental years (MacKenzie & Gibson, 1970).

The movements of young flounder populations along an estuary or tidal river can potentially be traced through their parasite load (Burn, 1980). Burn (1980) was able to discern the movement patterns of the smooth flounder *Liopsetta putnami* in the Great Bay Estuary, New Hampshire, from the infection prevalences of *Cryptocotyle lingua* and the protozoan *Myxobilatus* sp. Both these parasites were only present in the lower estuary, and found to be absent from the 0+ fish in the upper estuary. He concluded, therefore, that this age group of the fish population was restricted to the upper estuary during its first year of life.

There are relatively few studies on the helminth parasites of flounders in the tidal Thames (see for instance Munro *et al*., 1989; Munro, 1992) and, in particular, the digenean metacercariae harboured within them, which may be related to host population studies. The existence of metacercarial cysts in the gills of flounders, however, was observed during a general parasitological survey carried out on flounders collected from Lots Road in 1989 (unpublished observations). These were later identified as belonging to the digenean *Cryptocotyle concava* (Messoudi, 1993).

A parasitological survey of flounders in the tidal Thames was therefore undertaken to determine whether other metacercarial cysts utilized flounders as intermediate hosts in this region of the Thames. The results of this survey are presented in the present contribution, and are discussed in relation to host population structure and host movements in the tidal Thames.

Materials and methods

Sample collection

Flounder samples were collected from Lots Road Power Station (LRPS), Chelsea, Central London (NGR TQ 246 770) and West Thurrock Power Station (WTPS), West Thurrock, Essex (NGR TQ 592 770) during a sampling period between January 1992 and November 1994. The flounder samples retrieved in 1992 were collected intermittently from Lots Road between January and April 1992, before their numbers from this location began to drop at the onset of summer. Consequently collection was transferred to West Thurrock, where there was a relatively constant supply of flounders during the summer months of June, July and August. Sampling of flounders was resumed at Lots Road in October, but was only maintained for a further month before being suspended for a year. Sampling of flounders began again in November 1993 and was continued at regular monthly intervals for one full year ending in October 1994.

Flounders are drawn from the Thames River in water intakes at both LRPS and WTPS, but are then removed from the water flow on filter screens. Fish then accumulate in reservoirs from which they were caught using drop nets with a mesh size of 15 mm diameter. Retrieved flounders were placed into large sealed buckets containing cool river water, and transferred back to the laboratory. Here they were maintained live in recirculating freshwater aquaria prior to their examination. Flounders collected from WTPS were transported in larger containers to provide sufficient oxygen-saturated water for the longer trip. Fish sample sizes at both collection sites are summarized in table 1. In the 12 months of continuous sampling at Lots Road during 1993/94, the samples reflect the total number of fish that could be caught in two hours of drop net sampling.

Examination of flounders

Flounders were examined as soon as possible, usually no longer than one week, after capture. They were maintained live during this period, as this provided fresh parasite specimens ideal for further examination or preparations for light or electron microscopy. The flounders were killed individually, immediately prior to examination, by a blow to the head and a severing of the spinal cord at the base of the head. The Standard Length (S.L. = pedicle length) of each flounder was measured to the nearest mm.

Examination for metacercarial cysts

External body examination

The flounder's ocular and blind surfaces were inspected by eye using transmitted light through the body. Due to the thickness of many of the flounders only *Cryptocotyle concava* cysts could be discerned using this method since they caused a distinctive host reaction (a melanized host capsule) manifesting in a conspicuous black spot. The other types of metacercarial cysts could not be visualized using this method in any hosts. After examining the skin and body, the caudal, dorsal and ventral fins were removed and examined individually under a Kyowa binocular dissecting microscope.

Internal body examination

The gill arches of the flounders were removed individually and placed in a separate watch glass containing teleost 7‰ saline. Each gill arch was examined and the number of metacercarial cysts counted. The surrounding muscles and connective tissue were also examined for any cysts. The inner lining of the opercular chamber, the mouth and the oesophagus of each flounder in the preliminary sub-sample were examined for

Table 1. Individual monthly sample size of each main flounder sample.

	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
Lots Road '93/94	14	24	8	12	2	33	36	24	25	28	8	10	
West Thurrock '92								3	36	6			
Lots Road '92			16	20	24	26						2	33

Table 2. Mean flounder lengths and corresponding length range of the three flounder samples collected between 1992 and 1994.

Flounder sample	Mean S.L. (mm)	Range (mm)	SD
Lots Road 1992 (n = 121)	107.7	51–220	± 32.9
West Thurrock 1992 (n = 45)	133.7	48–232	± 32.2
Lots Road 1993/94 (n = 224)	123.2	51–250	± 32.4

metacercarial cysts. The eyes were also dissected out and examined, as well as the brain and the surrounding tissues.

The peritoneal cavity of the flounder was exposed, and the visceral organs were removed with the gut cut anteriorly at the oesophagus and posteriorly at the anal sphincter, and transferred into a petri dish containing teleost saline. The liver squashes were only examined for parasitic organisms in a preliminary sub-sample, and randomly in the remaining flounder samples. The mesenteries, the kidneys, the genital organs and the urinary bladder were only examined in this preliminary sub-sample, in addition to heart squashes. The blood was not examined. The gut was examined for metacercarial cysts in all flounder.

