Parasitization of a hydrothermal vent limpet (Lepetodrilidae, Vetigastropoda) by a highly modified copepod (Chitonophilidae, Cyclopoida)

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SUMMARY

The limpet *Lepetodrilus fucensis* McLean is very abundant at hydrothermal vents on the Juan de Fuca and Explorer Ridges in the northeast Pacific Ocean. This limpet is parasitized by an undescribed chitonophilid copepod throughout the limpet's range. The parasite copepodite enters the mantle cavity and attaches to the afferent branchial vein. The initial invasive stage is a vermiform endosome within the vein that develops an extensive rootlet system causing an enlargement of the afferent branchial vein. Subsequently, an ectosomal female body grows outside the vein to sizes up to 2 mm in width. Once a dwarf male attaches, egg clusters form and nauplii are released. In over 3000 limpets examined from 30 populations, prevalence averaged about 5% with localized infections in female limpets over 25%. After the establishment of limpet populations at new vents, copepod prevalence increased over the succeeding months to 3 years. Host effects were marked and included castration of both sexes and deterioration in gill condition which affected both food acquisition and the gill symbiont. There was a significantly greater parasite prevalence in larger females which likely modifies the reproductive and competitive success of local host populations.

Key words: Chitonophilidae, Lepetodrilacea, hydrothermal vent, parasitization, pathogenesis, parasitic copepod, castration, host condition, host sex bias.

INTRODUCTION

Despite the difficulties of developing marine community models that include parasites, their modifying effects on host success cannot be ignored (Mouritsen and Poulin, 2002). Gathering adequate information in poorly accessible deep-sea habitats such as hydrothermal vents is challenging; the minimal record of parasite effects on vent fauna likely reflects lack of study (de Buron and Morand, 2004; Terlizzi *et al*. 2004). Because of the dispersed and sometimes ephemeral nature of the vent habitat, study of the dynamics of disjunct populations in this ecosystem is attractive; a parasite has not only the requirement of finding a vent but also must rely on a host that has also successfully recruited. Community models of hydrothermal vent fauna usually identify an assemblage of low diversity dominated by a few species (Van Dover, 2002; Tsurumi, 2003). Examination of parasite abundance and effects in these dominant species is necessary to model the factors controlling community structure. Hydrothermal vents on the Juan de Fuca and Explorer Ridges of the northeast Pacific provide habitat for the limpet *Lepetodrilus fucensis* McLean (Vetigastropoda, Lepetodrilidae) that can be very abundant (up to 390 000 m^{-2}) (Sarrazin and Juniper, 1999; Bates *et al*. 2005). Many aspects of this limpet's life history traits and foraging strategy explain its high abundances (Metaxas, 2004; Kelly and Metaxas, 2007; Bates, 2007*a*); however, it is not clear what phenomena limit its numbers. *L. fucensis* has multiple feeding mechanisms and its gill morphology is specialized to process suspended food particles and host symbiotic bacteria that are ingested. Suspension feeding and symbiont farming are primary feeding mechanisms in active venting (5–25 °C), while grazing may be more important in peripheral locations and waning vents (Bates, 2007*b*). This study examines collections from the 600+ km extent of the Explorer/Juan de Fuca Ridge spreading ridge system. We also study new hydrothermal vents that were colonized by *L. fucensis* in the years after 1998, when the Axial Volcano erupted obliterating prior vents with extrusive lavas (Embley *et al*. 1999; Marcus and Tunnicliffe, 2002).

The family Chitonophilidae was erected by Avdeev and Sirenko (Avdeev and Sirenko, 1991); it is a small group of highly modified copepods infecting polyplacophorans and gastropods that produces

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Table 1. Locations of ventfields on the Juan de Fuca and Explorer Ridges from which limpet samples were examined

(Within each field collections may derive from several vents. Samples were used to examine geographical spread (G), or parasite establishment in annual samples of newly created vents (E), or parasitization of limpets on the periphery of vent influence (P) and at senescent vents (S).)

Field	Locality	Depth (m)	Latitude	Longitude	Samples
Einstein	Explorer	1790	49°45.6′N	130°15.6′W	G14
Heat Flow	Middle Valley	2420	48°25.8′N	128°40.9′W	G11
HiRise	Endeavour	2125	47°58.1′N	129°05.3′W	G9
Clam Bed	Endeavour	2192	47°57.8′N	129°05.5′W	G6, G7, G8, G15
Main	Endeavour	2193	47°56.8′N	129°05.9′W	G24
Floc	CoAxial	2230	46°18.6′N	129°42.5′W	G19, S5
CASM	Axial	1581	45°59.4′N	130°00.2′W	G3
ASHES	Axial	1546	45°56.0′N	130°00.8′W	G1
South Rift	Axial	1520	45°56.0′N	129°59.0′W	G16, E2, E4, E20–22, E26–28, E30–32, P18, P23, P25, S17
North	Cleft	2283	44°54.0′N	130°15.5′W	G12, S13

copious egg masses often mistaken for host eggs (Huys *et al*. 2002). There is little information on the effects of chitonophilid parasites on their hosts. Tissue penetration by the endosome can be very extensive and, presumably, can induce fatality in the host (see illustration in Avdeev and Sirenko, 2005). Egg development in some species likely affects host gill function (Franz and Bullock, 1990) and partial female castration (Huys *et al*. 2002) may occur. This paper describes the occurrence of a parasite in *Lepetodrilus fucensis*. The parasite resembles described copepods in the family Chitonophilidae but likely represents a new species currently under examination (R. Huys, personal communication). The objectives of this study are to (i) describe the nature of the infection including prevalence patterns in different host habitats, (ii) examine the rate at which the parasite establishes in new host populations, and (iii) determine the effects of the parasite on tissue condition and reproductive potential of the host. This study is the first to examine parasite prevalence across the range of a hydrothermal vent host and to document the effects of parasitization on that host.

MATERIALS AND METHODS

Limpet sampling

Lepetodrilus fucensis limpets were collected from August 1990 to August 2007 using manipulators or a suction sampler on a remotely operated vehicle. Collections were transferred to closed containers subsea and preserved in formalin or alcohol on board ship. We collected *L. fucensis* from most of the known vent field locations along the Explorer and Juan de Fuca Ridges (Table 1). An additional suite of samples came from vents visited annually after the Axial Volcano eruption and represented time since establishment of new limpet populations. We also examined samples from low venting conditions (both vent periphery and waning vents) to assess prevalence in suboptimal host conditions. Microhabitat gradients were steep especially when pairing samples: 'high flux' means a discrete fluid outlet venting water below about 25 °C, while 'periphery' means the zone within 1–2 m of that outlet where no fluid flow is visible and temperatures are below 5 °C. Subsamples of about 100 limpets taken from each collection at one time formed the basis of this work; because it was not possible to deduce parasite presence prior to host dissection, these subsamples are unbiased by the sample method. In addition, we examined small (20 limpets each) paired collections from high flux and peripheral areas of 11 vents (50–75 cm from fluid flow) as described by Bates *et al*. (2005) and Bates (2007*a*). Due to low numbers of available limpets, these data were pooled to examine the microhabitat difference. We limit the comparative examinations to limpets with a shell length of 5 mm or greater as these animals are capable of gametogenesis (Kelly and Metaxas, 2007). However, at the locality with the highest infection rate, we examined a set of individuals smaller than 5 mm to determine the minimum size at which a limpet can be infected. In summer 2007, live limpets from 2 vents (Marker 33 and Marker 113) on Axial Seamount were placed in separate wells of a culture tray and incubated in deep sea water at 3 °C. The wells were inspected for nauplii every 12 h. Several limpets were dissected to examine live parasites.

