Glochidia ecology in wild fish populations and laboratory determination of competent host fishes for an endemic freshwater mussel of south-western Australia.

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Abstract. Glochidia (parasitic larvae) of freshwater mussels generally require a fish as a host. *Westralunio carteri* Iredale, 1934 (Bivalvia: Hyriidae), the only freshwater mussel found in south-western Australia was listed as Vulnerable, but recently changed to Least Concern (International Union for the Conservation of Nature). Glochidia were found on four alien and seven native species of fish from 18 sites in the South West Coast Drainage Division. On alien fishes, prevalence of glochidia ranged from 0.0 to 39.5% and mean intensity (number of glochidia per infested fish) from 1.0 to 6.0, while on native fishes prevalence was 9.2-75.0% and intensity was 2.3-7.1. Glochidia infestation was greatest on benthic fishes, which may be a consequence of greater encounter rates, but other factors, such as host size, probably also influence glochidia prevalence and intensity. Glochidia were generally restricted to fins of infested fish, and rarely on gills or the body surface. In the laboratory, four native and one alien fish species were found to be competent hosts for their ability to produce juvenile *W. carteri*, but two alien fish species were not. The inability of some alien fishes to produce juvenile *W. carteri* could potentially reduce recruitment success in areas dominated by alien fishes.

Keywords: *Westralunio carteri*; Unionoida; Unioniformes; Hyriidae; metamorphosis.

Running Head: Host fishes for *Westralunio carteri* of south-western Australia

Introduction

Freshwater mussels (Unioniformes) are an ancient order of bivalves physiologically adapted to live in fresh water and are found on all continents apart from Antarctica. Many species have undergone population reductions resulting in threatened species listings (Bauer and Wächtler 2001; Bogan and Roe 2008). In terms of freshwater ecosystem function, they provide water filtration, nutrient cycling and biodeposition of sediments, structural habitat and micro-refugia for other benthic organisms and a food source for predators (Spooner and Vaughn 2008).

Unioniformes are dioecious and reproduce sexually with fertilisation occurring in specialised chambers of the females' gills known as marsupia, where the embryos are brooded to the larval stage (Bauer and Wächtler 2001; Strayer 2008). This stage is often used to distinguish the two unioniform superfamilies; Unionoidea (Hyriidae, Margaritiferidae and Unionidae) larvae are known as 'glochidia' compared to the 'lasidia' or 'haustoria' of the Etherioidea (Etheriidae, Iridinidae and Mycetopodidae), although Graf and Cummings (2006) placed the Hyriidae into the Etherioidea based on phylogenetic evidence. Most larvae are parasitic (Bauer and Wächtler, 2001; Strayer 2008). When released, larvae have a brief opportunity to attach to a host, which is usually a fish (Bauer and Wächtler 2001; Strayer 2008). Some larvae possess hooks, known as larval teeth, along the ventral edges of their valves, which assist with attachment to the body surfaces, fins, mouth or gills of their hosts (Bauer and Wächtler 2001; Strayer 2008). Shortly after attachment, larvae are encapsulated by host epithelial tissue and remain in a parasitic phase for a period of weeks to months, depending on species and climate (Bauer and Wächtler 2001; Rogers-Lowery and Dimock 2006; Strayer 2008).

Factors controlling successful attachment and metamorphosis to the juvenile stage are complex and multi-faceted. They include environmental factors, such as water temperature,

water depth and habitat composition, adult mussel density and host factors, such as behaviour, seasonal migrations, endemic distributions, abundance, and immune response (Arey 1932; Bauer and Vogel 1987; Rogers and Dimock 2003; Dodd *et al*. 2005; Strayer 2008).

Identification of host fish species and glochidia ecology is a crucial component of unioniform conservation (Haag and Warren 1998; Martel and Lauzon-Guay 2005). Nine of the 11 (81.8%) native freshwater fishes of the region are endemic (Morgan *et al*. 1998, 2011; Allen *et al*. 2002), which is the greatest degree of freshwater fish endemicity in Australia, and contributes to the classification of the region as one of the world's biodiversity hotspots (Myers *et al*. 2000).

Westralunio carteri Iredale, 1934, is endemic to the South West Coast Drainage Division, where it is the only freshwater mussel in the region and the only member the genus in Australia (McMichael and Hiscock 1958; Walker 2004). The species was listed as Vulnerable in 1996 (IUCN 1996), but recently changed to Least Concern(Köhler 2011) and as a Priority 4 species by the Western Australian government (DEC 2011), although there is a paucity of information on the life history of the species. Few studies have quantified glochidia ecology in wild populations of fishes in Australia nor other regions in the Southern Hemisphere, although a number of Northern Hemisphere studies have identified host fishes in a laboratory setting, few have examined glochidia ecology in the wild (Strayer 2008).

The aim of this study was to determine the host fish species of the glochidial stage of *W. carteri*, quantify the prevalence and intensity of glochidia infestation throughout its range, determine which fish species may be competent hosts to sustain the life-cycle of the mussel and discuss factors that may influence the distribution and abundance of glochidia in wild fish populations. This study will hopefully encourage others to study glochidia ecology in wild

populations at a regional scale and will have broad applications in the study of freshwater mussel biology nationally and internationally.