Muscle squashes were carried out on a small sub-sample of the flounders used for the epidemiological survey. The presence of metacercarial cysts of *Cryptocotyle concava* and *Timoniella* spp. were initially recorded in the preliminary sub-sample, but accurate cyst numbers were difficult to ascertain without resorting to the complete excision and examination of the somatic muscles of each fish. The mean intensity and intensity values for metacercariae, therefore, are related to the superficially observed metacercarial cysts in the skin, fins, gills and internal organs.

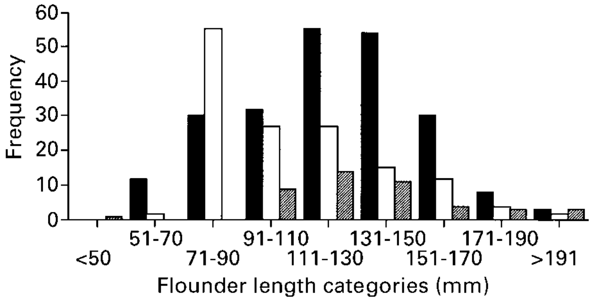

Fig. 1. The length composition of the three main flounder samples collected between January 1992 and October 1994 (0+ (< 70 mm); 1+ (71–120 mm); 2+ (121–170 mm); 3+ (171–215 mm), derived from Chen (1994)). ■ Lots Road 1993/94; □ Lots Road 1992; ▨ West Thurrock 1992.

Organization of data

The data accumulated from the epidemiological survey were organized into three distinct sample groups, depending on the year the flounders were retrieved, and the location from which they were collected. These samples were organized as 1992 Lots Road flounder sample (n = 121), 1992 West Thurrock flounder sample (n = 45), and 1993/94 Lots Road flounder sample (n = 224). Table 1 summarizes details of monthly sample sizes, and the months in which samples were retrieved, for each group. The 1993/94 Lots Road flounder sample provided the basis for the main epidemiological survey of the metacercarial cysts of flounders presented below. In conjunction with this, the remaining two samples were included whenever required to confirm these results or to compare variations in the parasitic infections at different locations in the tideway.

Results

Host population structure

All three flounder samples exhibited a range of lengths (S.L.) corresponding to 0+, 1+, 2+, and 3+ age groups (Chen, 1994). The mean S.L. of both Lots Road flounder samples, however, was generally lower than the West Thurrock sample (table 2).

The length composition of each sample group is illustrated in fig. 1. The figure shows the 1992 Lots Road sample group skewed to the right, the modal group being the flounders measuring between 71 and 90 mm S.L. corresponding to young 1+ flounders (Chen, 1994). The 1993/94 Lots Road data set has a modal class of 111–130 mm, but with some evidence of a shoulder in the 71–110 mm length category. These two length groups correspond to the 2+ and 1+ flounder age classes respectively. The fish from the 1992 West Thurrock sample also revealed a 111–130 mm modal class (fig. 1).

The monthly mean lengths of the 1993/94 Lots Road flounder sample were examined to determine any seasonal trends related to possible migratory patterns. The mean lengths remained relatively stable, ranging between 122·5 and 134 mm, at this site throughout most of the year, although values were dramatically reduced in July and September when mean lengths measured 96 mm and 112 mm respectively.

Table 3. Overall prevalence, mean intensity and intensity of *Cryptocotyle concava*, *Timoniella* spp. and *Labratrema minimus* metacercarial infections in the 1993/94 Lots Road flounder sample collected between November 1993 and October 1994.

Lots Road 1993/94 (n = 224)	Prevalence (%)	Mean intensity ($\bar{x}$)	Intensity (I)
Cryptocotyle concava	34.8	5.0	14.4
Timoniella spp.	18.8	1.3	6.0
Labratrema minimus	9.3	0.7	7.8

Table 4. Overall prevalence, mean intensity and intensity of *Cryptocotyle concava*, *Timoniella* spp. and *Labratrema minimus* metacercarial infections in the 1992 Lots Road flounder sample collected between January and November 1992 (samples were not retrieved between May and August).

Lots Road 1992	Prevalence (%)	Mean intensity ($\bar{x}$)	Intensity (I)
Cryptocotyle concava (n = 118)	42.4	14.5	34.2
Timoniella spp. (n = 97)	21.6	1.3	5.8
Labratrema minimus (n = 97)	13.4	1.1	8.4

Table 5. Overall prevalence, mean intensity and intensity of *Cryptocotyle concava*, *Timoniella* spp. and *Labratrema minimus* metacercarial infections in the 1992 West Thurrock flounder collected between June and August 1992.

