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Migration, site-specificity and development of *Benedenia lutjani* (Monogenea: Capsalidae) on the surface of its host, *Lutjanus carponotatus* (Pisces: Lutjanidae)

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SUMMARY

Life-history attributes of the capsalid monogenean *Benedenia lutjani*, a parasite of *Lutjanus carponotatus* from the Great Barrier Reef, Queensland, Australia, were investigated from experimental infections. Oncomiracidia of *B. lutjani* invaded and attached at any site on the fish, but more commonly invaded body surfaces. Immature specimens then migrated to the pelvic fins. Development of the reproductive organs of *B. lutjani* corresponded with migratory movements on the host. Parasite aggregation on the pelvic fins coincided with the development of functional male reproductive organs and some protandrous worms that possessed a vagina appeared to be inseminated. Migration to, and aggregation on, the branchiostegal membranes (membranous folds posterior to the opercula) coincided with the onset of sexual maturity and commencement of egg production by parasites. The rate of parasite development and the timing of migratory events on the host were influenced by water temperature. All specimens of *B. lutjani* reached sexual maturity between 12 and 14 days p.i. at 24 °C and between 8 and 10 days p.i. at 27 °C. Anterior hamuli grew continually during a 16-day experiment at 27 °C and 25-day experiment at 23 °C and their length appeared to provide a suitable index to estimate parasite age. The possible adaptive significance of the migratory behaviour, site-specificity and its link with changes in parasite development are discussed.

Key words: monogeneans, invasion, migration, site-specificity, development, reproductive biology.

INTRODUCTION

It is well established that many parasites undergo complex migrations in or on their hosts, from the site of invasion by the infective stage to the final site occupied by the adult (e.g. Ulmer, 1971; Crompton, 1976; Sukhdeo & Bansemir, 1996), especially in endoparasites with complex, multi-host life-cycles. There is evidence, however, that ectoparasites with simple, direct life-cycles such as monogeneans may migrate from the invasion site to the definitive microhabitat of the adult worms. Oncomiracidia of the gill monogenean, *Urocleidus adspectus* (Dactylogyridae), attach to the external surfaces of their round-bodied teleost host and move anteriorly to reach the gills where they mature (Cone & Burt, 1981). Larvae of *Entobdella soleae* (Capsalidae) move anteriorly after attaching to the upper surface of their flatfish teleost host and migrate to the lower surface where parasites reach sexual maturity (Kearn, 1984). Ogawa (1984) demonstrated that *Benedenia hoshinai* (Capsalidae) migrates posteriorly to the caudal fin, the posterior portion of the dorsal fin and the posterodorsal part of the body proper of its round-bodied teleost host. *Neobenedenia girellae* (Capsalidae) (see Whittington & Horton (1996) for an account of its likely synonymy with *N. melleni*) may migrate from the fins to the body of its flatfish teleost host (Bondad-Reantaso *et al*. 1995). The most bizarre migration known among Monogenea is that of *Pseudodiplorchis americanus* and *Neodiplorchis scaphiopodis* (Polystomatidae) infecting desert toads (*Scaphiopus*). Ciliated oncomiracidia invade the amphibian hosts via the nostrils; larvae enter the buccal cavity and glottis and migrate to the lungs where juvenile development occurs; juvenile *P. americanus* then migrate via the toad's intestine to the urinary bladder where parasites mature (Tinsley & Earle, 1983; Tinsley & Jackson, 1986).

When *Benedenia lutjani* (Capsalidae) was described, Whittington & Kearn (1993) reported that its distribution on the body skin and fins of the round-bodied teleost, *Lutjanus carponotatus* (Lutjanidae), was not random. Whittington & Kearn (1993)

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discovered more adult specimens of *B. lutjani* attached to the pelvic fins of freshly caught fish and this distribution was exaggerated for juvenile and adult specimens when a fish maintained in a tank for 2 to 3 weeks became heavily infected (see Fig. 9 in Whittington & Kearn, 1993). They proposed that the observed distribution of *B. lutjani* may be consistent with preferential settlement by larvae on the pelvic fins and either migration of other juveniles from body surface to the pelvic fins before parasites reached sexual maturity, or mortality of body surface parasites before they matured. A second species, *B. rohdei* (Capsalidae), reported from *L. carponotatus* by Whittington, Kearn & Beverley-Burton (1994) is known to be specific to the gills (Whittington & Kearn, 1993). The occurrence of 2 related monogeneans, *B. lutjani* and *B. rohdei*, on apparently specific, but different, sites on the same round-bodied teleost species has provided opportunities for comparative investigations of features of their biology. So far, studies have explored the photobiology of hatching (Ernst & Whittington, 1996) and the morphology and ultrastructure of the anterior adhesive areas (Whittington & Cribb, 1999) in these congeners and aspects of host-specificity in *B. lutjani* (see Ernst & Whittington, 2001). We developed a technique to remove capsalids from the external surfaces and gills of live *L. carponotatus*, which provided a method to manipulate infections experimentally to investigate site-specificity, possible migration on the host and parasite development.

MATERIALS AND METHODS

Collection and maintenance of hosts

Specimens of yellow stripey, *L. carponotatus*, ranging from 120 to 350 mm (length to caudal fork, LCF) were caught by angling at Heron Island, Queensland, Australia at the southern end of the Great Barrier Reef (23°27′S, 151°55′E). Fish were identified from the description given by Randall, Allen & Steene (1990). After capture, fish were transported in 60 l drums containing aerated seawater to aquaria at the Heron Island Research Station (HIRS) of The University of Queensland. Fish were transferred gently to large covered glass aquaria provided with a continuous flow of fresh seawater and fed fresh chopped fish every 2 days. Specimens of *L. carponotatus* were caught by angling using lures with barbless hooks (Diggles & Ernst, 1997) to ensure specimens were in good condition. Some specimens were maintained in captivity for more than 40 days.