For each limpet, the shell was measured to the nearest millimeter and then removed; the limpet gender was noted and parasite presence determined. When present, the copepod was removed along with the limpet mantle and the infection was categorized into 1 of 5 stages defined in a subjective manner but reproducible among the authors (see definitions in

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Results section). The parasite ectosome was measured at the widest point. After parasite removal, further dissection exposed the limpet gill and the gonad. A qualitative condition index of gill and gonad tissue was applied on a scale of 0 to 3 reflecting condition scores from 'very poor' to 'good'. For the symbiont-bearing gills, a zero score applied when over 90% of the gill filaments were diminished in depth thereby reducing the area of the bacteria-hosting region. For this score, dense bacterial colonies were not present on the gill and/or the distended branchial vein was attached to the gill and filaments underlying the parasite were misshapen. Remaining gill scores were assigned according to the number of filaments compromised: one, 50–90%; two, 10–50%; and three, under 10%. Gonad condition scores were based on the abundances of egg bundles and seminal fluid visible under a light microscope. Gonad fullness was scored: 0 = oocytes and seminal fluid (white shimmering) absent; 1 = gametes sparse and only present in part of the gonad; 2 = moderate abundance throughout; 3 = full gonads. We examined 111 unparasitized limpets from high flux vent flow to rate the health of the gill and gonad to determine the 'healthy' population condition. All scores were collected by one observer. For further examination of the anatomical detail, specimens were dehydrated in a graded ethanol series, critical-point dried and imaged using light microscopy. To generate scanning electron micrographs tissues were sputter-coated in gold and imaged on a Hitachi S-3500N microscope.

We tested whether parasites occur in limpets that are, on average, larger than would be expected by chance. For each sex, we compared the difference in the size class (mm intervals) of each infected animal to the median size class of uninfected individuals of that gender in the population from which it originated. The null expectation is a normal distribution centred on zero difference in size. We determined if the frequency distribution of differences for each sex deviated from normal using a Kolmogorov-Smirnov One-Sample Test (SPSS 15.0).

Histological techniques

For both infected and healthy limpets, a transverse cut was made at the neck to remove the head from the body. The body, containing the gonad and parasite (when present), was then prepared for histological analysis following standard techniques. Tissues were dehydrated in a series of graded ethanol solutions, cleared in xylene, and embedded in paraffin. Sections of gonad were cut at a thickness of 5 μm and stained with Harris' haematoxylin and eosin. Gametogenic maturity was measured from 2–4 replicate sections of gonad cut >200 μm apart. For female limpets, all oocytes sectioned through the nucleus were measured (theoretical spherical diameter) and classed as either mature (vitellogenic) or immature. Female gametogenic maturity was quantified as the number of mature (vitellogenic) oocytes expressed as a percentage of the total number of oocytes measured. Male gametogenic maturity was quantified as the percentage of total gonad cross-sectional area occupied by spermatozoa. All measurements were obtained using Adobe Photoshop CS and SigmaScan Pro 5.0 image analysis software. To increase sample size for statistical analysis, limpets were assigned to 1 of 3 infection levels: no parasite, early parasitization (Stages 1 to 3) or late parasitization (Stages 4 and 5). A one-way ANOVA was performed for males and females separately, to compare gametogenic maturity among infection levels. Tukey's HSD post-hoc tests were used to examine differences as detected by the ANOVA.

RESULTS

General description

The afferent branchial vein (*sensu* Fretter, 1988) runs inside the afferent axis and extends from the anterior of the mantle (atop the head of the limpet) to the posterior pericardial cavity. The mantle cavity overlies the entire gill for about 4/5 the body stopping before the pericardial cavity. The female copepod parasite is almost always located in the afferent branchial vein. An adult parasite has an endosomal portion that can occupy one-third to two-thirds of the vein length (Fig. 1A). In the most extreme example, the endosome 'rootlets', a bifurcated structure with lateral outgrowths, extended for 5·7 mm in the blood vessel of a host that was 9·3 mm in length. A parasitized vein is expanded between 5–10 times its normal diameter even at the earliest infection stages. The female parasite's ectosome is connected through the wall of the vein where it, and the attached eggs, lie on the dorsal surface of the gill (Figs 1A–C and 2). Occasionally, the parasite was found on the efferent branchial vein and, in 6 instances, an immature parasite occurred in the posterior pericardial cavity. Only once could we find the moulted head of the late-stage female copepodid embedded in the branchial vein (Fig. 1I).

In nearly all mature female chitonophilids examined, the most distal eggs had formed nauplii hatching into the mantle cavity (Fig. 1J). The male was present as a spherical, unsegmented body attached to the posterior region of the ectosome (Fig. 1B; G also shows copepodid moults); only 1 male was present on most females but up to 5 clustered males could occur. A detached male has 2 anterior 'lateral lobes' (*sensu* Lamb *et al*. 1996) (Fig. 1H).

Stages of parasitization

We define 5 'stages' in the parasitization of *Lepetodrilus fucensis* that were manifest in our dissections.