Materials and Methods

Field sampling of host fishes

In total, 1005 fishes from 11 species (four alien and seven native) were captured from 18 sites (Fig. 1) using two-winged fyke nets (11.2 m wide, 0.8 m deep, 2mm nylon mesh), seine nets (3.0 m wide, 1.0 m deep, 2 mm nylon mesh) and/or electro-fishing (Smith-Root® Model LR20) during November and December 2010. All fishes were identified to species (Morgan *et al*., 1998, 2011) and measured for total length (TL) to the nearest 1 millimetre.

For *Tandanus bostocki* Whitley, 1944 (n = 306), glochidia were easily identified in the field from whitish, bladder-like cysts on the surface of the fish (Klunzinger *et al*. 2011). A sub-sample (n = 5) of infested *T. bostocki* and other fishes (n = 699) were transported live to the laboratory, where they were anaesthetised in AQUI-S™ and examined for glochidia using a dissecting microscope. The fins, body, gill filaments, opercula, eyes and mouths were examined for glochidia and their location was recorded.

Glochidia exposure trials

To determine whether glochidia of *W. carteri* metamorphose to the juvenile stage on different hosts, during 19 September to 6 November 2011 seven species of fish (*Afurcagobius suppositus* (Sauvage, 1880), *Carassius auratus* Linnaeus, 1758, *Geophagus brasiliensis* (Quoy and Gaimard, 1824), *Gambusia holbrooki* (Girard, 1859), *Nannoperca vittata* (Castelnau, 1873), *Pseudogobius olorum* (Sauvage, 1880) and *T. bostocki*) were exposed to gravid female *W. carteri* in the laboratory. *Afurcagobius suppositus*, *G. holbrooki* and *T. bostocki* were sourced

from the Collie River; *C. auratus* and *N. vittata* were purchased from Veba's Aquarium Supply (O' Connor, Western Australia 6163); *P. olorum* and *G. brasiliensis* were sourced from Bennett Brook. Fishes were captured using fyke nets or seine nets, transported to the laboratory and maintained in aquaria with continuous bio-filtration and aeration several weeks prior to the exposure trial.

A sample (n = 10) of adult *W. carteri* were hand-collected from each of the 18 field study sites and identified as *W. carteri* based on McMichael and Hiscock (1958) and Walker (2004). Mussels were examined, macroscopically for gravidity by holding valves open and manipulating the foot and visceral mass so that marsupia could be scrutinised for mature glochidia. Maturity was determined by the presence of swollen red marsupia, similar to Stage IV Hyriidae elsewhere (Jones *et al*. 1986; Byrne 1998). For comparison, two gravid adult *W. carteri* from the Collie River site were anaesthetized in 0.01% benzocaine solution and dissected. Glochidia were removed and examined under a compound microscope or dehydrated in graded ethanols, placed on a glass cover slip attached to a specimen stub, critical point dried, sputter-coated with gold, and photographed in a Philips XL 20 Scanning Electron Microscope (SEM).

Mussels, which were observed to have released mature glochidia were exposed to fishes for two hours in continuously aerated 9 L plastic buckets which contained dechlorinated tap water (N.B. glochidia maturity was evident by the examination of a mucus sample which contained glochidia actively 'blinking' and free of their vitelline membrane). Each bucket contained 10 individual fishes of a particular species and 20 gravid individual mussels. Exposed fishes were transferred to individual plastic fish hatcheries (Resun® Model FH-01, Guangzhou, China) and mounted inside 45L aquaria containing continuously aerated sponge bio-filters and dechlorinated tap water. Fishes were fed a diet of bloodworms daily.

Commencing on the second day post-exposure, individual fishes were captured by hand and placed on 75 mm diameter round blank agar plates containing enough water to cover the gills of each fish, and the fins quickly (< 1 min) examined for glochidia under a dissecting microscope. Infested fishes were maintained in the isolation chambers and uninfested fishes were maintained in separate aquaria. Prevalence data for the laboratory trials were not recorded because the aim of the experiment was to determine whether glochidia metamorphose to the juvenile stage, rather than a quantified analysis of infestivity. The water from the bottom of each isolation chamber was siphoned and transferred into another agar plate and scrutinised for the presence of either glochidia or juvenile *W. carteri* microscopically. Immediately following examination, fishes were transferred back into the chambers and placed into their respective aquaria. This process was repeated every second day post-exposure until glochidia were no longer found attached to the fins of infested fishes and juveniles appeared in the isolation chambers.

Statistical analysis

For each fish species at each locality, and for each fish species over all localities, we calculated mean glochidia prevalence (percentage of fish infested) and intensity (number of glochidia per infested fish). Ninety five percent confidence limits were calculated for prevalence, assuming a binomial distribution and intensity, from 2,000 bootstrap replications, using Quantitative Parasitology 3.0 (Rozsa *et al*. 2000). Differences in prevalence among fish species or sites and the tissue site of infestation among species were tested by Chi-square analysis and differences in intensity by a non-parametric Kruskal-Wallis test. The effect of TL on prevalence was tested by comparing the TL of infested and uninfested fish using a Mann-Whitney U-test and the relationship between TL and intensity was tested by Spearman's correlation analysis. A

Bonferroni adjustment was made for multiple comparisons of prevalence and intensity, to ensure an experiment-wide error rate of 5%. We defined significance at the $P < 0.05$ level and a statistical trend was defined as $0.05 < P \leq 0.10$.