Collection and maintenance of parasites and eggs

Specimens of *L. carponotatus* at Heron Island are infected with relatively small numbers of *B. lutjani* and *B. rohdei* (see Whittington & Kearn, 1993; Whittington *et al*. 1994). To obtain large numbers of adult parasites for egg laying and to reduce the number of fish required, a technique using pieces of netting described previously by Ernst & Whittington (1996) was applied to increase infection intensity in tanks containing up to 8 freshly caught fish. The netting snared eggs laid by both parasite species and prevented eggs from being washed out of the tanks. Exposure to the large numbers of larvae hatching from these snared eggs greatly increased the infection intensities of the captive fish and, between 2 and 5 weeks later, provided large numbers of adult *B. lutjani* and *B. rohdei*. Live parasites were removed from freshly killed fish using fine needles and *B. lutjani* and *B. rohdei* were kept separately and permitted to lay eggs. Eggs of each monogenean species were collected and maintained in Perspex egg wells following Ernst & Whittington (1996), who reported that eggs hatched after 4–10 days depending on species and temperature, only during periods of daylight. Recently hatched, free-swimming oncomiracidia were used in experiments to infect groups of *L. carponotatus* cleaned of all capsalids by chemical treatment (see below). The natural infection of *L. carponotatus* by *B. lutjani* and *B. rohdei* could therefore be uncoupled to provide host specimens infected with either *B. lutjani* or *B. rohdei*.

Experimental procedures

Benedenia spp. were removed from live *L. carponotatus* by immersing fish for 2 h in seawater containing 20 mg/l Praziquantel (Sigma). A stock solution was made by dissolving 80 mg of Praziquantel/ml of ethanol, which was then added to an appropriate volume of seawater in an aquarium to provide the required dose. During treatment, the flow of seawater to the tanks was disconnected and an air stone provided aeration. Fish were then transferred to a separate aquarium containing fresh flowing seawater. Within 48 h, the treatment was repeated. Dissections of a sample of 6 *L. carponotatus* treated as described above demonstrated that the double bath in Praziquantel successfully removed all specimens of both capsalid species.

Four infection experiments were performed between September 1994 and August 1995. Ambient seawater temperature around Heron Island varies seasonally and mean monthly water temperatures on the reef flat during our study ranged from 19·8 to 30·5 °C (data recorded manually by HIRS staff daily). We conducted infection experiments at 3 temperatures within this range: 23 °C in May and June 1995 (these data were grouped); 24 °C in October 1994; 27 °C in March 1995. Before exposure to free-swimming oncomiracidia of either monogenean species, uninfected fish were kept in aquaria containing fresh flowing seawater for at least 48 h following the second Praziquantel treatment. Groups

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of up to 9 uninfected *L. carponotatus* were exposed to freshly hatched larvae of either *B. lutjani* or *B. rohdei* for 24 to 36 h. The continual flow of fresh seawater into the aquaria (volume: 100–140 l) was stopped prior to and during exposure to larvae. The water level in the tanks was reduced so that uninfected fish were exposed to oncomiracidia in a volume of between 50 and 70 l of seawater. Aeration for fish was provided from an air stone. Seawater in the treatment tank was maintained at a constant temperature by placing it within a large plastic tray (10 cm deep) with a constant flow of fresh seawater through it. At the first signs of hatching, dishes containing eggs of either *B. lutjani* or *B. rohdei* were placed at the bottom of aquaria so that swimming oncomiracidia had access to uninfected hosts. After 24 to 36 h (1 or 2 periods of daylight, depending on estimations of egg hatching success), the dish containing parasite eggs was removed from the aquarium. When possible, hatching success was monitored by examining the dish for unhatched eggs. After exposure to larvae, groups of fish were removed from the tank and placed in a clean aquarium with a continual flow of fresh seawater where they were maintained.

Each group of experimentally infected fish was transferred to a clean aquarium every 4 days to keep aquaria free of fully embryonated eggs laid by parasites reaching sexual maturity. This procedure is effective in breaking the life-cycle of parasites on fish held in aquaria for prolonged periods because the fastest development time for *B. lutjani* eggs is 4 days and for *B. rohdei* eggs is 6 days at temperatures > 26 °C (Ernst & Whittington, 1996). To prevent contamination by eggs, hand nets were soaked and washed in hot tap water after use to kill any adhering eggs. A total of 31 fish was exposed to larvae of either *B. lutjani* or *B. rohdei* and these fish were killed by pithing at intervals ranging from 1 to 25 days post-infection (p.i.). A longer-term infection experiment to determine the longevity of *B. lutjani* was discontinued due to contamination with *B. lutjani* eggs.

Recovery of parasites

Immediately after pithing, the head was removed and each region of interest (interior and exterior head surfaces including crevices such as lip folds, jaw and branchiostegal membranes (Whittington, 1996, 1998), body flanks, fins and gills) was dissected from adjacent parts, placed in separate dishes and examined thoroughly for parasites using a stereomicroscope. Oncomiracidia of these capsalid species are approximately 240 μm long (Whittington & Kearn, 1993; Whittington *et al*. 1994) and it was possible that some post-larvae could be overlooked on fish only recently exposed to infective larvae (e.g. dissections 1–3 days p.i.). To ensure recovery of all parasites from all infected fish, after the initial examination described above, each dissected part was placed in a dish of seawater containing 100 mg/l Praziquantel which quickly removed any worms that had been overlooked. The site of recovery for each parasite specimen from each fish was recorded. After location, parasites were preserved in 10% buffered neutral formalin beneath slight cover-slip pressure (sufficient in most cases to ensure haptoral sclerites lay flat enough to measure; see Whittington & Horton, 1996; Whittington, Deveney & Wyborn, 2001), dehydrated in an ethanol series, cleared in cedar wood oil and mounted in Canada balsam. Measurements were made using a computerized digitizing system similar to that described by Roff & Hopcroft (1986). Where possible, total length (including haptor) and anterior hamuli (AH) length (terminology for haptoral sclerites follows Whittington & Horton, 1996) were measured. Obvious anatomical features such as presence of sperm within the vaginal seminal receptacle and presence of the vitellarium were also noted to assign specimens into 1 of 5 developmental categories. These were defined as: immature (neither male nor female reproductive organs functional); protandrous (male reproductive organs fully developed and sperm present within seminal vesicle and vas deferens); protandrous inseminated (as for protandrous, but sperm clearly present in vaginal seminal receptacle); adult(?) (female reproductive organs regarded as fully developed based on presence of vitelline cells in vitelline reservoir and oocytes in germarium, but no clear evidence of insemination and no signs of egg production); adult (male and female reproductive organs fully functional; worms inseminated and/or egg production underway).