West Thurrock 1992 (n = 45)	Prevalence (%)	Mean intensity ($\bar{x}$)	Intensity (I)
Cryptocotyle concava	51.1	7.4	14.4
Timoniella spp.	20.0	1.9	9.3
Labratrema minimus	13.3	1.2	9.2

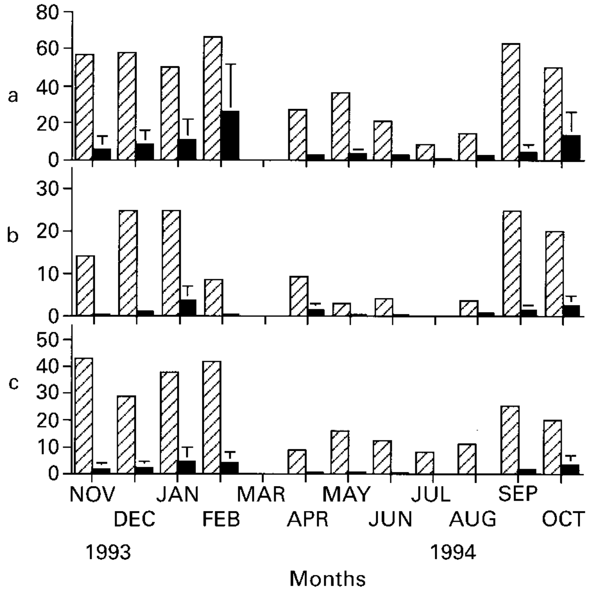

Fig. 2. The observed monthly percentage prevalences (▨) and mean intensities (■) of the three types of metacercarial cyst species infecting flounders retrieved from Lots Road, Chelsea, between November 1993 and October 1994. a, *Cryptocotyle concava*; b, *Labratrema minimus*; c, *Timoniella* spp. (The observed prevalences and mean intensities for March are omitted due to the small host sample size (n = 2).)

Digenean metacercariae

Overall infection characteristics

During this investigation four digenean species were discovered utilizing flounders as a second intermediate host and acting as carriers of their metacercarial cyst stage (table 3). The most prevalent of these metacercarial infections in flounders retrieved from Lots Road in 1993/94 was the heterophyid species *Cryptocotyle concava*. Also present were two congeneric species belonging to the acanthostomatid genus *Timoniella* (identification of these and other parasite species confirmed by Dr R. Bray, The Natural History Museum), namely *T. imbutiforme*, which has not been recorded in British flounders prior to this date, and *T. praeterita*, which is believed to be a newly recorded parasitic infection of this species of flatfish. The latter two species are referred to by their generic name *Timoniella* spp. in this investigation as they were not differentiated during the course of the epidemiological examination. The last metacercarial species, which was the least prevalent in the examined flounders, belongs to the order Gasterostoma and was identified as *Labratrema minimus* (tables 3–5). This species also represents a new record for a parasitic infection of flounders.

The overall infection characteristics of each of the three types of metacercariae in each sample group are presented in tables 3–5, and show *C. concava* to be the most common of the three types of larvae.

Temporal variations in infection levels

The monthly prevalences and mean intensities (total

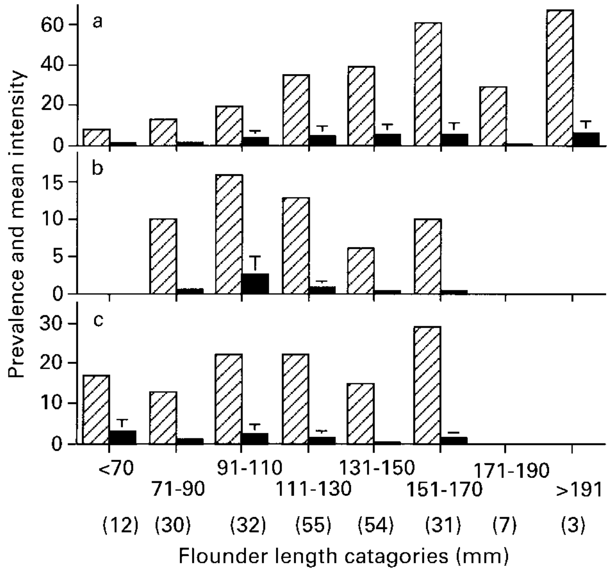

Fig. 3. The percentage prevalences (▨) and mean intensities (■) exhibited by the three metacercarial cysts in each flounder host size category (sample size indicated in parenthesis). a, *Cryptocotyle concava*; b, *Labratrema minimus*; c, *Timoniella* spp. (Data derived from the 1993/94 Lots Road sample.)

number of cysts/ total number of fish examined) of the three types of metacercarial cysts in the 1993/94 flounder sample from Lots Road are graphically presented in fig. 2a–c.

From these figures it is evident that the infection prevalences of metacercarial cysts of *C. concava*, *L. minimus* and *Timoniella* spp. were relatively high in late autumn and winter (September–February), and generally lower in spring and summer (April–August) (fig. 2a–c). Cysts were, nevertheless, present all year round with the exception of those of *L. minimus* which were absent in the flounders retrieved in July 1994 (fig. 2b). The sample size for March was too small (table 1) for infections with *Timoniella* spp. and *L. minimus* metacercariae to be reliably detected, and was therefore omitted.

The mean intensity of each metacercarial infection resembled to an extent the pattern observed in the prevalences, i.e. a peak in the cooler months, and a decrease coinciding with the summer (fig. 2).