Results

Prevalence and intensity of glochidia on fish hosts

In wild systems, glochidia were attached to and encysted on 10 of the 11 fish species examined, with mean prevalences over all sites ranging from 0 to 90.5% and mean intensities from 1 to 6 glochidia per infested fish (Table 1). Because different fish species were captured at different sites, Table 1 confounds differences in glochidiosis among species with differences among sites. In seven sites where two or more species of fish were collected in sufficient numbers (i.e. $n \geq 10$ fish), there were significant differences in prevalence among species in two sites and significant differences in intensity among species in one site (Table 2). In five sites, both native and alien fishes were captured and there were no consistent differences in prevalence or intensity among these groups (Table 2). For six species which were sampled from two or more sampling sites in sufficient numbers (i.e. $n \geq 10$ fish), there were significant differences in prevalence among sites for four species, and significant differences in intensity among sites for two species (Table 3).

There were no significant differences in the TL of fish infested and uninfested with glochidia for most species (Figs. 2 and 3; Table 4). However, infested *T. bostocki* were significantly larger than uninfested *T. bostocki* ($n = 306$, $U = 1799.0$, $P < 0.001$), as were *B. porosa* ($n = 76$, $U = 373.5$, $P = 0.04$) and a significant positive relationship was found between TL and glochidia intensity in *T. bostocki* ($n = 28$, Spearman's correlation, $P < 0.0001$). No relationships between TL and glochidia intensities were observed in any other fishes. Infested *G.*

holbrooki tended to be larger in TL than uninfested *G. holbrooki*, appearing as a trend (n = 114, U = 1229.0, P = 0.09). Generally, infestations occurred most often on the fins, but occasionally on other tissues (Table 5) and there were significant differences among species; fins were most heavily infested (χ^2 = 235.7, d.f. = 54, P < 0.001).

Glochidia metamorphosis

Glochidia were released on tan-coloured mucus strings from the exhalent siphons of *W. carteri*. Glochidia were visible through translucent host tissue as brown, sub-triangular shaped shells attached to the fins of infested fish, similar to those obtained from the gill marsupia of adult female *W. carteri* (Fig. 4), with the exception of *T. bostocki*, in which cysts were not translucent, but contained glochidia as reported by Klunzinger *et al*. (2011).

Two species of native euryhaline fishes (*A. suppositus* and *P. olorum*), two species of native freshwater fishes (*N. vittata* and *T. bostocki*) and one alien fish species (*G. holbrooki*) were found to be competent hosts for glochidia of *W. carteri*. Glochidia were encysted on these fishes and underwent metamorphosis to the juvenile mussel stage in the laboratory. Although attachment may have occurred briefly, glochidia were not encapsulated on two other alien fish species (*C. auratus* and *G. brasiliensis*). Time to metamorphosis ranged from 20 to 27 days (Table 6).

Discussion

This study suggests that *W. carteri* is a host generalist for native fishes (*A. suppositus*, *B. porosa*, *G. occidentalis*, *L. wallacei*, *N. vittata*, *P. olorum* and *T. bostocki*), although *B. porosa*, *G. occidentalis* and *L. wallacei* need to be tested to confirm whether they are competent host species. *W. carteri* does not appear to utilise the alien *C. auratus* or *G. brasiliensis*. However,

the alien *G. holbrooki* was confirmed as a competent host. The alien *P. caudimaculatus* may be a potential host based on field studies, but we did not test the species in the laboratory; its distribution in Western Australia is very restricted (Morgan *et al*. 2004).

Other hyriids are also native endemic host generalists (Bonetto and Ezcurra 1963; Humphrey 1984; Widarto 1996; Walker *et al*. 2001).

We found differences in glochidia prevalence among different host species, both overall and in comparisons of different species within localities. Whether this was due to differences in contact rate or in host compatibility is unclear, but is probably a combination of both factors (see Strayer 2008). Contact rate may be influenced by site-specific factors, such as water depth and adult mussel density (Strayer 2008). Although these were not measured in the current study, we did find significant differences in glochidia prevalence on the same (four of six) species of fish in different localities, which suggests that environmental factors influenced contact rate between glochidia of *W. carteri* and its hosts, although larger and more uniform sample sizes would help solidify statistical arguments. Contact rate is also likely to be influenced by host-specific factors, such as habitat utilisation; fishes more closely associated with mussel beds and the sediments may have an increased likelihood of encountering glochidia (Humphrey 1984; Widarto 1996; Strayer 2008). In the current study, prevalence of glochidia infestation was greatest on the two native euryhaline goby species. These are both dense-bodied, negatively buoyant benthic-feeders with modified pelvic fins for clinging to the benthos (Pen *et al*. 1992; Pusey and Bradshaw 1996; Morgan *et al*. 1998, 2011; Allen *et al*. 2002).

The method of glochidia release in *W. carteri* however, may facilitate contact with hosts other than strictly benthic species. The mucus strings had active glochidia attached to them and *W. carteri* has been observed expelling material from its exhalent siphon up to a distance of 5 cm

vertically (Klunzinger 2011). This illustrates the potential of the species to distribute glochidia onto other surfaces in a 'cob-web-like' manner as described by Matteson (1948) and could also target fishes that feed and occupy mid-water and at least very shallow surface areas of streams. Moreover, mid-water species, such as *N. vittata* tend to occupy shallower habitats near sloping river banks, as do *W. carteri*, based on our observations.

Contact rate is unlikely to provide a complete explanation of differences in prevalence of glochidia infestation among host species. *Tandanus bostocki*, which is also a species closely associated with the benthic regions of streams, had only a low to moderate prevalence of infestation. This species also appeared to have reacted to attached glochidia, encapsulated within a much thicker cyst that contained 'pus-like' material, a feature which was absent in other host species. Studies on other Unioniformes show evidence of innate and adaptive immune responses from fish hosts (Bauer and Vogel 1987; Rogers and Dimock 2003; Dodd *et al*. 2005), but we cannot substantiate any claims of immune response in *T. bostocki* or other host species without immunological study.