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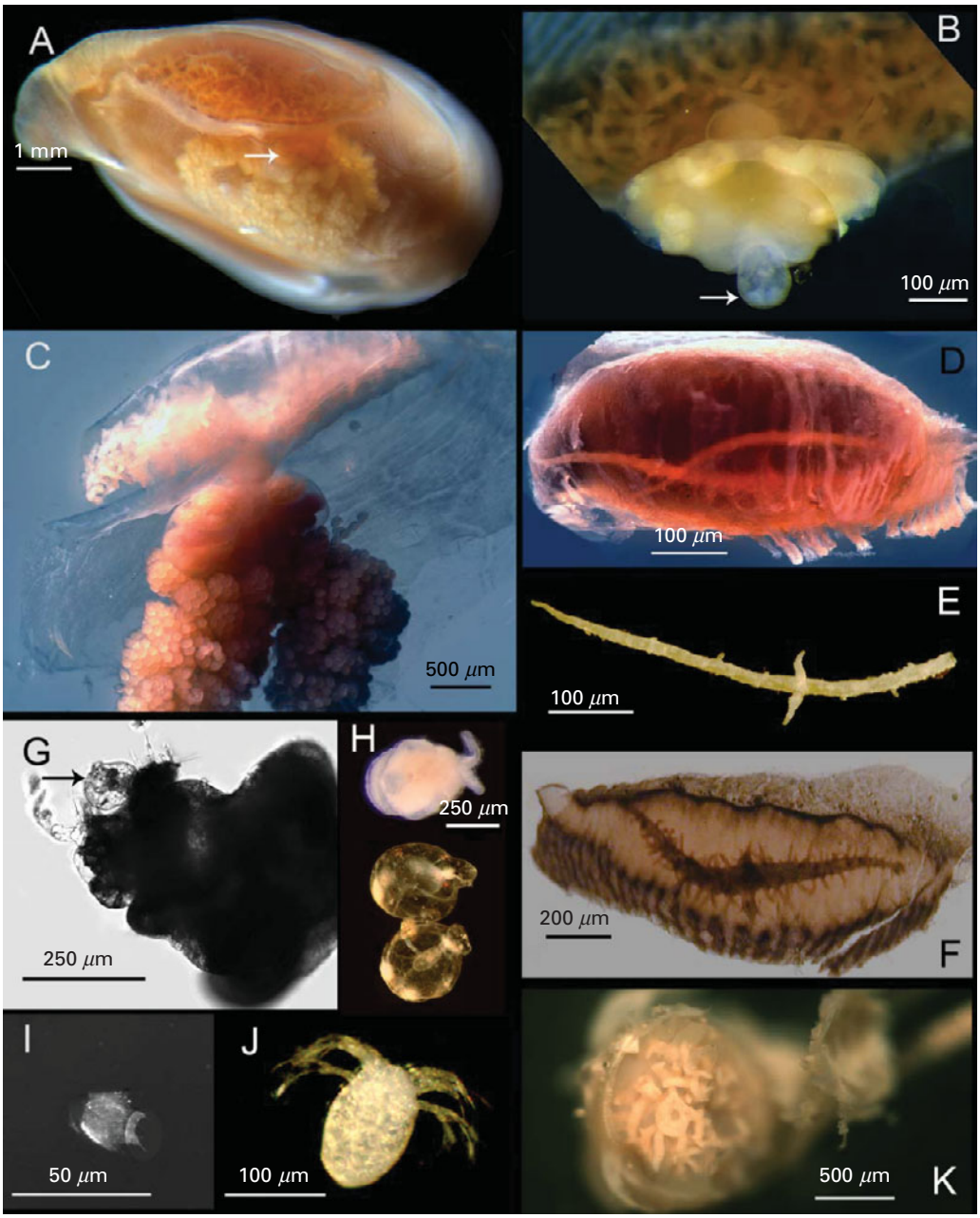

Fig. 1. Cyclopoid chitonophilid copepod (putative new species) infecting the hydrothermal vent limpet *Lepetodrilus fucensis* McLean. (A) *L. fucensis* with shell and mantle removed. Branchial vein is infiltrated with copepod endosome at the top of the image. Copepod ectosome (arrow) has masses of yellow eggs attached overlying the limpet gill. (B) An early Stage 4 chitonophilid showing a bulbous, ovoid attachment point through the branchial vein to the branching endosome. A male (arrow) has attached and ova are maturing inside the female. Two images in different focal planes were merged leaving the faint semicircular artefact on the ectosome. (C) A Stage 4 chitonophilid removed from the limpet host to show the 2 large clusters of eggs. (D) A distended branchial vein with 2 Stage 1 vermiform copepods. (E) A Stage 1 endosome removed from vein showing 2 central extensions. (F) A Stage 2 endosome that has begun to branch. (G) A high-contrast image of female endosome with eggs removed to show an attached male (arrow) and 2 copepodite moults embedded in the female tissue. (H) One male found free in limpet mantle shows the lateral lobes that presumably attach in the female tissue; 2 additional males to illustrate the simplified body structure. (I) Head of a female copepodite removed from a Stage 1 endosome inside a branchial vein; the remainder of the body was not evident. (J) Chitonophilid nauplius removed from limpet mantle cavity. (K) Critical-point dried branchial vein of limpet cracked to show the cross-sectional structure of the endosome. When observed live, both the rootlets and the main stem display rapid wriggling movements.

Stage 1 (Fig. 1D, E): the parasite is a single rootlet ranging from 0·5 mm to 3 mm in length. It has a worm-like or 'vermiform' appearance usually with 2 small central protrusions and appears lightly chitinized. It lies free within the afferent branchial vein.

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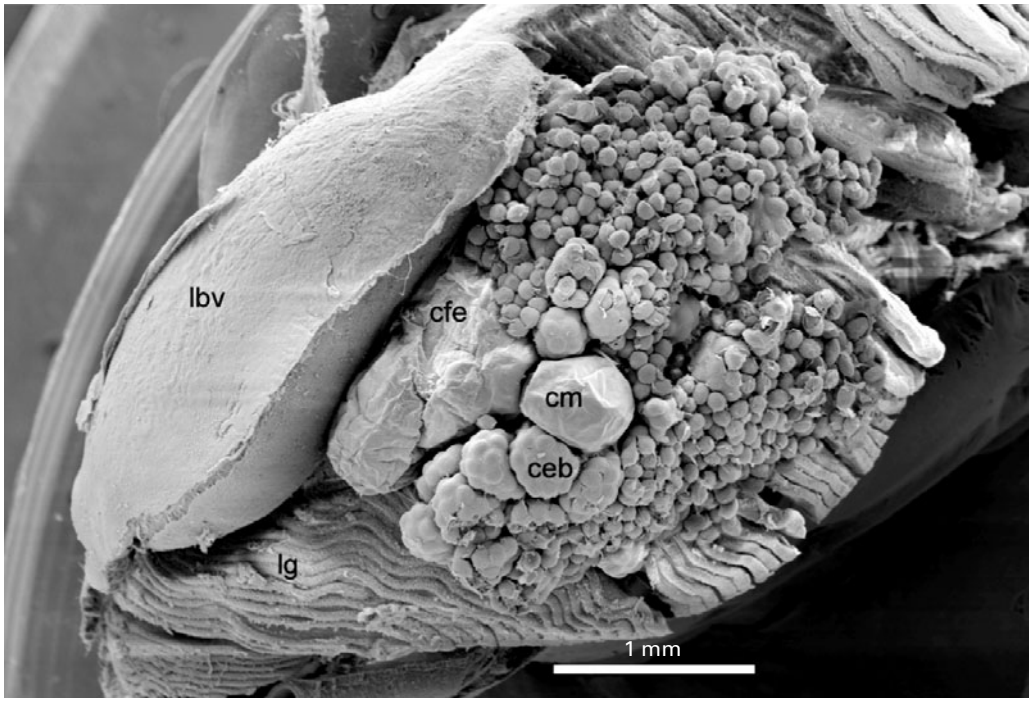

Fig. 2. SEM micrograph showing a Stage 4 chitonophilid copepod female ectosome (cfe) attached to a distorted branchial vein (lbv) overlying the limpet gill (lg). The male (cm) is attached at the apex of the female ectosome and surrounded by bundles of eggs (ceb).

Stage 2 (Fig. 1F): the initial endosome now has many side rootlets extending radially along the entire length although it is still entirely unattached within the blood vessel. One central extension has increased in length.

Stage 3: the extension is anchored to the blood vessel wall and a small ectosomal body is developing on the outer surface of the vessel. The rootlet system increases in complexity with tertiary structures forming. No male is present.

Stage 4 (Fig. 1A, C, Fig. 2): the ectosome is a large heart-shaped body anchored to the blood vessel. No appendages or segmentation are evident. The mature female has a dwarf male(s) attached usually at the apex between 2 large egg clusters. The endosome has an extensively branching structure that remains within the blood vessel.

Stage 5: More than one parasite present in a host in any combination of stages.