Infection levels related to host size

To examine the relationship between infection levels with metacercarial cysts and host fish size, prevalences and mean intensities of infections were calculated for a range of host length categories for the 1993/94 Lots Road sample group (< 70 mm, 71–90 mm, 91–110 mm, 111–130 mm, 131–150 mm, 151–170 mm, 171–190 mm, > 190 mm) (fig. 3).

Figure 3a shows a steady increase in the prevalence of the metacercarial cysts of *C. concava* with increasing host length. Smaller flounders measuring 50–70 mm long (S.L.), corresponding to the older 0+ age class (Chen, 1994), exhibited an overall prevalence of 8%, which steadily increased with each subsequent host size category (fig. 3a). The observed prevalences in the two highest categories (171–190 mm and > 190 mm), however, have to be regarded as provisional as the sample sizes were only 7 and 3 respectively. The variation in mean intensity resembled the observed prevalence pattern (fig. 3a).

The infection characteristics, and in particular the prevalences, of the other two types of metacercarial cysts, *Timoniella* spp. and *L. minimus*, however, did not fit any recognizable pattern (fig. 3b and c). *Labratrema minimus* cysts were generally more prevalent in the 1+ flounder age class, and slightly less in older flounders, but were completely absent from the 0+ group (fig. 3b). This latter observation, however, may well have resulted from the sample size, which consisted of only 12 flounders. The mean intensity of this metacercarial infection, on the other hand, decreased with flounder length after a peak in the 1+ flounders (fig. 3b). Similarly, *Timoniella* spp. showed no discernible pattern in the prevalence related to host size, and the mean intensity decreased with increasing flounder length. It was, however, the 0+ and not the 1+ flounders that displayed the highest mean intensity values.

Frequency distributions of metacercarial cysts

The frequency distributions, as exemplified by the variance to mean ratio ($S^2/\bar{x}$)), for each of the three metacercarial cyst infections, *C. concava*, *L. minimus*, and *Timoniella* spp. in the 1993/94 flounder sample, are presented in table 6. Overdispersion was exhibited by all the metacercarial infections, although *C. concava* showed the highest $S^2/\bar{x}$) ratios of the three types.

Interrelationship between the three types of metacercarial cysts

The intensity of the metacercarial cysts of each species in each individual flounder host were plotted against each other in an attempt to identify any correlation

Table 6. The percentage frequency distribution of *Cryptocotyle concava, Labratrema minimus* and *Timoniella* spp. cysts in the 1993/94 flounder sample, and the observed mean intensity, variance and variance/mean ratio for each of the species (mean and variance calculations based on absolute parasite numbers).

	Parasite number categories							
	0	1–10	11–20	21–30	> 31	$\bar{x}$	S^2	$S^2/\bar{x}$
Cryptocotyle concava	64.7	22.5	5.8	3.1	3.6	5.0	240	48.0
	0	1–5	6–10	11–20	> 20	$\bar{x}$	S^2	$S^2/\bar{x}$
Labratrema minimus	91.0	6.0	1.3	1.4	0.9	0.7	14.7	21.0
Timoniella spp.	81.3	10.7	4.5	2.6	0.9	1.3	17.7	13.7

Table 7. The inter-relationship between the intensity of each of the metacercarial cyst species infecting flounders in the Thames tideway.

	Best fit line for linear relationship	n	r	*P*
Cryptocotyle concava & *Timoniella* spp.	y = 0.262 + 0.210 x	224	0.772	< 0.001
C. concava & *Labratrema minimus*	y = 0.410 + 0.064 x	224	0.259	< 0.01
Timoniella spp. & *L. minimus*	y = 0.091 + 0.490 x	224	0.538	< 0.001

between the levels of the parasite infections. The results of these regression analyses as well as the 'r' and *P* values for the cysts are displayed in table 7. All the regression analyses were positively significant.

Discussion

Host population structure

The predominant length groups of flounders retrieved from the upper and middle Thames tideway during the present study were consistent with the flounder lengths that are expected to be present in the upper estuarine reaches of many rivers in the British Isles. Hardisty & Badsha (1986) studying flounders in the Severn Estuary, observed the population to consist mainly of 0+, 1+, and 2+ age classes in the upper tidal reaches of the estuary. Fish migrated annually to the lower estuary in late summer (usually August and onwards), returning again in April the following year. The corresponding length measurements for these flounders ranged from a mean 76 mm T.L. (total length) in the 0+ age group, to 183 mm in the 2+ age group. Slightly higher lengths were recorded by flounders in the Medway Estuary, suggesting more favourable conditions (Van Den Broek, 1980; Hardisty & Badsha, 1986).

In comparing the flounder lengths of the fish sampled in this present study with those of an earlier study by Chen (1994) collected from the same sites in the tidal Thames, it is confirmed that the flounders in the present study are representative of the 0+, 1+, and 2+ age classes. Judging from the mean lengths of each of the flounder samples and comparing the expected ages observed by Chen (1994) for these lengths, the greatest proportion of fish in the 1992 Lots Road sample were 1+ years old. The 1992 West Thurrock sample and the 1993/94 Lots Road samples consisted mainly of 2+ fish. The mean length of the 1993/94 flounder sample could also correspond to the late 1+ year class (table 2).