Survey of wild specimens of L. carponotatus *for parasites*

Wild fish from waters around Heron Island were surveyed to compare the distribution and size of their parasites with those of parasites from experimental infections. In May 1995, 10 *L. carponotatus* ranging from 175 to 290 mm LCF were caught from the same locality described above. All these fish were killed, dissected and examined for capsalid monogeneans (see above) within 24 h of capture. Parasites were preserved, processed and mounted as described earlier.

Estimation of external surface area of L. carponotatus

Approximate external surface areas of body, head, branchiostegal membranes and fins, but excluding gills, of 1 specimen of *L. carponotatus* (220 mm LCF) were estimated. After dissection, each part was placed onto a piece of graph paper and the outline

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Table 1. Numbers of *Benedenia lutjani* recovered from a variety of sites on *Lutjanus carponotatus* in experimental infections

Temperature (°C)	Days p.i.	Total no. of specimens recovered	Body	Fins					Head	Branchiostegal membranes	Gills
				Anal	Caudal	Dorsal	Pectorals	Pelvics			
23 ($n = 216$ specimens)	1	7	1	—	—	1	4	1	—	—	—
	2	25	17	—	—	4	2	2	—	—	—
	3	57	48	1	—	5	—	2	—	1	—
	4	40	16	4	7	5	—	8	—	—	—
	6	71	14	—	4	3	—	50	—	—	—
	15	11	—	1	—	1	—	—	5	4	—
	20	2	—	—	—	—	—	—	—	2	—
	25	3	—	—	—	—	—	—	—	3	—
24 ($n = 52$ specimens)	6	4	—	—	—	—	—	4	—	—	—
	9	11	—	1	—	1	—	9	—	—	—
	12	9	—	—	2	—	—	7	—	—	—
	14	9	—	1	—	—	—	8	—	—	—
	20	9	—	—	—	—	—	2	—	7	—
	25	10	—	3	—	—	—	—	—	7	—
27 ($n = 303$ specimens)	1	4	3	—	—	—	1	—	—	—	—
	2	10	8	—	—	—	2	—	—	—	—
	3	22	17	—	—	—	2	3	—	—	—
	4	47	13	—	—	19	1	14	—	—	—
	6	56	1	—	8	19	—	28	—	—	—
	8	18	5	—	1	1	—	10	1	—	—
	10	75	3	—	—	—	—	15	6	50	1
	13	46	3	—	—	—	—	4	3	36	—
	16	25	1	—	—	—	—	—	4	20	—
		$n = 571$									

was traced. Fins and branchiostegal membranes were opened out during this procedure. Areas were calculated in cm^2.

RESULTS

Levels of experimental infection achieved by B. lutjani *and* B. rohdei

In a series of 3 experiments using a total of 23 *L. carponotatus* exposed to a total of approximately 6700 *B. lutjani* eggs (hatching success not assessed), a total of 571 parasite specimens was recovered (Table 1). Parasites ranged in total length from 155 μm for a specimen from the body of a fish killed 1 day p.i. at 27 °C to 2780 μm for a specimen removed from the branchiostegal membranes of a fish killed 25 days p.i. at 23 °C. Due to contraction when fixed, some recently attached oncomiracidia were smaller than the live, slightly compressed freshly hatched larvae reported by Whittington & Kearn (1993). In 1 experiment using a total of 8 *L. carponotatus* exposed to approximately 970 *B. rohdei* eggs (hatching success approximately 85%), no parasite specimens were recovered from any site from any fish killed 1, 2, 3, 4, 6, 9, 12 and 15 days p.i. Therefore, results presented below represent data for experimental infections by *B. lutjani* only.

Recovery of B. lutjani *from experimentally infected fishes*

Table 1 summarizes the data from experimental infections. Parasites were recovered from all fish exposed to hatching larvae ranging from 2 specimens at 20 days p.i. at 23 °C to 75 specimens at 10 days p.i. at 27 °C. These data indicate that at the 3 temperatures investigated, *B. lutjani* larvae invade and attach at any site on the fish, but parasites are more commonly found on body surfaces 1–3 days p.i. At each temperature 6 days p.i., most monogeneans were on the pelvic fins. The majority of parasites were also on the pelvic fins 14 days p.i. at 24 °C and 8 days p.i. at 27 °C. However, most parasites were located on the outer surface of the branchiostegal membranes (i.e. the surface continuous with the external surface of the operculum) 20 and 25 days p.i. at 23 °C and 24 °C and 10, 13 and 16 days p.i. at 27 °C (Table 1). A single worm recovered from the gills 10 days p.i. at 27 °C (Table 1) was no different from other specimens found on other regions of the same fish specimen.

Fig. 1 illustrates the parasite distribution on their hosts (expressed as a percentage; total number of parasites = 303) in the 27 °C experiment at various intervals p.i. In general, these data indicate that