From 167 limpets, we extracted 198 chitonophilid copepods. Of these parasites, about 20% were in the first vermiform stage (Table 2) while nearly 60% (including many Stage 5 infections) were egg-bearing females. The Stage 3 ectosome varies little in size (Fig. 3A) and shows no relationship to host size. Development of egg masses occurs at about 0·7 mm ectosome width. This Stage 4 ectosome ranges up to 2·2 mm across (Fig. 3A), and it is positively related to host limpet size (Spearman correlation, $P<0{\cdot}01$; Fig. 3B). The smallest limpet found harbouring a parasite (Stage 1) was 2·8 mm length.

Table 2. Stages of parasitization by chitonophilid copepod within the limpet *Lepetodrilis fucensis* as defined in this study

(From 3211 limpets, we extracted 198 parasites which is the sum of Stages 1 to 4; Stage 5 designates limpets with more than 1 parasite.)

Stage	Description	Number observed
1	Vermiform body with only minor central extension; distended host blood vessel	39
2	Initial secondary branching on endosome contained entirely within blood vessel	19
3	Extensive endosomal branching; small ectosome attached through blood vessel wall	22
4	Extensive endosome; male present; large ectosome with eggs	118
5	Multiple parasites present	20

Field observations

Live chitonophilid nauplii emerged from several limpets cultured at surface pressure. These nauplii have 3 pairs of appendages and large lipid reserves. They did not survive beyond 60 h although moulting to a second stage was suggested by the presence of exuviae discarded in the dish. The live adult chitonophilids removed from 3 limpets were remarkable in the mobility of the endosome. In each case,

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Table 3. Prevalence of chitonophilid copepods in *Lepetodrilus fucensis* along the Juan de Fuca and Explorer Ridges

(Prevalence of parasitization is the proportion of limpets sampled with 1 or more copepods at any infection stage. Relative prevalence takes into account the limpet sex ratio in samples that range from 40% to 50% male. * = Clam Bed field samples; the other Endeavour samples are different ventfields. 'All' represents all specimens examined including those in Table 4.)

Sample	Locality	Number of limpets	Prevalence of parasitization	95% Confidence interval (±)	Relative prevalence in males	Relative prevalence in females
G14	Explorer	113	0·053	0·042	0·038	0·066
G11	Middle Valley	101	0·050	0·043	0·020	0·080
G09	Endeavour	109	0·037	0·035	0·000	0·071
G06	Endeavour*	258	0·143	0·043	0·085	0·191
G07	Endeavour*	100	0·260	0·086	0·277	0·245
G08	Endeavour*	102	0·118	0·063	0·083	0·148
G15	Endeavour*	52	0·154	0·099	0·000	0·276
G24	Endeavour	137	0·000	0·000	0·000	0·000
G19	CoAxial	106	0·009	0·018	0·000	0·016
G03	Axial	102	0·020	0·027	0·000	0·033
G01	Axial	106	0·038	0·036	0·000	0·069
G16	Axial	107	0·028	0·031	0·000	0·061
G12	Cleft	116	0·017	0·024	0·019	0·016
ALL	**ALL**	**3211**	**0·052**	**0·008**	**0·031**	**0·074**

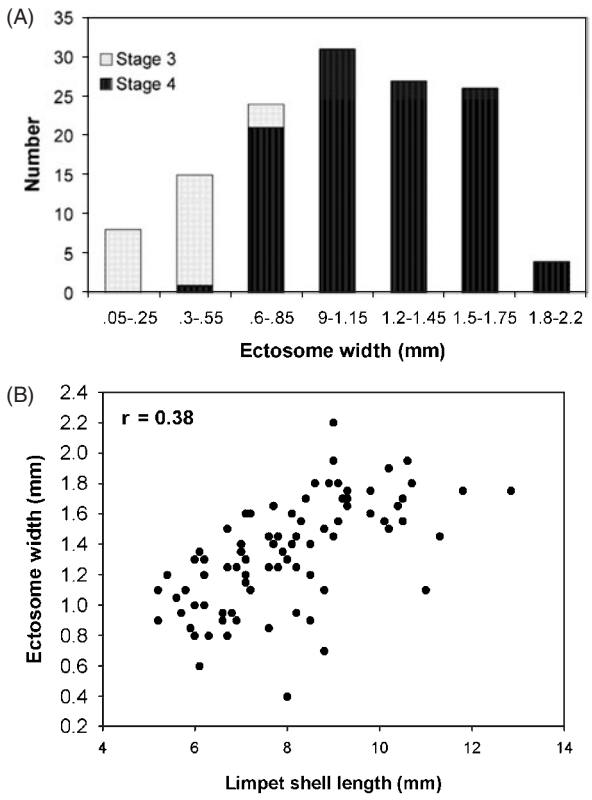

Fig. 3. (A) Size frequency histogram of chitonophilid ectosome width in Stages 3 and 4. Stage 3 ectosome widths are less variable than in Stage 4, which can exceed 2 mm. (B) Ectosome width (mm) of chitonophilid parasites in Stage 4 infection ($n = 81$) versus shell length (mm) of the host *Lepetodrilus fucensis*. Spearman correlation is significant at $P < 0{\cdot}01$.

the entire rootlet system writhed vigorously during and after excision from the branchial vein. The ectosome remained motionless.

Geographical range, prevalence and intensity of the chitonophilid infection

The parasite was present in limpets at all 6 major hydrothermal sites along Juan de Fuca and Explorer Ridges (Table 3) representing the full geographical range of the host *L. fucensis* over 600 km. Overall, we examined 3211 limpets to find 167 parasitized individuals (5·2% prevalence, 95% CI ± 0·8%). Three collections from 1999 at one field of the Endeavour locality (Clam Bed) returned prevalence values between 11·8% and 26·0% (Table 3: G07, G08, G15). To determine if these prevalence levels persisted at this locality, a small sample from 2001 ($n = 52$ limpets) was examined and yielded 15·4%. The overall parasite prevalence for this vent field is 16·4% (95% CI ± 3·2%). Mean abundance of the parasite in the 30 host populations studied ranges from 0 to 0·18 (S.E. 0·05). The overall abundance in the 3211 limpets was 0·06 (S.E. 0·01).

Multiple parasites can occur in a single limpet. Up to 5 parasites occurred in a single host and one limpet hosted 4 fully mature females with eggs. The afferent branchial vein may have multiple parasites but, in one case, the secondary infection was located on a different vessel. Of the 20 limpets with multiple parasites, 18 were female. 'Intensity' of infection represents the number of parasites in an infected host (Bush *et al.* 1997). Mean intensity for the 167 parasitized limpets is 1·19 (S.D. ± 0·57) parasites per host. Mean intensity in male limpets (1·02 S.D. 0·22) is significantly lower than in females (1·23 S.D. 0·65) ($t = 2{\cdot}07$, $P < 0{\cdot}05$).