The age and length composition of flounder populations at a particular location, however, is variable throughout the year and is dependent on the immigration of the 0+ fish, as well as the emigration of older age groups (Beaumont & Mann, 1984). This was evident in the observed monthly mean lengths of the 1993/94 flounder samples from Lots Road which remained relatively constant during the winter and spring, but dropped dramatically in July coinciding with the immigration of the 0+ young-of-the-year fish from the lower estuary. The mean lengths of fish in the West Thurrock sample on the other hand remained high in summer indicating that the 0+ age classes were not prevalent at this location, but were probably travelling to higher, less saline locations in the tidal Thames during that period. The 1992 Lots Road sample, however, was dominated by very young 1+ flounders during late winter, early spring, more so than the 1993/94 sample. These flounders may, however, correspond to the winter immigration of 0+ fish down into the tidal reaches from freshwater locations further upstream (Hutchinson & Hawkins, 1993). If this were so, then the parasites harboured in these fish should give an indication of from where they had originated, an analysis which can also be employed on fish travelling up from the lower estuary during spring and summer (MacKenzie, 1987).

Various studies have proposed that a range of metacercariae might be ideal indicators of fish movements. The easy detectability of the cysts, coupled with their prolonged presence in the host, and in most cases their site specificity, increases their utility in this respect (MacKenzie, 1983, 1987). Cercarial stages, and therefore the chance of becoming infected with metacercarial stages of digenean parasites, are often restricted in spatial distribution in their external habitat, due to the location of their first intermediate hosts which in most cases are either a marine, brackish, or freshwater gastropod or lamellibranch (MacKenzie, 1983; Williams & Jones, 1994). Where the first intermediate host has quite defined habitat requirements and is limited by these to restricted locations, the determination of the region in which the host has acquired a metacercarial infection becomes simplified, although other factors do come into play, including host feeding behaviour and cercarial emergence cycles.

Digenean metacercarial cysts of flounders in the tidal Thames

After a detailed examination of flounders in the tidal Thames, four digenean metacercarial infections were identified as occurring consistently in this flatfish species. These were *Cryptocotyle concava* (Creplin, 1825), a representative of the Heterophyidae (Wootton, 1957), *Timoniella imbutiforme* (Molin, 1859), and *Timoniella praeterita* (Looss, 1901) belonging to the Acanthostomatidae (Maillard, 1973, 1974), and *Labratrema minimus* (Stossich, 1887), from the Bucephalidae (Matthews, 1973; Maillard, 1976).

Of the metacercariae discovered in this study *C. concava* is the only one to have been previously reported from flounders in the British Isles, and in particular the tidal Thames (Messoudi, 1993). Its generic relative *C. lingua*, however, is a much more common parasite of flounders and other fish species in Britain and other regions of the Northern hemisphere (MacKenzie & Gibson, 1970; Van Den Broek, 1979; Køie, 1983; Scott, 1985; Lysne *et al*., 1994). These two species of *Cryptocotyle* appear to demonstrate low host specificity with respect to their final hosts, both of them apparently utilizing a wide range of marine birds and mammals (Stunkard, 1930; Wootton, 1957) (table 8).

Timoniella imbutiforme (synonym *Acanthostomum imbutiforme*) has also been observed in flounders, *Platichthys flesus*, but only in two regions of the Black Sea, namely the Asov Sea (Solonchenko, 1982), and Egorlytsky Bay (Parukhin *et al*., 1983). The common dab, *Limanda limanda*, was reported as a host to this metacercaria in Danish waters (Køie, 1983), but the acknowledged intermediate host of this parasite in British waters is the common goby, *Pomatoschistus microps* (Krøyer) (McDowall & James, 1988) (table 8). The present study is probably the first reported infection of flounders by *T. imbutiforme* in British waters.

The second species of *Timoniella*, *T. praeterita*, has not been reported in flounders, *Platichthys flesus*, in any location either in Britain or elsewhere prior to this date. Flounders are, therefore, a new second intermediate host species for the metacercarial cyst stage of this parasite. The previously reported host of *T. praeterita* was *Pomatoschistus microps* from the Mediterranean and Caspian Seas (Maillard, 1974; Brooks, 1980). The definitive host of both species is reported as the sea bass, *Dicentrarchus labrax* (Maillard, 1976). Both *T. imbutiforme*, and *T. praeterita* appeared similar when encysted in the fins and flesh of the host, and therefore could not be differentiated during the epidemiological examination of the flounders. They were consequently combined under their generic name, *Timoniella* spp., for the duration of the survey and described accordingly.

The fourth digenean metacercarial species, *L. minimus* (synonym *Bucephalus haimeanus*), similarly, has not been reported from flounders, *Platichthys flesus*, prior to this investigation, although they were found in plaice, *Pleuronectes platessa,* and the common goby, *Pomatoschistus microps*, from Cardigan Bay, Wales (Matthews, 1973). They were also reported from the latter host in the Mediterranean (Maillard, 1976). Flounders are, therefore, also a new host species for the metacercarial stage of this parasite. The definitive host is also the sea bass, *Dicentrarchus labrax* (Maillard, 1976) (table 8).