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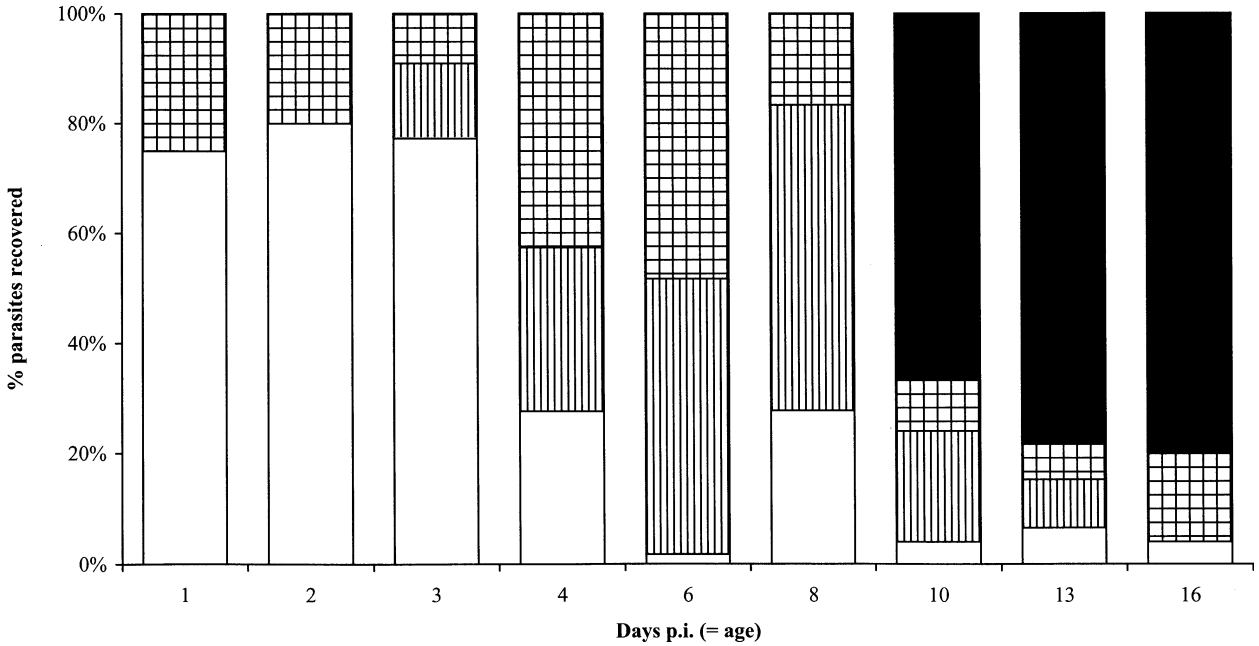

Fig. 1. Distribution of specimens of *Benedenia lutjani* recovered at intervals (days p.i.) from an experimental infection of 9 specimens of *Lutjanus carponotatus* at 27 °C. Parasites recovered from the body (□), pelvic fins (▥) and branchiostegal membranes (■) are shown separately, as a percentage of the total parasite numbers from each fish (Table 1). Figures for all other sites (Table 1) have been added together to calculate percentage recovered (▦).

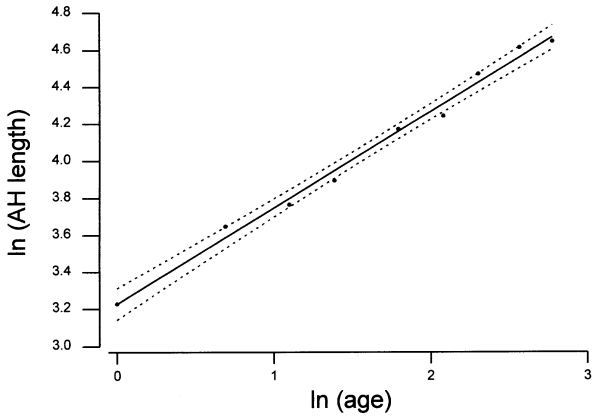

Fig. 2. Relationship between the natural logarithm of age (= days p.i.) and the natural logarithm of mean anterior hamulus (AH) length (in μm) from an experimental infection of 9 specimens of *Lutjanus carponotatus* with *Benedenia lutjani* over 16 days at 27 °C. AH length was measured for a total of 234 *B. lutjani* specimens and the mean was calculated for each of the 9 ages shown at 27 °C in Table 1. The regression line is defined by the equation: ln(AH length) = 0·552 ln(age) + 3·17 ($P < 0{\cdot}001$; $r^2 = 0{\cdot}925$). Dashed lines represent 95 % confidence limits.

invading and newly established larvae occurred mostly on body surfaces with few at other sites (pectoral and pelvic fins) from days 1 to 3 p.i. (Fig. 1). From 4 to 8 days p.i., parasite numbers on body surfaces decreased and numbers on the pelvic fins increased. From 10 days p.i., most parasites were recovered from the branchiostegal membranes.

The relationship between the age (= days p.i.) of *B. lutjani* and AH length was investigated using measurements from 234 mounted specimens collected in the 27 °C infection experiment. The AH of *B. lutjani* grew continually during this 16 day experiment. The relationship between the natural logarithm of age and the natural logarithm of mean AH length (Fig. 2) weighted for number can be described by the following linear equation: ln(AH length) = 0·552 ln(age) + 3·17 ($P < 0{\cdot}001$; $r^2 = 0{\cdot}925$). A similar relationship was found for the 23 °C infection experiment over 25 days: ln(AH length) = 0·46 ln (age) + 3·32 ($P < 0{\cdot}001$; $r^2 = 0{\cdot}866$). Too few data were collected at 24 °C (Table 1) to run a regression analysis.

Observations on the reproductive status of B. lutjani *specimens*

Developmental characteristics of *B. lutjani* specimens recovered from experimental infections at 24 °C and 27 °C are summarized in Table 2. At 27 °C, all specimens collected between 1 and 4 days p.i. and most at 6 days p.i. were immature. At 8 days p.i., no immature stages were found, most were protandrous, and even 1 inseminated adult was observed (Table 2). Most parasites at 10 days p.i. were adult(?). At 13 and 16 days p.i., most specimens were mature inseminated and/or egg-laying adults (Table 2). At 24 °C, reproductive organs developed more slowly: some immature specimens remained 9 days p.i (cf. no immature specimens > 8 days p.i. at 27 °C) and some protandrous specimens remained 12 days p.i. (cf. no protandrous specimens > 10 days p.i. at 27 °C) (Table 2).

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Table 2. Developmental stages of *Benedenia lutjani* specimens recovered from groups of *Lutjanus carponotatus* infected experimentally at 24 and 27 °C

(An explanation of each developmental stage is given in the text. The 'predominant site(s)' on the host is/are the site(s) from which > 50 % of parasites were recovered. Loss of, or damage to, some specimens precluded measurements being made of all material referred to in Table 1 (e.g. days 1 and 2 p.i. at 27 °C). Abbreviations: branch., branchiostegal membranes; imm., immature; ins., inseminated; protand., protandrous.)