The effect of host size was evident in *T. bostocki* and *B. porosa* in which infested fishes were larger in TL than uninfested fishes, which is not surprising considering the wide range in TL for these species, which are two of the largest of the region's 11 native freshwater fishes (Morgan *et al*. 1998, 2011). Although introduced fishes such as *G. brasiliensis* and *C. auratus* are deep bodied and can grow to relatively sizeable lengths (≥ 200 mm), none larger than 140 mm were examined in this study and from the almost complete lack of glochidiosis in these fishes, no inference on size can be made. In the case of *G. holbrooki*, larger fish tended to become infested more so than their smaller counterparts, although the trend was not significant. Bauer and Vogel (1987) support the idea that larger fish have a larger fin area and filter more

water through their gills, resulting in a greater chance of glochidia infestation, a concept which supports our observations for *T. bostocki*, *B. porosa* and *G. holbrooki*. Other authors (e.g. Blažek and Gelnar 2006) also reported a positive relationship with host size on glochidia infestation elsewhere. On the contrary, smaller and younger fish sometimes carry greater glochidia loads than older and larger fish, which could be attributed to the immune naivety concept (Young and Williams 1984; Klunzinger *et al*. 2010).

Although previous studies have shown that attachment and metamorphosis of glochidia is more successful on native than on alien fishes (Bauer 1987; Rogers *et al*. 2001; Wächtler *et al*. 2001), successful transformation has sometimes been observed on alien host fishes (Hiscock 1951; Atkins 1979; Walker 1981; Widarto 1996; Watters 1997; Watters and O' Dee 1998; Strayer 2008). The alien *G. holbrooki* had a moderately high prevalence of infestation in the field and exhibited successful metamorphosis in the laboratory, a finding also observed for other hyriids of eastern Australia (Walker 1981; H. Jones pers. comm. 2010). Infested *G. brasiliensis* were never found either in the field or in laboratory trials, although only 20 fish were examined. Although six glochidia were found on a single *C. auratus* in the field, it is uncertain whether they were viable and successful attachment and metamorphosis did not occur in the laboratory. Furthermore, wild *C. auratus* examined in our study appeared to be undergoing fin fragmentation, possibly as a result of glochidia attachment, an observation supported by Rogers-Lowery and Dimock (2006), who found that resistant fish slough epithelial cells in response to glochidia. Laboratory trials (Hiscock 1951; Walker 1981; Widarto 1996) have previously suggested that *C. auratus* and carp (*Cyprinus carpio* Linnaeus, 1758) are unsuitable hosts for Australian Hyriidae, possibly because the mucus produced by their epithelial tissues is too thick

to allow glochidial attachment, even though hyriid glochidia can be induced to attach in a laboratory setting, the glochidia are usually shed within 2-3 hours (Walker 1981).

The time required for transformation of glochidia to the juvenile stage is largely dependent on temperature (Walker 1981; Humphrey 1984; Hastie and Young 2003). In this study, we placed temperature data loggers in a few aquaria to estimate whether transformation time of *W. carteri* glochidia might be affected by temperature during the trial period. In some cases, different species of fishes had to be collected from different systems at different times because there was a difference in glochidia maturity in wild populations from those sites. Fishes from more northerly localities were exposed in mid-September (early-spring) and others from more southerly locations were exposed in mid-October (mid-spring) 2011. Mean temperatures were lower during the September exposure period than those in the October exposure period which may help explain why the former glochidia took 26-27 days to produce juvenile mussels and the latter fishes took 20-21 days to produce juveniles. Because we cannot separate the effects of fish species and post-hatching age of glochidia, we cannot say for certain that differences in transformation time were due to temperature alone, although preliminary findings are suggestive.

The findings of this study have a number of implications for the conservation of *W. carteri* and other Australasian hyriids which remain to be studied. First some fish species may be more important than others in maintaining connectivity between patches of mussels. Although quantifying native fish migration patterns has been accomplished for some species in the south-west of Western Australia (Chapman *et al*. 2006; Beatty *et al*. 2010), more information will be useful in predicting the ability of host fishes to maintain connectivity between populations of *W. carteri.* The widely distributed surface feeding *G. occidentalis*, for example,

is a strong swimmer (Pen and Potter 1991; Keleher 2010) and tends to travel great distances during annual spawning migrations; *G. occidentalis* could therefore be a key host species in maintaining connectivity among mussel populations. The relatively high prevalence of glochidia infestation on the euryhaline species, *A. suppositus*, *L. wallacei* and *P. olorum* could become detrimental to mussel populations in some cases. For example, if these fishes release metamorphosed juvenile mussels after they have migrated into more saline reaches of rivers, the survival of juveniles in these environments would be unlikely assuming *W. carteri* juveniles follow salinity tolerances similar to other Australian juveniles which are intolerant of salinities much greater than 3.0 g L^{-1} (Walker 1981; Widarto 1996). In systems where estuaries connect to freshwater river reaches, the movement patterns of these species between fresh and saline waters are largely unknown and further information is needed to more accurately predict the fate of attached glochidia. Nevertheless, the gobies are common and found well inland from estuaries and are supported by breeding populations in freshwater rivers and lakes of the region (Morgan *et al*. 1998, 2011) and *P. olorum* is commonly reported as hosts for glochidia elsewhere (e.g. Walker 1981; Humphrey 1984; Widarto 1996).