Spatial and temporal patterns of metacercarial infection

In attempting to understand the factors which may have generated the spatial and temporal distribution patterns of metacercarial infections in flounder revealed by this study, it is clear that, potentially, the dynamics of flounder infections are exceptionally complex. Fish are undergoing, probably repetitive, migrational movements up and down the Thames tideway between more and less saline regions. Sources of infection (i.e. infected molluscan first intermediate hosts) are distributed non-randomly in the estuary. The susceptibility of flounder to infections with the four different digeneans is likely to vary in a parasite-specific manner and the longevity of metacercarial infections of the four species is likely also to be species-specific.

All of these factors (and doubtless others not listed here) will influence the level of metacercarial infection to be found in an individual flounder collected from a specific site in this sampling programme. In attempting to come to terms with this complexity, the approach taken here has been to concentrate on the aspects of the epidemiological data set which are likely to be the most robust. These are as follows:

1. All these infections, at both sites, increase on average in the winter, and decrease in the summer.
2. *Cryptocotyle concava* infection increases with host length (and probably host age).
3. The other species do not demonstrate such a relationship.
4. Prevalences and intensities of *C. concava* are higher than those observed in *Timoniella* spp. and *L. minimus* infections.
5. The prevalences of *C. concava* were higher in the West Thurrock sample than in both the Lots Road samples.

Table 8. The recorded life-cycles of the four digenean species found utilizing flounders as second intermediate host for their metacercarial stages in the tidal Thames.

Digenean (metacercarial) species	First intermediate host	Second intermediate host	Final (definitive) host	References
Cryptocotyle concava (Creplin, 1825)	*Amnicola longinqua* in N. America *Hydrobia ulvae* in northern Europe	Estuarine and freshwater fish species	Various fish eating birds and mammals	Wootton (1957) Køie (1983)
Labratrema minimus (Stossich, 1887)	*Cardium edule* and *Ostrea edulis*	Goby, *Pomatoschistus microps* Plaice, *Pleuronectes platessa*	Sea bass, *Dicentrarchus labrax*	Maillard (1976) Matthews (1973)
Timoniella imbutiforme (Molin, 1859)	*Hydrobia ventrosa* and *H. acuta* in Europe, *H. ulvae* in British and Danish waters	Goby, *Pomatoschistus microps* Turbot, *Scophthalmus maximus* Sole, *Solea solea* Flounder, *Platichthys flesus*	Sea bass, *D. labrax.*	Maillard (1973) McDowall & James (1988) Køie (1983)
Timoniella praeterita (Looss, 1901)	*Hydrobia ventrosa* and *H. acuta*	Gobiidae and other small teleosts	Wolf-fish, *Anarhichas lupus* Sea bass, *D. labrax*	Dawes (1946) Maillard (1974)

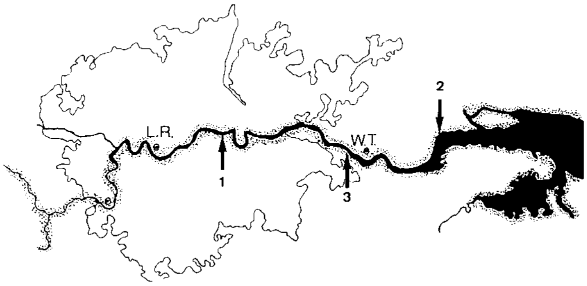

Fig. 4. Distribution of *Hydrobia ulvae* and *Cerastoderma edule* in the Thames tideway. (W.T.) West Thurrock, (L.R.) Lots Road, (1) South Bank, upstream limit of *Hydrobia ulvae*, (2) Mucking, upstream limit of *Cerastoderma edule*, (3) Purfleet, regular occurrence of *H. ulvae*.

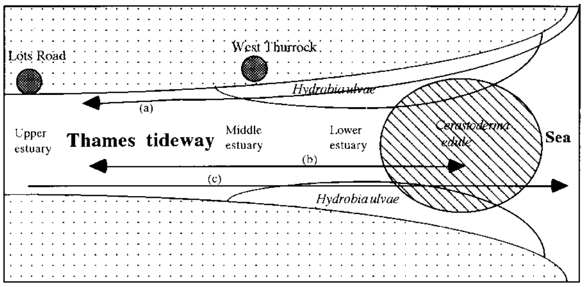

Fig. 5. Diagrammatical representation of the assumed movement patterns of the flounders in the tidal Thames, and the possible 'infective zones' encountered by the fish during their migration into the lower estuary: (a) represents the initial immigration of metamorphosed flounders from the sea into the estuary; (b) represents repeated up and down movements by subsequent year classes within the estuary for a period of around three years (Chen, 1994); (c) represents the final emigration of pre-spawning flounders out to sea.

6. All three infections were positively correlated with each other.

In addition to these relatively firm conclusions derived directly from this study, it also seems reasonable to deduce from other work that:

7. The likeliest first intermediate hosts of *C. concava* (Lauckner, 1987), and *Timoniella* spp. in British waters is the estuarine gastropod *Hydrobia ulvae* (McDowall & James, 1988), while that of *L. minimus* is the marine lamellibranch *Cerastoderma edule* (synonym *Cardium edule*) (Matthews, 1973).
8. Flounders are likely to be a better-suited second intermediate host for *C. concava*, than perhaps for *Timoniella* spp. and *L. minimus*.