Variation in prevalence in host genders

Over all the collections, 7·5% of female limpets hosted parasites compared to 2·8% of males. Over

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Table 4. Prevalence of chitonophilid parasite in *Lepetodrilus fucensis* at vents formed on Axial Volcano after the January 1998 lava eruption

((A) Year that we observed limpets recruiting at new vents varied; the lack of replicates in the early months is due to very low limpet abundance anywhere on the new lavas. Limpets were not seen at Cloud N6 vent until 30 months post-eruption. Bolded samples are paired with those in part B of the table. (B) These 3 samples are paired with samples in the upper part of the table (E02, E20 and E27) where limpets were collected within high fluid flow around tubeworms; the P series limpets were scattered on basalt about 50–100 cm distant from fluid flow.)

Sample	Vent	Months post-eruption	Number of limpets	Parasite prevalence	95% Confidence interval (±)	Relative prevalence in males	Relative prevalence in females
(A) Within vent							
E21	Snail vent	8	14	0·000	0·000	0·000	0·000
E04	Mkr 113	18	137	0·007	0·014	0·000	0·014
E02	**Nascent**	30	110	0·018	0·025	0·018	0·018
E22	Cloud N6	30	102	0·029	0·033	0·031	0·027
E28	Mkr 113	30	116	0·026	0·029	0·038	0·016
E20	**Cloud N6**	42	109	0·028	0·031	0·033	0·021
E30	Mkr 33	42	109	0·092	0·054	0·077	0·105
E31	Mkr 33	54	109	0·000	0·000	0·000	0·000
E32	Mkr 33	66	98	0·051	0·044	0·022	0·075
E26	Mkr 33	104	106	0·075	0·051	0·000	0·136
E27	**Mkr 113**	104	109	0·037	0·035	0·048	0·021
(B) Vent periphery							
P18	**Nascent**	30	103	0·049	0·042	0·018	0·085
P23	**Cloud N6**	42	112	0·054	0·044	0·000	0·109
P25	**Mkr 113**	104	50	0·120	0·042	0·038	0·278

Table 5. Proportions of male and female host *Lepetodrilus fucensis* with parasitic chitonophilid copepods at each infection stage

(e.g. 33% of parasitized males hosted Stage 1 parasites. In total, 167 limpets were parasitized. Mean gill and gonad health (±1 S.D.) decline with progressive stages of the infection; maximum 'healthy' score is 3.)

Infection stage	Proportion of males $n=43$	Mean gill health	Mean gonad health	Proportion of females $n=124$	Mean gill health	Mean gonad health
1	0·33	2·5±0·6	2·0±1·0	0·17	2·6±0·6	2·0±0·8
2	0·11	2·2±0·8	2·0±0·7	0·06	1·8±0·7	1·3±0·8
3	0·17	2·3±0·5	2·0±0·0	0·08	2·0±0·8	0·9±0·8
4	0·33	1·7±0·5	1·5±0·7	0·56	1·7±0·5	0·8±0·7
5	0·04	2·0±0·0	1·0±0·0	0·14	1·7±0·5	0·7±0·5

20% of the females at Clam Bed had 1 or more parasites. After the data are adjusted for the gender ratios in each collection, parasite prevalence still shows a strong female bias (Tables 3, 4); a paired sample *t*-test is significant ($t=3{\cdot}42$, D.F. $=29$; $P=0{\cdot}001$). The proportions of the parasitized genders in each of the 5 stages are significantly different ($\chi^2=14{\cdot}31$, D.F. $=4$, $P<0{\cdot}01$). Male limpets tend to have a higher proportion of infection in Stages 1 and 2 (Table 5).

There is a marked difference in parasite prevalence between female and male limpets at smaller host sizes (Fig. 4A): up to 7 mm host size, prevalence is nearly 3 times higher in females. We examined whether copepod occurrence is related to host size. Because limpet genders are not equally represented across the size classes, we compared the size of each parasitized host to the median limpet size for its collection. With no size bias, the resultant plot (Fig. 4B) should show a normal distribution centered around zero difference from the median collection size. Overall, 19% of infected limpets are 2–4 mm larger than the uninfected populations median while only 6% are 2–4 mm smaller than the median (Fig. 4B). Furthermore, the differences between the sizes of parasitized limpets and the population median (both sexes) are significantly larger than would be predicted from a normal curve (K-S One-Sample Test: $Z>1{\cdot}75$, $P<0{\cdot}01$).

Colonization of new host populations

Vent fauna developed at numerous new vents on the Axial Volcano South Rift zone after the January 1998

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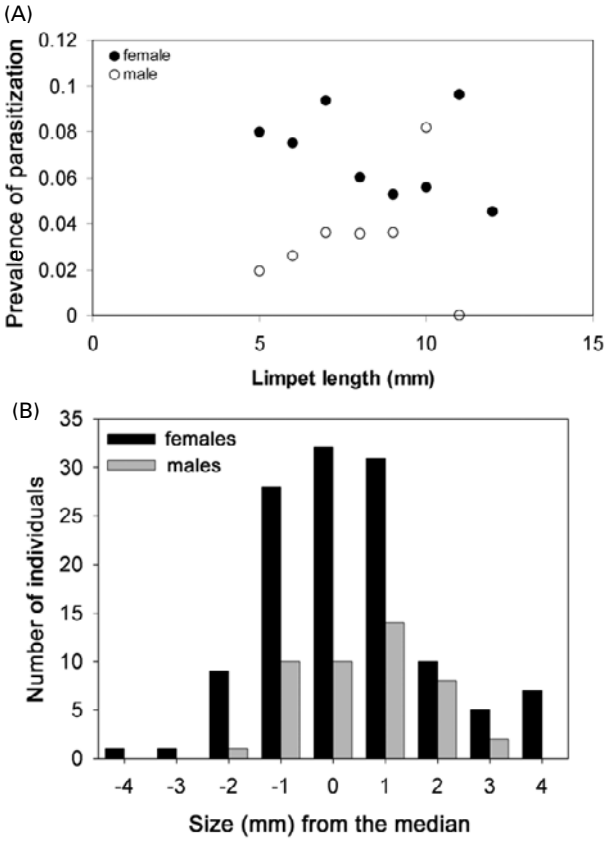

Fig. 4. (A) Prevalence of parasitization by chitonophilid copepods for each size interval of male and female host limpets. In the '11 mm male' and the '12 mm female' size categories, less than 25 limpets were available for examination. (B) Size distribution of parasitized limpets relative to the median size of the population from which they were collected. Limpets are larger (+1 to 4 mm), the same (0), or smaller (−1 to −4 mm) than the population median. For both sexes (females, $n=124$; males, $n=45$), there is a skew to larger sizes.

eruption. Limpets were rare at 8 months post-eruption and still rare at several vents at 18 months. Table 4A reflects low parasite prevalence in these first limpet recruits. Rate of increase with time is variable but, by 4 and 5 years post-eruption, prevalence of the parasite is similar to the Ridge average. For comparison, sample G16 (Table 2) limpets were sampled in 1998 within 50 m of Mkr 113 at a vent unaffected by the eruptive lavas to yield a 2·5% prevalence of the copepod.