Although host fishes have been identified for glochidia of a number of species in the Northern Hemisphere, the Southern Hemisphere fauna has received far less research attention (Walker *et al*. 2001). Furthermore, the majority of these studies have been on the identification of suitable hosts under controlled laboratory conditions (Strayer 2008), but this study is one of the first to investigate glochidia infestation in wild populations of fishes (also see Kelly and Watters 2010). It confirms fish species vary in their suitability as hosts based on the ability of *W. carteri* to transform to the juvenile mussel stage. Broadly speaking, this study has shown that differences in prevalence and intensity occur that would be due to multiple factors in various

river systems and host species. Further research is necessary to elucidate those factors that may influence the level of glochidia infestation, juvenile survival and preferred habitats and thus recruitment of *W. carteri* and other Unioniformes.

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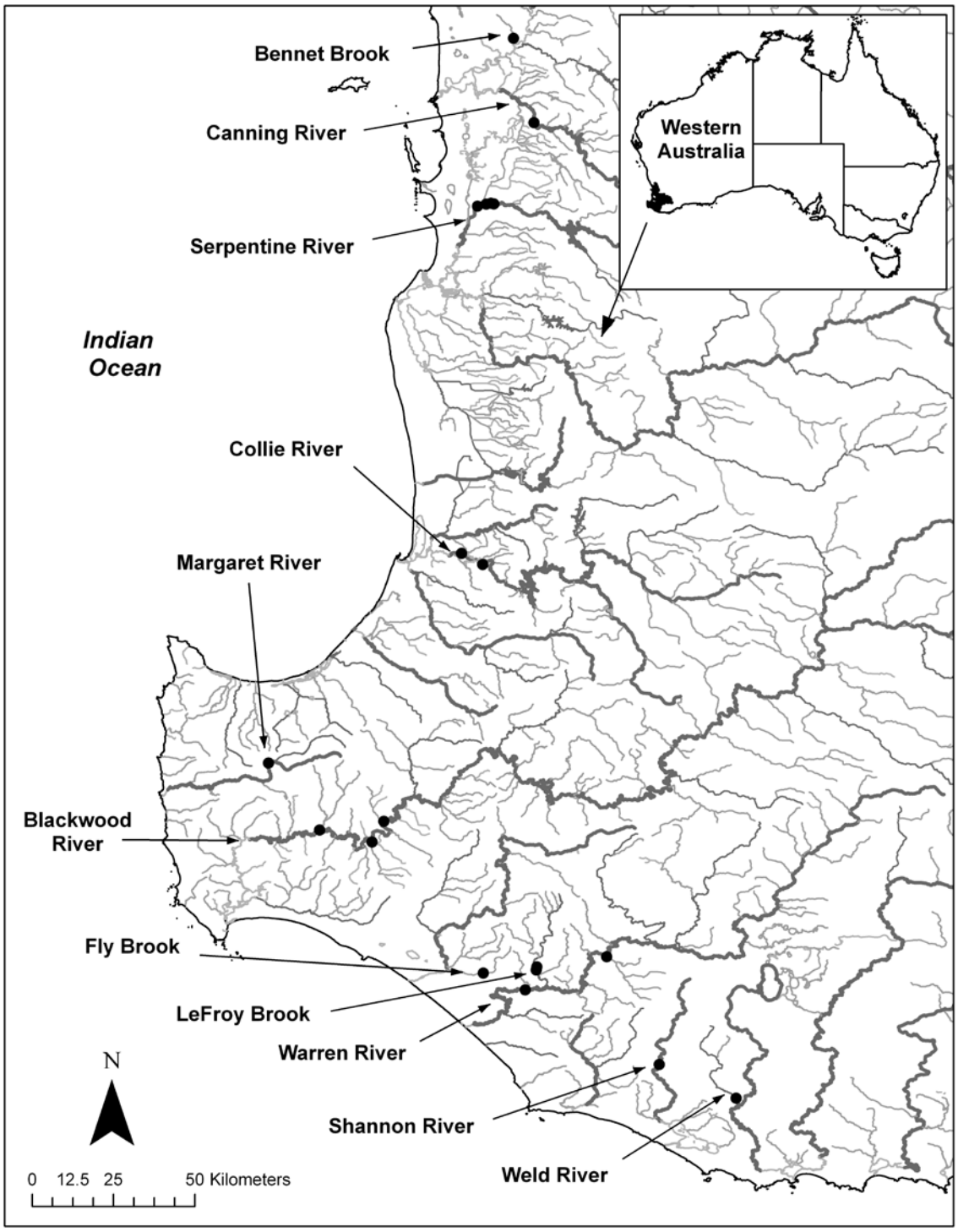

Fig. 1. The South West Coast Drainage Division of Western Australia, showing the locations of sampling sites for fishes examined for glochidia of *Westralunio carteri*.