Temperature (°C)	Days p.i.	Predominant site(s)	Imm.	Protand.	Protand. (ins.)	Adult (?)	Adult (ins./eggs)	Total no. of specimens
24	6	Pelvics	4	0	0	0	0	4
	9	Pelvics	5	6	0	0	0	11
	12	Pelvics	0	6	0	1	1	8
	14	Pelvics	0	0	0	6	3	9
	20	Branch.	0	0	0	5	4	9
	25	Branch.	0	0	0	1	7	8
27	3	Body	22	0	0	0	0	22
	4	Dorsal and pelvics	47	0	0	0	0	47
	6	Pelvics	31	3	0	0	0	34
	8	Pelvics	0	8	1	1	1	11
	10	Branch.	0	0	0	33	16	49
	13	Branch.	0	0	0	8	29	37
	16	Branch.	0	0	0	2	17	19

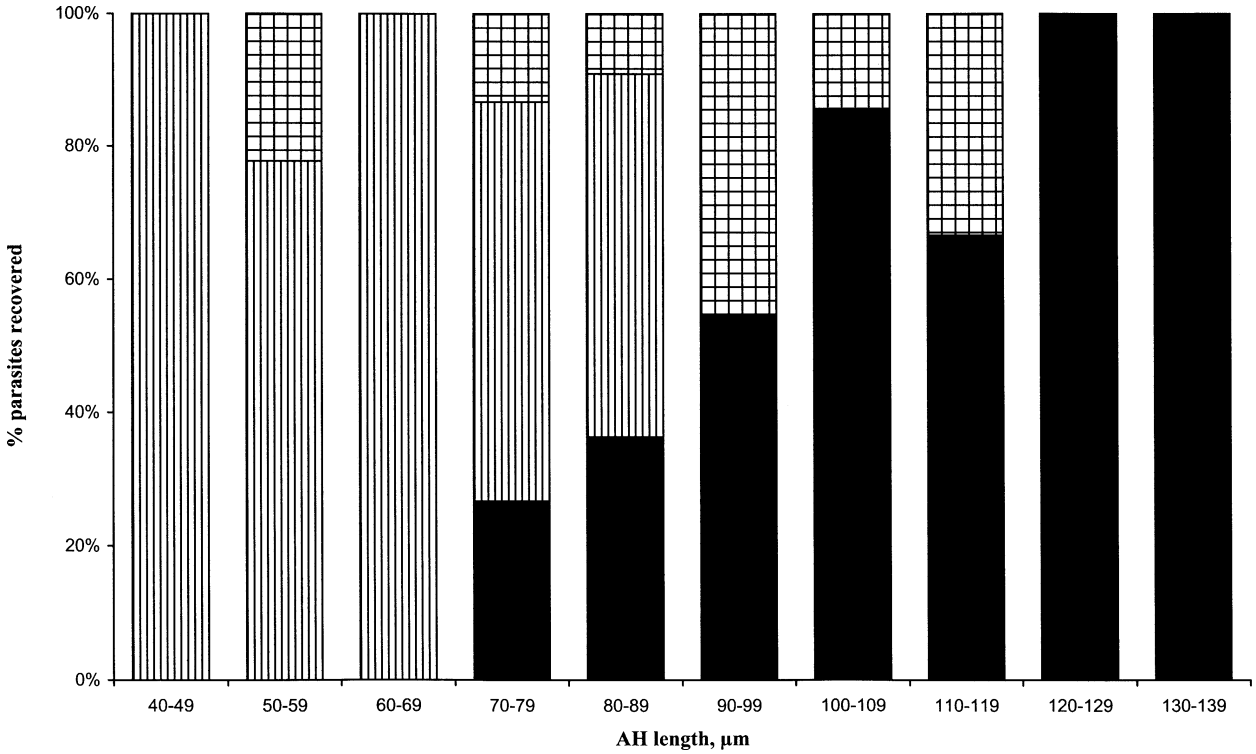

Fig. 3. Relationship between anterior hamulus (AH) length (in μm) and distribution of *Benedenia lutjani* specimens from a sample of 10 wild *Lutjanus carponotatus* from waters around Heron Island in May 1995 (see Table 3). Parasites in each 10 μm AH length range recovered from pelvic fins (▥) and branchiostegal membranes (■) are shown separately, as a percentage of the total parasite numbers (Table 3). Figures for other sites (Table 3) have been added together to calculate percentage recovery (▦).

Survey of wild specimens of L. carponotatus *for* B. lutjani *and* B. rohdei

Specimens of *B. lutjani* and *B. rohdei* were recovered from various external sites from a sample of 10 *L. carponotatus* (175–290 mm LCF) from waters around Heron Island in May 1995 (ambient seawater temperature, 23 °C). Infection details: for *B. lutjani*, prevalence 100 %, intensity 2–35, mean 10; for *B. rohdei*, prevalence 80 %, intensity 1–13, mean 5·5.

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Table 3. Distribution and developmental stages of specimens of *Benedenia lutjani* and *B. rohdei* recovered from 10 *Lutjanus carponotatus* caught directly from the wild around Heron Island in May 1995

(Ambient seawater temperature was 23 °C. Abbreviations as for Table 2.)

Site on host	Imm.	Protand.	Protand. (ins)	Adult (?)	Adult (ins./eggs)	Total no. of specimens	Estimated age† in days (mean (range))
Pelvics	12	14	3	1	12	42	8·1 (2·3–12·4)
Branch.	0	0	0	8	31	39	17·0 (8·1–31·4)
Other sites*	4	1	0	0	8	13	12·9 (4·3–23·4)
All sites (*B. lutjani*)	16	15	3	9	51	94	12·7 (2·3–31·4)
Gills (*B. rohdei*)	0	0	0	0	44	44	N/A

* These parasites were recorded from anal, caudal and dorsal fins only.
† Age estimates were calculated using the regression equation ln (AH length) = 0·46 ln (age) + 3·32 for experimental infections at 23 °C (see text for details).