The suggestion that *H. ulvae* is the first intermediate host of *C. concava* in British waters is based on the already established fact that this hydrobid gastropod is recognized as the first intermediate host of *C. concava* in European waters (Reimer, 1970 cited in Køie, 1983; Lauckner, 1987), and is also an additional first intermediate host of *C. lingua* in Russia (Ginetsinskaya, 1961). The gastropod *Hydrobia ulvae* was also reported to contain sporocysts of *Cryptocotyle jejuna* in the Ythan Estuary in the north-east of Scotland (Huxham *et al.*, 1995), confirming that species of the genus *Cryptocotyle* are already utilizing hydrobid snails in British waters. In addition, Van Den Broek (1977) regularly found *H. ulvae* in the diet of flounders collected from the Medway Estuary. *Hydrobia ulvae* is also widespread in the Thames Estuary and has been reported as far upstream as the South Bank, although it is more regularly found at Purfleet and below (Mr M. Thomas, National Rivers Authority, personal communication) (fig. 4).

The sporocysts and rediae of *T. imbutiforme* are also found in *Hydrobia ulvae* in British waters (McDowall & James, 1988), and possibly those of *T. praeterita* which is also known to infect the same hydrobid snails in Europe and the Mediterranean as the latter species, including *H. ventrosa*, and *H. acuta* (Maillard, 1973, 1974, 1976).

Several first intermediate hosts are known to harbour sporocysts of *L. minimus* in European waters, including *Ostrea edulis* and *Cerastoderma edule* (Maillard, 1976; Matthews, 1973). *Cerastodemia edule* is believed to be the host in British waters, although *O. edulis* is the type host (Matthews, 1973). The presence of *C. edule*, the common edible cockle, in the Thames estuary is an established fact as this mollusc is fished commercially here, as well as in The Wash, Morecambe Bay, the Dee Estuary and the Llanrhidan sands in South Wales (Tebble, 1966). Presently it is abundant in the lower estuary, but is not found any further upstream than Mucking (Mr M. Thomas, National Rivers Authority, personal communication) (fig. 4). This host is perhaps more likely to be the first intermediate host than *O. edulis*, the common European oyster, based on its habitat and distribution. The common edible cockle can be found in coastal bays and in the lower estuaries on a substratum that is preferred by flounders, i.e. muddy sand (Beaumont & Mann, 1984), below the low water tide-mark, where it is able to withstand brackish water salinity levels, but no lower than 20‰. The common European oyster, on the other hand, is only found offshore in depths ranging between 25 and 80 m on firm immobile substrata (Tebble, 1966).

The infection of flatfishes and particularly the flounder, *Platichthys flesus*, by metacercariae of *C. concava* is of importance to the continuation of the fluke's life-cycle, as these fish constitute an important food source of the final hosts, marine birds and mammals (Ginetsinskaya, 1961; Yamaguti, 1975). Summers (1979) also reported that 85% of the fish consumed by migrating cormorants in the Ythan Estuary were flounders.

This is in contrast to *Timoniella* spp. and *L. minimus*, which are both parasites of the sea bass, *Dicentrarchus labrax* (Matthews, 1973; Maillard, 1976), a fish that does not feed on flounders, but predates the common goby, *Pomatoschistus microps* (Wheeler, 1969). The latter host is also recognized as the principal second intermediate host of these digenean species (Matthews, 1973; Maillard, 1976).

Putting these central facts and/or assumptions together produces a working hypothesis which links movement and infection events in a way which can begin to explain the patterns of infection seen in this study. It is assumed that the infected populations of *Cerastoderma edule* and *Hydrobia ulvae* are found in the lower, more saline reaches of the estuary. It is further assumed that juvenile flounders in the first year of their life pass through this 'infective zone' (figs 4 and 5) during their first movement from offshore waters into the estuary (Chen, 1994). Thereafter, it seems probable that most will intermittently return to the lower estuary and therefore to higher chances of new infections during the first 3 years of their life (Summers, 1979; Hardisty & Badsha, 1986; Chen, 1994). How could this pattern of movements produce the infection levels described here?

Temporal infection prevalences for all the metacercarial infections were higher in autumn and winter, corresponding to a period in which young flounders normally occupied lower estuarine positions as opposed to the upper estuary (Hardisty & Badsha, 1986; Chen, 1994). The reduced prevalences in late summer, also demonstrated by each of the metacercarial infections, can similarly be correlated with the immigration of 0+ juveniles in late spring and summer (Chen, 1994) into the upper estuary, thus causing a dilution of the pre-existing infected population with a considerably less infected cohort.

The longevity of *C. concava* has not been examined previously but is assumed here to be similar to its congeneric relative *C. lingua*. *Cryptocotyle lingua* is known to survive for several years within certain hosts (Stunkard, 1930), including the dab in which accumulation of cyst numbers and prevalences was found to occur over consecutive age classes (Køie, 1983). A similar finding was observed here for *C. concava* infections in flounders, where prevalences and mean intensity increased consistently with consecutive host length categories (and probably host age), thus implying metacercarial survival over at least one if not more years.