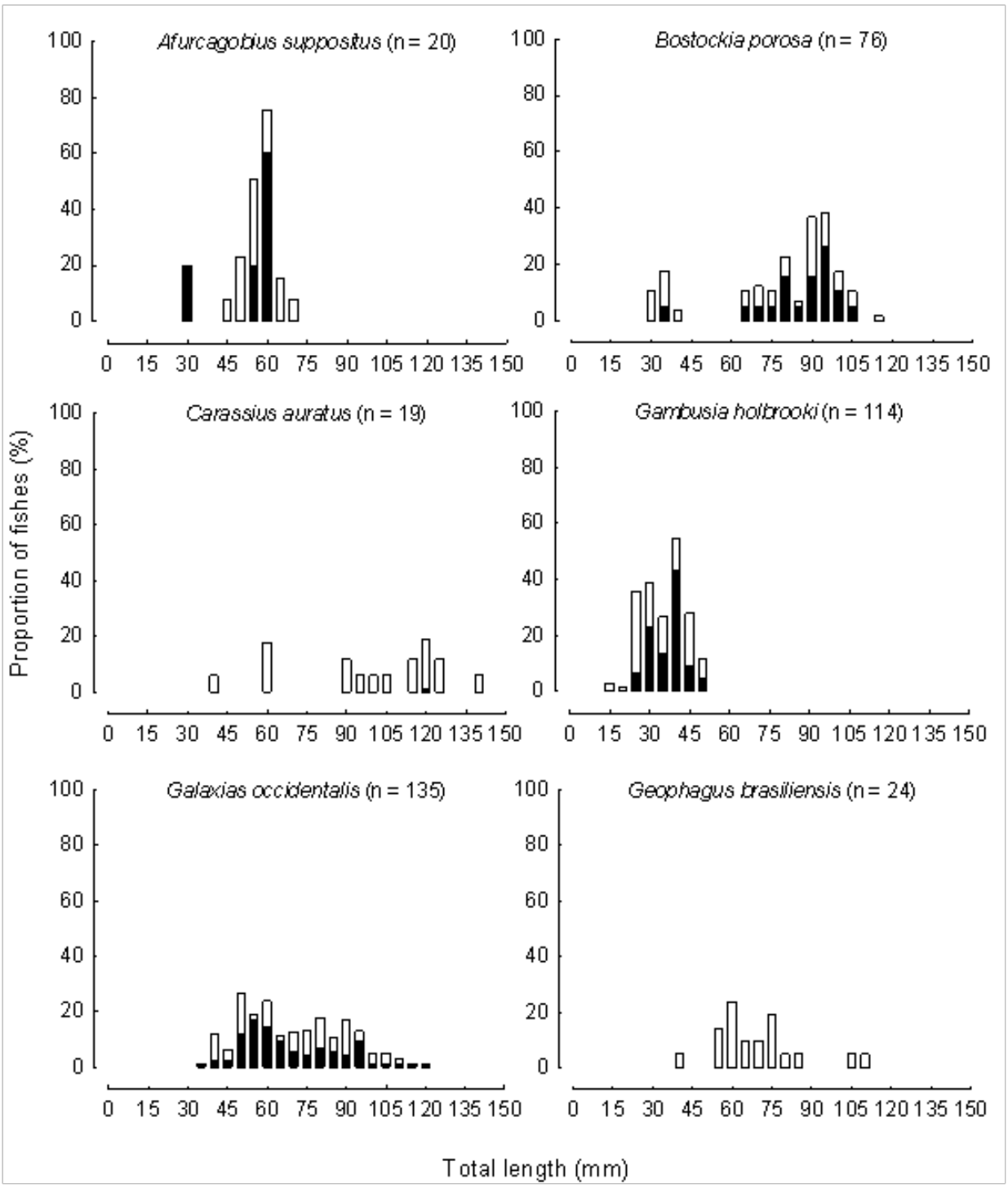

Fig. 2. Length–frequency histograms of six fish species infested (black bars) and uninfested (white bars) with glochidia of *Westralunio carteri*.