Prevalence of parasitization in low venting influence

Three paired samples compared copepod prevalence in limpets collected from high fluid flow to those at the same vent but in peripheral areas of no visible flow (Table 4B). In each case, prevalence is greater on the periphery overall, but particularly in females where prevalence of 28% occurs. In the 11 small, paired collections, limpets near visible vent flow were compared to limpets between 50 and 75 cm distant from that flow. Combined, 4 of 215 limpets in (or near) fluid flow hosted parasites, while parasites occurred in 22 of the 215 peripheral limpets. In all peripheral samples examined, only 6 males were infected compared to 37 females. In a different setting of low flow, we examined limpets from senescing vents that had nearly ceased flow. Prevalence was minimal: among 318 limpets from 3 vents, only 1 limpet had a chitonophilid parasite.

Host gill and gonad condition

The parasite and egg masses nearly always lie between the dorsal surface of the gill and the mantle. While they would not impede fluid flow into the mantle cavity (see Bates, 2007*b*, Fig. 7), they likely reduce fluid flow and particle delivery between the ctendial filaments and across the dorsal surface of the gill. The least impact of the copepod would be reduced fluid flow for gas exchange and suspension feeding and/or symbiont farming. Further, in ∼10% of cases with Stage 4 infections, the dorsal surface of gill lamellae were depressed and misshapen, and the bacteria-hosting region on the gill lamellae and the frontal cilia were absent. In the worst case of multiple parasites, the infection can occlude most of the gill surface.

In a survey of unparasitized limpets from high flux vent flow (52 females and 59 males), average gill score was 2·92. Gill condition deteriorates as stage of parasitization advances (Table 5). In animals hosting early infection stages, a small percentage of gill filaments (<10%; mean score = 2·5) had reduced depths underlying the distended branchial vein. At later infection stages, more than 50% of gill filaments (mean score = 2·0) were compromised.

Nearly all unparasitized limpets over 5 mm long contained full gonads (average condition score 2·99) and mature gametes (Fig. 5). In the presence of the parasite, 65% of the 167 infected limpets had few or no gametes and often no gonadal duct development. There is an immediate impact on the gonad at the earliest infection stages as the mean gonad score registers 2·0 for both sexes. Gamete abundance declines in later infection stages for both sexes, but especially for females (Table 5). By Stage 4, seminal fluid in the gonad was sparse (mean gonad score = 1·5) and oocytes were sometimes absent entirely (mean score = 0·8).

Histological sections reveal more detail of poor condition of limpet gonadal development (Fig. 6A). Limpets with Stage 4 parasites (gonad score = 0) have few gametes that are small and underdeveloped (Fig. 6B, C) compared to unparasitized limpets (Fig. 6D, E). Gametogenic maturity of *Lepetodrilus fucensis* is significantly lower when parasites are present in both males (ANOVA: $F(2,60)=27{\cdot}15$, $P<0{\cdot}001$) and females (ANOVA: $F(2,52)=38{\cdot}53$, $P<0{\cdot}001$) (Fig. 5). For males, mean spermatozoa relative area was lower in late infection stages,

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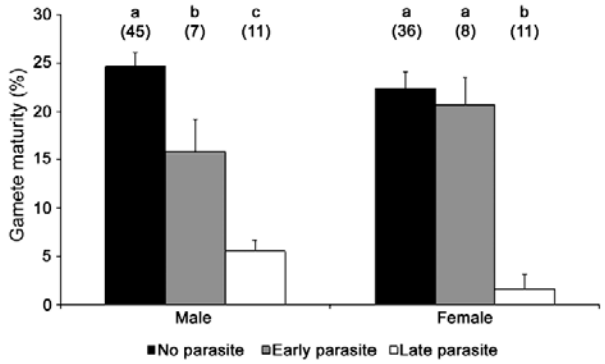

Fig. 5. Mean (+S.E.) gamete maturity of host male and female *Lepetodrilus fucensis* in unparasitized and parasitized states. 'Early parasite' includes Stages 1 to 3 while 'late parasite' includes Stages 4 and 5. Gametogenic maturity for males is the percentage of total gonad cross-sectional area occupied by spermatozoa, and for females, it is the percentage of mature oocytes. Numbers in parentheses indicate sample sizes. Significant differences between stages within a gender are indicated by different letters as identified by Tukey's HSD test ($P<0{\cdot}05$).

intermediate in early infection stages, and higher in unparasitized limpets (Tukey's HSD, $P<0{\cdot}05$). Even in early stages of infection, the spermatozoa are spread more thinly across the gonad as reflected by an average score of 2 (Table 5): the gonad had moderate abundances of seminal fluid with less densely packed sperm. Females in early infection stages have a similar oocyte size range (15–105 μm *vs* 10–110 μm) and percentage of vitellogenic oocytes (0–40% *vs* 10–40%) as unparasitized females (Fig. 7A and B). In contrast, females with Stage 4 or 5 infections have poorly developed oocytes with over 70% less than 40 μm and only a few ranging to 90 μm (Fig. 7C). Only a small pool of vitellogenic oocytes occurs in late-stage parasitization; in 3 of 4 animals, vitellogenic oocytes were absent from the ovary. The mean percentage of mature oocytes in late infection stages was significantly lower than in early infection stages and unparasitized limpets (Tukey's HSD, $P<0{\cdot}001$). However, while the mean percentage of mature oocytes was similar for unparasitized limpets and those in the early infection stages (Tukey's HSD, $P>0{\cdot}1$), the gonad fullness scores (above) indicate a parasite effect because there were fewer oocytes present.

DISCUSSION

The Chitonophilidae comprise highly modified copepods recently assigned to the Cyclopoida by Huys *et al.* (2002). Of the 16 described species, 13 parasitize chitons, 2 parasitize cocculiniform limpets, and 1 a nucellid snail (Avdeev and Sirenko, 2005). Our report extends the habitat range of the family Chitonophilidae from intertidal to deep-sea hydrothermal at 2400 m depth. The species parasitizing *Lepetodrilus fucensis* has morphological features similar to those in limpet hosts described by Jones and Marshall (1986) and Huys *et al.* (2002) but both males and females differ substantially in size, shape and other features from these described species. Because the genus *Lepetodrilus* occurs at vents worldwide, a search for other chitonophilids may reveal host-parasite co-evolution patterns.

Chitonophilid life cycle

Lamb *et al.* (1998) described chitonophilid parasite development for *Nucellicola holmanae*: the hatching nauplii develop in the host until the metanaupliar stage is released to develop as free-swimming copepodid stages. The final larval stage infects a new host and metamorphoses to the adult. From this description and our observations, we propose the following infection cycle in *L. fucensis*. A female copepodite enters the mantle cavity of the limpet on the incurrent respiratory current. It passes through the gill lamellae with the dominant fluid current (Bates, 2007*b*) and presumably attaches to the afferent branchial vein. A vermiform developmental stage is injected into the vein. For 3 reasons, we suggest that this stage is motile: (i) it has no connection to the vein wall after injection; (ii) observations of live parasites confirm that the endosomal rootlets can move vigorously, and (iii) occasionally, the vermiform endosome occurs in parts of the vascular system that have no exposure to the mantle cavity; it likely moved there from the injection site. The host vein diameter enlarges followed by extensive development of the branching endosome. The lightly chitinized rootlets appear to have a porous surface that probably facilitates nutrient absorption. The late Stage 2 attaches to the inner vein wall and an amorphous spherical body forms outside the blood vessel as the ectosome. The male copepodite attaches at the apex of the ectosome and moults to the adult form that manifests as a globular, unsegmented body fused to the female. Eggs form in clusters that are pushed further from the ectosome. Those at the distal end hatch nauplii into the mantle cavity and apparently are expelled on the respiratory current of the limpet. We can only assume there are developmental stages outside the host as described for *Nucellicola holmane* (Lamb *et al.* 1998; Huys *et al.* 2002).