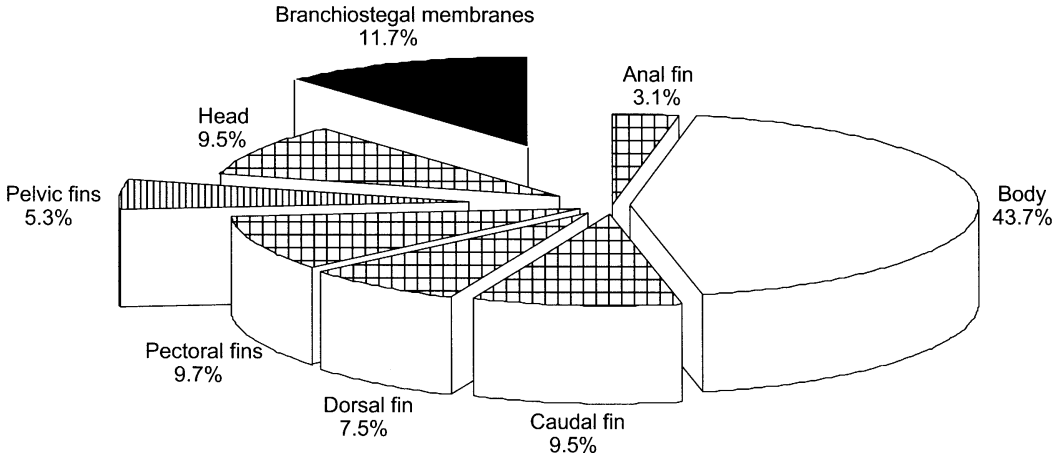

Fig. 4. Pie chart depicting the surface area of each external site examined (excluding gills) for specimens of *Benedenia lutjani*, expressed as a percentage of the total surface area of *Lutjanus carponotatus* estimated from a specimen 220 mm LCF. Top shading as for Figs 1 and 3.

The distribution of *B. lutjani* specimens on wild *L. carponotatus* is shown in Fig. 3. Parasites with smaller AH length occurred on the pelvic fins and parasites with larger AH length were found on the branchiostegal membranes. The age of worms was estimated using the length–age relationship generated from the 23 °C infection experiment (see above and Fig. 2). The distribution, developmental stage and estimated age are shown in Table 3 and correspond with those in experimental infections (e.g. Table 2). Most *B. lutjani* specimens on the pelvic fins of wild-caught *L. carponotatus* were immature or protandrous with an estimated age of 8·1 (2·3–12·4) days (Table 3). Parasites from the branchiostegal membranes were all adult(?) or adult with an estimated age of 17 (8·1–31·4) days. A small proportion of *B. lutjani* specimens of various developmental stages were recovered from the anal, caudal and dorsal fins (Table 3). No *B. lutjani* specimens were recovered from the body surfaces or gills of these wild fish.

All *B. rohdei* specimens recovered were from the gills and all were adults (Table 3) judged using the same scheme for *B. lutjani*.

Estimation of external surface area of L. carponotatus

The surface area of each external site (excluding gills) examined for parasites in this study was expressed as a percentage of the total external surface area of the fish estimated from a specimen of *L. carponotatus* 220 mm LCF (Fig. 4). The 2 sites that appear to be 'preferred' by *B. lutjani* represent relatively small proportions of the total surface area of *L. carponotatus*: the pelvic fins comprise 5·3% and the branchiostegal membranes (including their inner and outer surfaces) comprise 11·7% compared with 43·7% for the body proper (i.e. flanks) (Fig. 4).

DISCUSSION

Whittington & Kearn (1993) observed that adult specimens of the capsalid monogenean, *B. lutjani*, displayed a preference for the pelvic fins of their round-bodied teleost host, *L. carponotatus*, whether caught directly from the wild or when maintained in

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aquaria to promote heavy infections. To determine the infection dynamics of this monogenean species, Whittington & Kearn (1993) suggested further study using experimental infections. Questions posed were: (i) is there preferential settlement by larvae on the pelvic fins; (ii) do parasites that settle elsewhere migrate to the pelvic fins before sexual maturity is reached, or is there mortality of parasite stages on the body surfaces before sexual maturity is attained? Our infection experiments demonstrate that oncomiracidia of *B. lutjani* invade predominantly the host's body surfaces (i.e. the flanks of the body proper), but many attach to some fins. Postoncomiracidia then migrate to the pelvic fins and by 6 days p.i. at water temperatures between 23 and 27 °C, most parasites were recovered here. This finding is consistent with observations by Whittington & Kearn (1993). Unlike their study, however, we determined that the largest, sexually mature specimens of *B. lutjani* occur on the branchiostegal membranes, a site just anterior to the pelvic fins that was not examined by Whittington & Kearn (1993).

Contrary to the success achieved for *B. lutjani*, similar infection experiments using larvae of *B. rohdei*, which is specific to the gills of *L. carponotatus*, were unsuccessful. No *B. rohdei* specimens were recovered during dissections of 8 fish. Eggs of *B. rohdei* appeared to develop normally. We estimated that 85% of the eggs had hatched and swimming oncomiracidia were observed. Thorough examinations of experimentally infected fish were performed because it was considered possible that juvenile *B. rohdei* may inhabit an unusual site such as the nares, lip folds or beneath scales. These investigations failed to reveal any *B. rohdei* on fish exposed to freshly hatched oncomiracidia. Furthermore, an examination of *B. rohdei* specimens from wild fish provided little information on the life-history of this species because only adult specimens were found and all were located on the gills. Further work is required to determine how and where *B. rohdei* larvae invade *L. carponotatus*.

Kearn (1984) determined experimentally that *Entobdella soleae* (Capsalidae) larvae migrate anteriorly on the upper surface of their flatfish teleost host, *Solea solea* (Soleidae), and by 9 days p.i., some individuals had found their way to the lower surface where parasites reach sexual maturity. Ogawa (1984) provided evidence that *B. hoshinai* (Capsalidae) oncomiracidia attach broadly over the body and fins of their round-bodied teleost host, *Oplegnathus fasciatus* (Oplegnathidae), but fewer were reported ventrally and none was noted from pelvic fins. Migration of *B. hoshinai* occurs in a posterior direction because adults are most abundant on the caudal fin, the posterior part of the dorsal fin and the posterodorsal region of the body proper (Ogawa, 1984). For *Neobenedenia girellae* (Capsalidae) (probably *N. melleni*; see Whittington & Horton, 1996) on Japanese flounder (*Paralichthys olivaceus*) (Paralichthyidae), there is some indication that older parasites move from fins, the main site of larval settlement, to body surfaces (Bondad-Reantaso *et al.* 1995). West & Roubal (1998) noted that larvae of *Anoplodiscus cirrusspiralis* (Anoplodiscidae) settled on the head, skin and fins of snapper, *Pagrus auratus* (Sparidae), moving to the fins on maturity; adults appeared to prefer the caudal fin.