The increasing infections with host size could also account for the differences in prevalences observed in flounders collected from West Thurrock compared to flounders from Lots Road. Flounders from the former location corresponded mainly to the 2+ age class, hence the higher prevalences compared with the lower levels observed in flounders collected from Lots Road in the same year (1992), which corresponded to the 1+ age class.

The longevity of *Timoniella* spp. has also not been examined before but, unlike the case of *C. concava*, there are no closely related species which have been previously examined with which to compare the present findings. However, the erratic infection prevalences observed in each host length category implies that the cysts are not surviving long enough to produce any identifiable positive association between prevalence and increasing host age. This could be a direct result of the incompatibility of this parasite with this host, or simply mean that the cysts are naturally short-lived.

The longevity of *L. minimus*, on the other hand, has been examined by Matthews (1973) in both the principal second intermediate host, the common goby, and in an unsuitable flatfish host, the plaice. The results indicated that metacercariae survived at least 10 months in the former host, but only 2 weeks in the latter. Only 2% of the metacercariae detected survived more than 2 weeks in the plaice, and none reached the developmental stage observed in cysts found in the common goby. The continuous presence, however, in this study of cysts in flounders throughout the year (with the exception of July), implies that this species survives much longer in this host than in plaice. The infection peaks in autumn and winter when the flounders are assumed to migrate to the lower estuary and are exposed to the infection, but is also present throughout summer at a time when the flounders are found in the upper reaches of the estuary and distant from the first intermediate host, *Cerastoderma edule*. Their survival rate is therefore sufficiently long enough to span a period of at least 6 months, and possibly a little longer.

According to Chubb (1979), seasonal acquisition rates can only be reliably estimated in the first year of exposure to cercarial invasion. In the case of *L. minimus*, the 1+ flounders would then be the reliable age class to be examined, since no infection was detected in the 0+ age class. The 0+ flounders, however, were infected with *C. concava* and *Timoniella* spp. metacercariae, suggesting that young-of-the-year flounders are exposed to cercariae of the latter two types during their migration from the sea into the estuary but are not exposed to *L. minimus* cercariae. This result, however, can possibly be explained when the behaviour of 0+ flounders is considered closely. Hutchinson & Hawkins (1993) found that post-larval and juvenile 0+ flounders aggregated at the water margin of the River Itchen during their primary migration upriver, and generally avoided saline water (20‰) unless disturbed. As mentioned earlier, the proposed first intermediate host of *C. concava* and *Timoniella* spp. in the tidal Thames is *Hydrobia ulvae* which is an intertidal inhabitant of the estuary, and normally found on the mud-flats at the water margin (Barnes, 1994; Frid & James, 1988). Thus, the youngest 0+ flounders come into contact with this gastropod at these sites, and consequently become exposed to the cercariae of these species. On the other hand, they avoid the high saline (20‰) tidal region where the first intermediate host of *L. minimus*, *Cerastoderma edule*, occurs, even though the cockle is present on a substrate that is generally preferred by older flounders.

The association between the two metacercarial infections, *C. concava*, and *Timoniella* spp., is further emphasized by the strong, positively correlated relationship in their observed intensities in flounders. The relationships, however, were positive and significant between all the metacercarial infections. Several factors may generate this positive association. Those factors need not be related to direct interactions between the metacercarial species themselves, but instead possibly arise from shared ecological habitats or hosts. Such indirect factors include: (i) the close spatial proximity of the two suggested first intermediate hosts, *Hydrobia ulvae* and *Cerastoderma edule*, in the lower estuary; (ii) the shared use by *C. concava*, and *Timoniella* spp. of *Hydrobia ulvae* as first intermediate hosts; and (iii) the shared use by *L. minimus* and *Timoniella* spp. of *Dicentrarchus labrax* as the final host. The fact that the acquisition of all the infections probably occurs during a relatively restricted time during the annual winter migration to the lower estuary may also contribute to the positive parasite associations.

To summarize, therefore, it is evident from the results that:

1. The young-of-the-year flounders contract infections of *C. concava* and *Timoniella* spp. on their initial migration into the estuary when they first encounter the shared first intermediate host, *Hydrobia ulvae*, at the margins of the estuary. They presumably avoid the cockle beds of *Cerastoderma edule*, the first intermediate host of *L. minimus*, during this primary immigration into the estuary, whilst avoiding the high salinity levels that they are normally associated with (fig. 5).
2. An increase in infection prevalences in autumn and winter, found in subsequent flounder age classes, corresponds to the annual winter migration undertaken by these fish from the upper and middle estuary into the lower estuary. This migration is repeated several times throughout the duration of their presence within the estuary (fig. 5). The prevalences are reduced in summer due to the influx of less infected young-of-the-year flounders.
3. The possible long-term survival of metacercarial cysts *C. concava* in flounders, in addition to the observed higher overall prevalences compared to the other metacercarial infections suggest that this host is a better suited second intermediate host for this species than for the others studied.

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