Poulin (1996*a*) documented only a few other copepod parasites of benthic invertebrates with a female to male size ratio as large as the 4·5 ratio in this chitonophilid. Although the selection pressures driving male dwarfism are not well understood, one hypothesis is that smaller, more mobile males may be selected for when the probability of locating females is low (Poulin, 1996*b*). Conversely, female parasites tend to display larger body size and are

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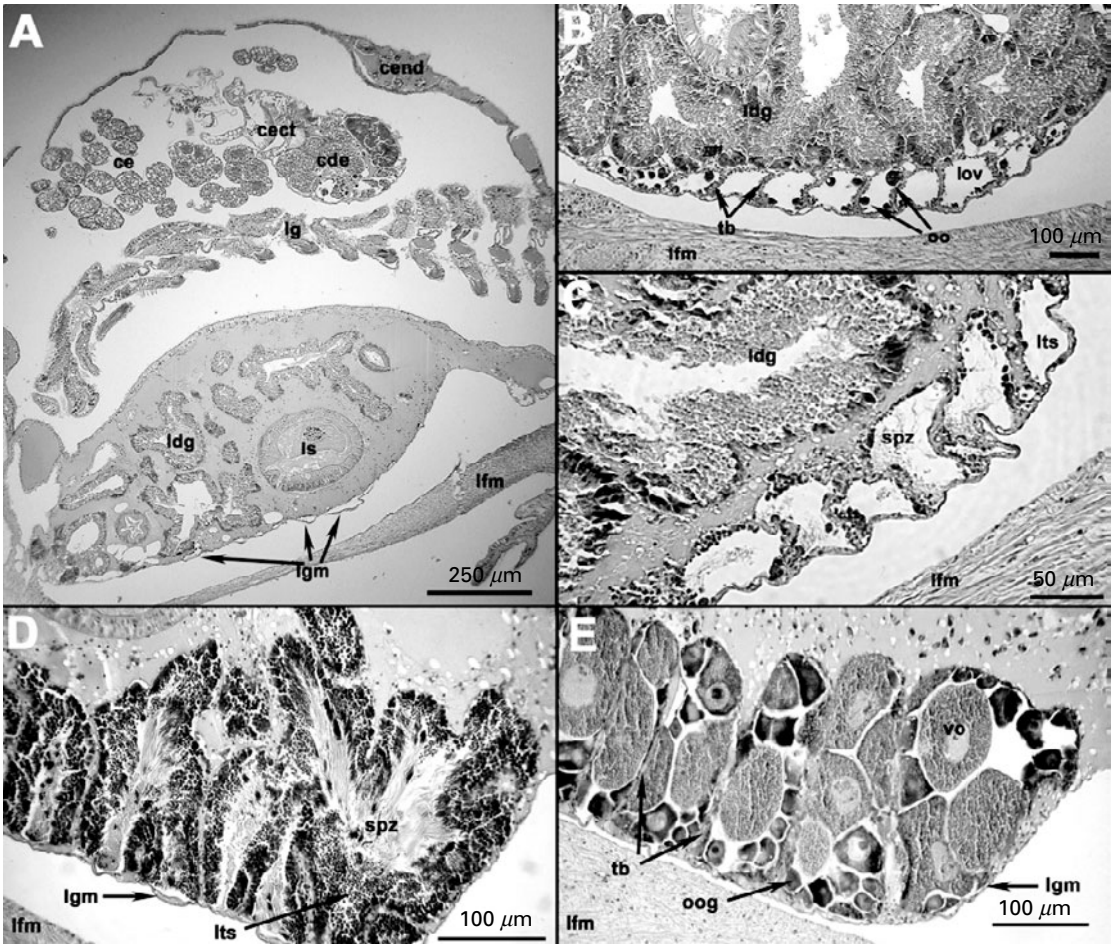

Fig. 6. Micrographs of histological sections of host limpet *Lepetodrilus fucensis* with Stage 4 infection, stained with haematoxylin and eosin. (A) Transverse section of parasitized limpet showing copepod eggs (ce) and developing eggs (cde) above limpet gill (lg) along with copepod endosome (cend) in limpet blood vessel and part of the ectosome (cect); an emaciated limpet gonad membrane (lgm) lies below the digestive gland (ldg) and stomach (ls), and above the foot musculature (lfm). (B) Effect of stage 4 infection on ovary (lov) of limpet female and reduction in oocyte production (oo); trabeculae of ovary (tb). (C) Effect of stage 4 parasitic infection on testis (lts) of male limpet and reduction in spermatozoa (spz) production. (D) Section of the testis in an unparasitized male limpet illustrating abundant spermatozoa. (E) Section of the ovary of an unparasitized female limpet illustrating abundant vitellogenic oocytes (vo) and oogonia (oog).

correspondingly more fecund when their host is difficult to find (Gotto, 1962; Poulin, 1995). Hence, the strong dimorphism reported here may reflect a life-history strategy that can successfully compensate for the difficulty of locating a vent endemic host as larval stages must recruit to dispersed, isolated sites on a deep ridgecrest. Males are faced with the additional challenge of finding a female where prevalence is typically about 5%.

Prevalence and host relationship

The variability in prevalence of the chitonophilid among habitats, vents and ventfields is high (from 0 to 0·28). Relatively high infection rates in localities such as Clam Bed may persist. This localized phenomenon suggests that the chitonophilid may develop quickly, as suggested for *Lepetellicola brescianii* (Huys *et al*. 2002), and is not advected out of the general vicinity. In peripheral areas of discrete vents, adult limpets are not abundant (Bates *et al*. 2005) yet chitonophilid prevalence is high relative to high flux conditions. Parasitized limpets may occur in a suboptimal habitat as a consequence of their parasite burden. For example, reduced fitness in the preferred venting habitat may cause the host to leave conditions of high space competition or the parasite may alter host behaviour (e.g. Moore, 2002). Although the parasite does not appear to recruit to limpets at dying vents (low flux vent 'cues' may not attract the copepodids), it does have an effective dispersal stage that establishes the infection in populations that have recently colonized new vents. While sample size and within-locality variability are confounding factors, our examination of prevalence with time suggests a lag period of months to 2 years.