We have established that reproductive development in *B. lutjani* corresponds with its migratory movements on *L. carponotatus*. Parasite aggregation on the pelvic fins coincides with the development of functional male reproductive organs. Aggregation on the branchiostegal membranes, apparently the definitive site for adult *B. lutjani*, corresponds with the onset of sexual maturity and egg-laying. Furthermore, the distribution and developmental stages of *B. lutjani* specimens in natural and experimental infections are similar, indicating that experimental data mirror events in the natural environment.

In the original description of *B. lutjani*, Whittington & Kearn (1993) noted that it was a small species. With the benefit of hindsight, it is apparent that its morphometrics were based on comparatively small, sexually mature specimens from the pelvic fins. Whittington *et al.* (2001) measured a different sample of adult *B. lutjani* from the branchiostegal membranes of *L. carponotatus* dissected immediately after capture and provided revised morphometrics. Not surprisingly, mean values for most parameters measured were larger than those presented by Whittington & Kearn (1993) and reflect the fact that the definitive site for adults was unknown when *B. lutjani* was described.

There are previous reports where site-specificity has been correlated with monogenean development, but these relate mostly to polystomatids from tetrapod hosts. The most striking example is *Pseudodiplorchis americanus* from North American spadefoot toads, *Scaphiopus* spp. Free-swimming larvae invade, and migrate over, skin and gain entry to the nostrils of the toad (Tinsley & Earle, 1983). Migration continues via the buccal cavity to the lungs where juveniles continue to develop and *P. americanus* do not become sexually mature until they reach their final site, the urinary bladder (Tinsley & Earle, 1983; Tinsley & Jackson, 1986). The final migratory phase from lungs to urinary bladder occurs via the alimentary canal (Tinsley & Jackson, 1986).

Among monogeneans of fishes, juvenile and adult *Calicotyle kroyeri*, a monocotylid that parasitises several species of rajid elasmobranchs (e.g. Llewellyn, Green & Kearn, 1984; Chisholm *et al.* 1997, 2001), occupy different sites. The larval invasion route is unknown, but early development of *C. kroyeri* can occur in the rectal gland whereas most

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parasites in the cloaca are adult (Kearn, 1987). Most monogeneans, however, parasitize the 'skin', fins or gills of teleosts. The present account for *B. lutjani* is unusual because, to our knowledge, it represents the first report of a monogenean species from the external surfaces (excluding gills) of a teleost with clearly separate microhabitats for juvenile and adult parasites. Pelvic fins support juveniles and some sexually mature *B. lutjani*, whereas sexually mature worms occur almost exclusively on the branchiostegal membranes. Recent studies indicate that a similar developmental demarcation may be the case for a polyopisthocotylean gill monogenean. Chigasaki *et al*. (2000) demonstrated experimentally that more larvae of *Heterobothrium okamotoi* (Diclidophoridae) settle on the host's body surfaces and fins than on the gills. Migrations by the parasites occur because Ogawa & Inouye (1997*a*, *b*) reported that juveniles inhabit the gills and adults live on the walls of the branchial cavity.

The adaptive significance of migration by *B. lutjani* towards the pelvic fins of *L. carponotatus* after larval invasion anywhere on the external surfaces most likely concerns enhanced opportunities for individuals to meet and exchange sperm as proposed by Whittington & Kearn (1993). Data presented here appear to reinforce this 'mating hypothesis'. Protandrous individuals are able to exchange sperm on the pelvic fins providing the vagina is functional. As Kearn, James & Evans-Gowing (1993) noted for *E. soleae*, sperm exchange early in life effectively maximizes reproductive output because parasites can begin laying fertile eggs as soon as they become sexually mature. The pelvic fins are clearly 'chosen' in some way by *B. lutjani* because they represent approximately 5% of the total surface area of *L. carponotatus*. A likely mechanism may be innate programming or fixed action patterns shaped by evolution which, in predictable conditions, confer significant adaptive value on organisms (Sukhdeo, 1997). The adaptive significance for convergence of protandrous or recently matured *B. lutjani* on the pelvic fins likely relates to reproduction, because mating chances between individuals will be promoted before adults leave to migrate to the branchiostegal membranes. Segregation of mature individuals on the branchiostegal membranes, representing approximately 12% of the total surface area of the host (~ 6% when one considers that *B. lutjani* was found only on their external surfaces), may further support successful mating because all specimens here can not only donate, but also receive, sperm. Some alternative reasons for segregation of juveniles and adults are discussed below.

Hypotheses above are speculative because specific information is lacking about mating behaviour in *B. lutjani*. Despite maintaining large populations in small dishes containing seawater, no individuals have been observed to mate (previously unpublished observation) unlike, for example, specimens of *E. soleae* (see Kearn, 1970), *B. rohdei* (as *Benedenia* sp. 1; see Kearn & Whittington, 1992) and *B. seriolae* (see Kearn, 1992). Nevertheless, many inseminated individuals of *B. lutjani* were found on pelvic fins and branchiostegal membranes of experimentally infected fish and hosts caught directly from seas around Heron Island. Insemination probably occurs either by intromission (as reported for *B. rohdei* and *B. seriolae*; see Kearn & Whittington, 1992 and Kearn, 1992 respectively) or by spermatophore exchange (as described in *E. soleae* by Kearn, 1970) and, as in these examples, it is probably a mutual event. No structures likely to be spermatophores have been observed in dishes containing large numbers of *B. lutjani* nor inside the vagina of inseminated individuals. However, self-insemination in *B. lutjani* cannot be ruled out and evidence that this occurs on some occasions is available for other capsalids (e.g. *Benedeniella* spp., see Kearn & Whittington, 1992; *Neobenedenia melleni*, see Whittington & Horton, 1996). Kearn *et al*. (1993), however, provided evidence that isolated individuals of *E. soleae* fail to self-inseminate.