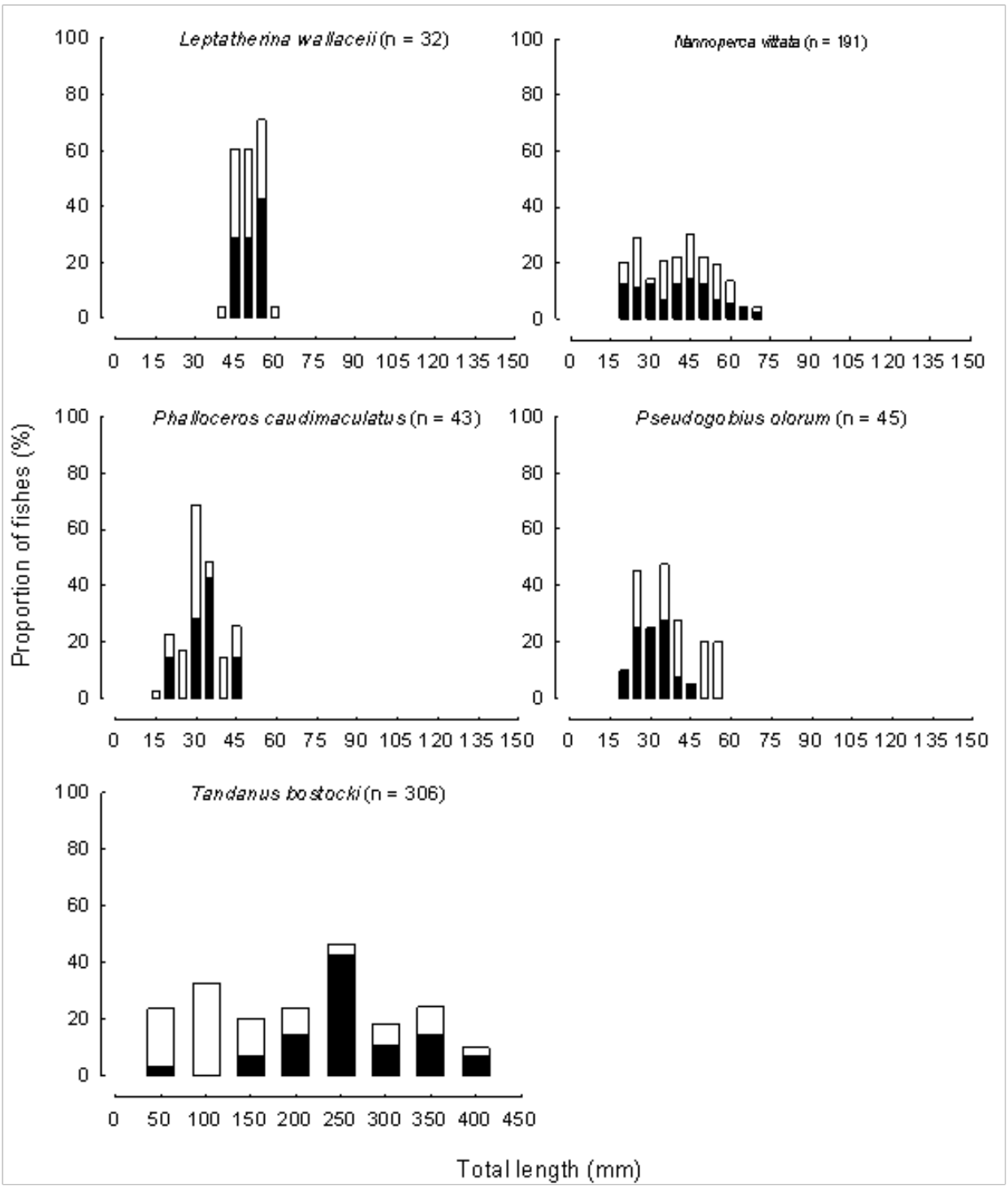

Fig. 3. Length–frequency histograms of five fish species infested (black bars) and uninfested (white bars) with glochidia of *Westralunio carteri*.

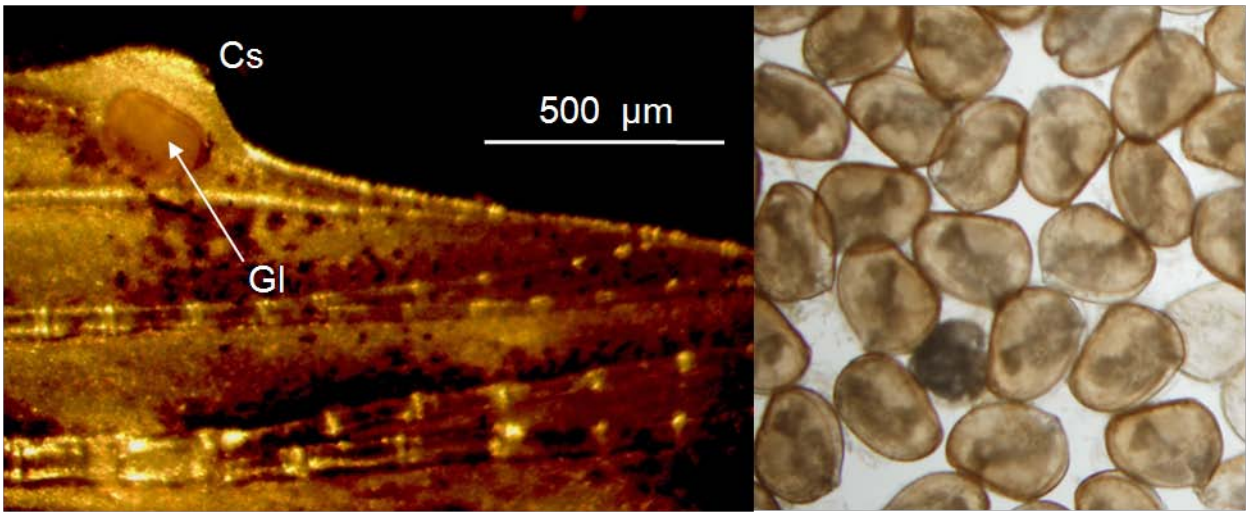

Fig. 4. (left) Glochidia (Gl) attached to and encysted (Cs) on the dorsalfin of a SwanRiver goby, *Pseudogobius olorum*. (right) Free glochidia obtained from the marsupia of an adult freshwater mussel, *Westralunio carteri*.