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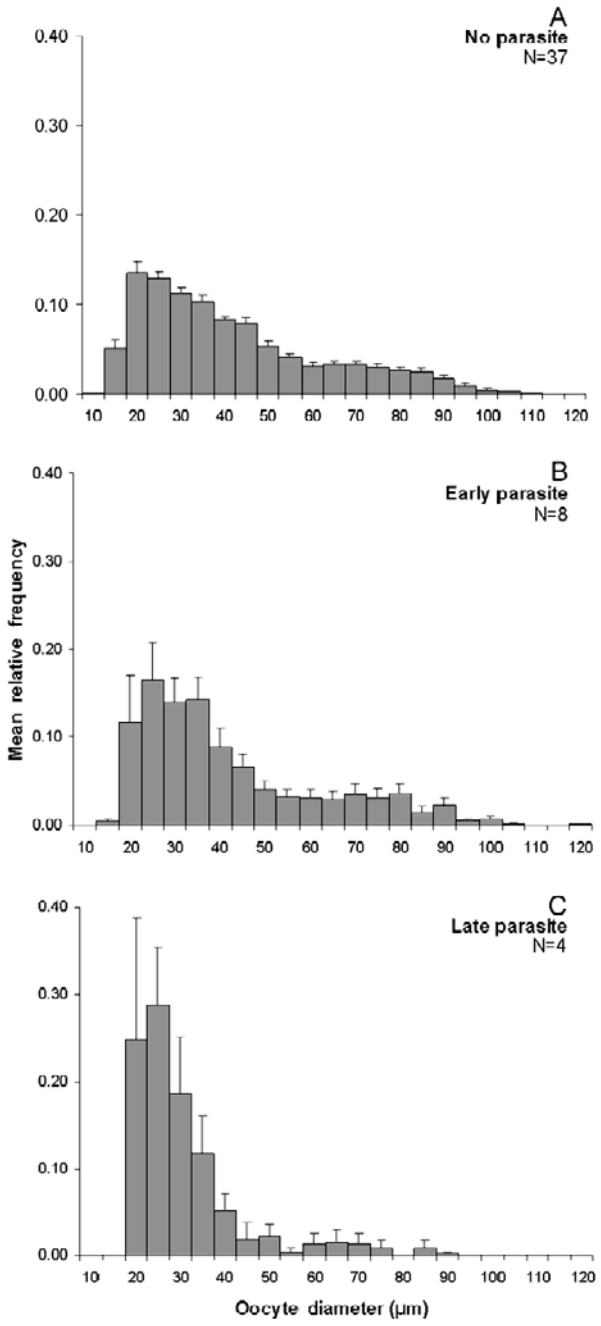

Fig. 7. Oocyte size-frequency histograms (mean + S.E.) for female limpet *Lepetodrilus fucensis* in unparasitized (A) and parasitized condition; 'early parasite' (B) includes Stages 1 to 3, while 'late parasite' (C) includes Stages 4 and 5. *N* = number of females examined.

Females are more than twice as likely to be parasitized than males and intensity of infection is also higher in females. Sex-biased parasitization is not unusual (Poulin, 1996*b*; Sheridan *et al*. 2000; Moore and Wilson, 2002). Female host bias may occur for reasons that include parasite choice and/or because female hosts more frequently contact the parasite as a consequence of differential host behaviour or development (e.g. Robb and Forbes, 2006). Infection of female *L. fucensis* may be advantageous to the parasite because they are more abundant and tend to be larger than male limpets in active vents (10–15 °C) where overall host tissue condition and feeding potential are maximized (Bates, 2007*b*). Alternatively, parasitized males may have lower survivorship. There is also a tendency for larger limpets of both sexes to be parasitized; the mechanism of host size selection is unclear and may simply represent an accumulative effect over time. However, a recent model that follows energy flow through hosts and parasites predicts that hosts parasitized by castrators should be larger than uninfected individuals (Hall *et al*. 2007). Such an outcome may be adaptive for the chitonophilid as it can grow larger in larger hosts and may be more fecund as a result.

Host effects

There are several likely effects of the chitonophilid on host function: on the vascular system, on the respiration efficiency of both host and its symbiotic bacteria, on suspension-feeding capability, and on reproduction. A major vein is occluded by the endosome; the dilated vein may be a host response to increase flows as the rootlets expand. Although hosts can compensate for nutritional loses to parasites by additional feeding (e.g. Slansky, 1986), we observed a decline in tissue condition with the development of the parasite that may be linked to uptake of host nutrients. The presence of the parasite also likely reduces the host's ability to suspension feed because the invasive mass blocks fluid flow on the dorsal gill surface. In addition, the size of the gill region with episymbiotic bacteria shrinks in response to a reduction in fluid flow (Bates, 2007*a*). The bacteria-hosting region of the gill was diminished where the parasite was present, implicating reduced flow due to the parasite presence; the net result is a greatly reduced ability of the limpet to gain nutrition from its symbionts and from suspension feeding. Additionally, the pathology likely has substantial effects in suboptimal limpet habitats such as the vent periphery; low survivorship in marginal conditions is known for parasitized intertidal mollusks (Mouritsen and Poulin, 2002).

The presence of late-stage or multiple parasites causes complete castration or severe reduction of gametogenesis in both male and female hosts. Huys *et al*. (2002) also described strong influences of another chitonophilid species on gonad development in cocculiniform limpets that include delay of maturation and sometimes castration. This phenomenon may be merely a non-adaptive by-product of the association (Brown *et al*. 2001; Hurd, 2001) in which limpet gametic output diminishes as it does in low nutritional condition in many invertebrates (Ramirez Llodra, 2002). However, both sexes of limpets exhibit diminished gamete production (sperm count and oviduct fullness measures) in early infection stages suggesting that partial castration

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occurs even before the effects of nutritional competition are felt. This chitonophilid may induce castration to access more host resources during a long life in a single host. Longevity is suggested by the continuous brooding in Stage 4 parasites that represent the majority of infections present. Ebert *et al.* (2004) proposed such an adaptive strategy for parasites with low requirements in early developmental stages but greater demands during an extended reproductive phase. The mechanism may be a direct inhibitory 'factor' on host gametogenesis (Webb and Hurd, 1999).

Lepetodrilus fucensis is cosmopolitan on the Juan de Fuca Ridge and is the most abundant macrofaunal vent animal on Juan de Fuca Ridge at an overall abundance of 41% of all animals present (Tsurumi and Tunnicliffe, 2003). Thus, a parasitic infection level around 5% appears low enough that the parasite might have little effect on its ability to dominate these communities. However, the deleterious effects on reproductive output may be substantial if infection rates are high for larger female hosts. This effect could be particularly marked in localized populations, such as at Clam Bed, where relative prevalence in females exceeded 15%. Reduction in *L. fucensis* larval output would reduce recruitment at isolated vent sites that primarily depend on local supply to maintain populations, particularly where prevalence of infection is high. Female fecundity increases exponentially with size (Kelly and Metaxas, 2007). Thus, high infection rates in large-sized female hosts would result in a decreased contribution to the next generation. Unlike *Lepetodrilus* species at other vent systems (e.g. Micheli *et al.* 2002), the role of predators in modifying *L. fucensis* success is small (Tsurumi and Tunnicliffe, 2003), thus the presence of a castrating parasite – even at low prevalence levels – probably has a substantial impact. Terlizzi *et al.* (2004) identified rickettsia-like and bacterial 'inclusions' in gill and gut tissue of *L. fucensis*. The compounding effects of multiple parasites in a host could be felt strongly, especially under deteriorating conditions in the habitat.

The chitonophilid parasite has a highly debilitating effect on its host through physical disruption, and castration; effects of haematophagy remain to be demonstrated. Few limpet populations are free of the parasite. A further study should examine the ability of limpets to compete and recruit at sites where infection levels are high. Our initial attempts to isolate the developing stages of the parasite were promising and it may be possible to examine infection and host response in more detail *in vitro*.

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