The importance of mating in the biology of parasites in general, and for monogeneans in particular, has been highlighted previously (e.g. Rohde, 1991, 1993, 1994; Lo, 1999). There is a general perception that parasites with good locomotory ability or those that can maintain 'large' populations on a host individual have a broader microhabitat (e.g. Rohde, 1991, 1993, 1994). Present knowledge of *B. lutjani* indicates individuals are highly mobile on host surfaces (Whittington & Cribb, 1999) and from data in the present study, this mobility allows parasites to move from one microhabitat to another during development. Indeed, the benefits of mobility, if shaped by fixed action patterns, may contribute significantly to the mechanism of habitat selection (Sukhdeo, 1990, 1997) by *B. lutjani*. Concerning population 'size', *B. lutjani* infected all of 10 host specimens caught directly from the wild (mean intensity, 10; range 2–35). There is much to understand about mating biology among the Monogenea and the approach proposed by Sukhdeo & Bansemir (1996), based on comprehensive studies at the level of individual parasites, has much to offer. For monogeneans from fish, the most complete picture is for *E. soleae* (see Kearn, 1970; Kearn *et al*. 1993).

Data that are lacking, but which would contribute significantly to an understanding of mechanisms that shape parasite distributions and site-specificity, include the duration of mating events, the quantity of sperm exchanged and the relationship between insemination, age, microhabitat, population size and density-dependent interactions. Kearn (1992) noted that brief but regular matings between *B. seriolae* specimens observed *in vitro* may reflect many

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opportunities to copulate in nature. *Entobdella soleae*, however, exchanges a larger volume of sperm via spermatophores (Kearn, 1992) and has not been observed to mate more than once. Arguments based on these observations apply only if events observed for parasites *in vitro* or in laboratory experiments mirror those *in vivo*. As Kearn (1992) stated, there are no indications to suspect otherwise for mating by *E. soleae* and *B. seriolae*. This notion is supported here because records of inseminated individuals of *B. lutjani* were congruent from fish in experimental infections and from fish caught directly from the sea.

We hypothesize that one reason for parasite site-specificity is to improve mating success following ideas of Rohde (e.g. 1991, 1994), but mate finding may have little bearing on the 'choice' by a *B. lutjani* individual to relocate to the branchiostegal membranes because it involves migration from a site already populated by potential partners. For *B. lutjani*, it is unknown whether there is a requirement (or a 'choice') for individuals to mate repeatedly. Perhaps a single mating event on the pelvic fins provides an individual with sufficient sperm to produce fertilized eggs for the rest of its life (duration unknown; see below).

It is possible that separate microhabitats for juvenile and adult specimens of *B. lutjani* confer other adaptive benefits. Spatial separation may provide distinct feeding grounds or spatial resources for each developmental cohort and therefore may reduce any intraspecific competition or interference. As parasites grow, it is also possible that larger specimens will require a different and more suitable substrate for attachment. Whittington (1996) suggested that site-specificity by some parasites may provide a selective advantage because some microhabitats on fish may receive less attention than others by cleaner organisms. Perhaps pelvic fins and branchiostegal membranes of *L. carponotatus* are inspected less by cleaners and if this is so, each site may represent a relatively safe, or safer, haven for *B. lutjani*.

Monogenean body length can vary due to the degree of flattening or the amount of muscle contraction during fixation and is, therefore, an unreliable index of age. Kearn (1990) determined that the anterior hamuli of *E. soleae* grow continuously throughout the life of the parasite and the length of these haptoral sclerites, seemingly unaffected by compression or preservation, provides a reliable index of age. From infection experiments, we have also found a close relationship between anterior hamulus length and age for *B. lutjani*. For *E. soleae*, the anterior hamuli grow continually but at a progressively decreasing rate (Kearn, 1963, 1990) whereas the relationship determined for *B. lutjani* is linear. One possible reason for this difference is that Kearn (1990) obtained growth data for the duration of the life-time of *E. soleae*, including 1 specimen that survived for 182 days. The maximum duration of our infection experiments was 25 days at 23 °C and 24 °C and although this is beyond the age when sexual maturity is reached, it is possible that anterior hamuli growth in 25-day-old parasites had not yet slowed.

Attempts to determine the longevity of *B. lutjani* were unsuccessful, but our data provide some clues. The maximum anterior hamulus length among specimens recovered from 10 wild *L. carponotatus* caught around Heron Island (ambient seawater temperature, 23 °C) was 135 μm whereas the maximum anterior hamulus length among worms in an infection experiment at the same temperature was 136 μm for a specimen recovered 25 days p.i. If anterior hamuli of *B. lutjani* continue to grow until death, the largest specimens from experimental infections can be assumed to be of equivalent age to the largest parasites found on wild hosts. Further study is required to refine knowledge of *B. lutjani* longevity, but a life-span equivalent to that of *E. soleae* (112–182 days in temperate European seas at approximately 12 °C according to Kearn, 1990) for this subtropical species seems highly unlikely.

We found marked variability in the intensity of *B. lutjani* specimens on experimentally infected *L. carponotatus*. Infections of 23 captive specimens of *L. carponotatus* by *B. lutjani* larvae led to the recovery of between 2 and 75 parasite specimens. This variability can be explained neither by fish size nor differences in exposure to oncomiracidia, because similarly sized fish were chosen for infection and variability of infection occurred between fish in the same experiment. It appears, therefore, that the variation of infection observed is due to differences in susceptibility between individual specimens of *L. carponotatus* and may have an immunological basis (e.g. see Buchmann, 1999). However, all 10 *L. carponotatus* caught directly from the wild were infected by *B. lutjani* (mean intensity, 10 [2–35]). One can only wonder at the odds of successful encounters between stripeys and *B. lutjani* larvae at the mercy of predators and ocean currents and faced with a diversity of fish species.

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