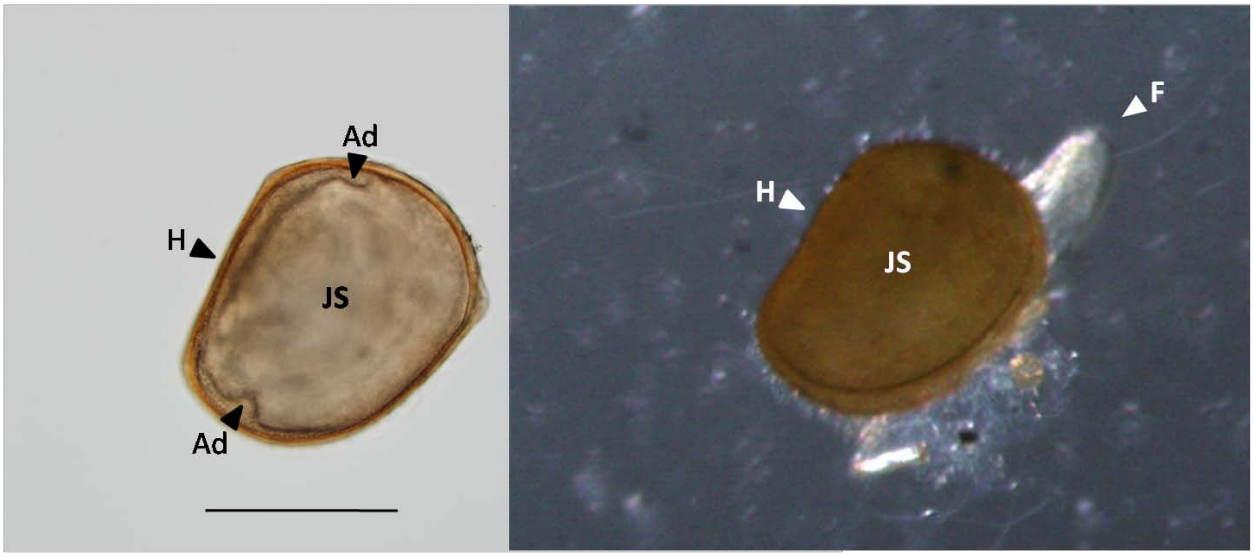

Fig. 5. Juvenile *Westralunio carteri* two days after detachment from a *Pseudogobius olorum*. F, foot; H, hinge ligament; JS, juvenile shell; Ad, rudimentary adductor muscles. Bar = 250 mm.

Table 1. Overall mean glochidia prevalence and intensity (given in bold) in 11 different fish species from the South West Coast Drainage Division of Western Australia, with 95% confidence limits (C.I.) calculated from 2,000 bootstrap replications given in brackets. Reported values were calculated from the overall mean of the mean glochidia prevalences and intensities for each fish species within each sampling site. Fish species are listed in order of greatest to least glochidia prevalence.

Family	Species	No. of individuals examined	Mean Prevalence (%)	Mean Intensity (No. glochidia per infested fish)
Gobiidae	Swan River goby, *Pseudogobius olorum* (Sauvage, 1880)	45	**90.5** (75.9-96.3)	**3.7** (0.0-9.6)
Gobiidae	South-western goby, *Afurcagobius suppositus* (Sauvage, 1880)	20	**75.0** (58.5-96.4)	**2.3** (1.3-3.2)
Galaxiidae	Western minnow, *Galaxias occidentalis* Ogilby, 1899	135	**62.6** (50.4-67.6)	**2.7** (1.3-4.0)
Percichthyidae	Western pygmy perch, *Nannoperca vittata* (Castelnau, 1873)	191	**44.8** (39.4-53.9)	**2.4** (1.2-3.6)
Poeciliidae	Eastern gambusia, *Gambusia holbrooki* (Girard, 1859)†	114	**41.0** (30.4-49.0)	**1.7** (1.1-2.3)
Percichthyidae	Nightfish, *Bostockia porosa* Castelnau, 1873	76	**29.1** (4.8-30.2)	**3.9** (0.0-8.4)
Atherinidae	Western hardyhead, *Leptatherina wallacei* (Prince, Ivantsoff & Potter, 1982)	32	**21.9** (9.3-39.9)	**1.1** (0.8-1.5)
Poeciliidae	One-spot livebearer, *Phallaceros caudimaculatus* (Hensel, 1868)†	43	**18.6** (8.3-33.4)	**1.5** (0.9-2.1)
Plotosidae	Freshwater cobbler, *Tandanus bostocki* Whitley, 1944	306	**9.2** (6.1-12.6)	**2.5** (0.0-8.2)
Cyprinidae	Goldfish, *Carassius auratus* Linnaeus, 1758†	19	**5.3** (0.0-26.0)	**6.0*** ---
Cichlidae	Pearl cichlid, *Geophagus brasiliensis* (Quoy & Gaimard, 1824)†	24	**0.0** ---	---
Total		1005		

†Alien fish species; *Reported intensity is based on one single infested fish; 95% C.I. could not be calculated; Dashes indicate intensity and 95% C.I. could not be determined because no fishes were infested.

Table 2. The number of fishes of each species where 10or more fish were collected, within each river and examined for glochidia of *Westralunio carteri*, the proportion of each species infested by glochidia and the mean intensity (number of glochidia per infested fish) of each species within each river (95% confidence intervals in parentheses). Comparisons were made between species within rivers; differences in prevalence were analysed by Chi-square and differences in intensity by Mann-Whitney or Kruskal-Wallis tests. Test statistics in bold with an asterisk indicate significance at the $P<0.05$ level, with the Bonferroni correction.

River Name (site)	Fish Species	N	Mean Prevalence (%)	Prevalence χ^2 (d.f.)	Mean Intensity	Intensity Statistic (d.f.)
Bennett Brook	*G. occidentalis*	21	100.0 (83.9-100.0)		5.9 (5.0-7.2)	
	P. olorum	17	94.1 (71.3-99.9)	**26.7 (3)***	5.6 (3.8-8.5)	H = 6.6 (2)
	N. vittata	17	35.3 (14.2-61.7)		2.7 (1.0-7.0)	
	G. brasiliensis†	24	0.0 (0.0-14.25)		-	
Canning River	*N. vittata*	15	26.7 (7.8-55.1)		1.8 (1.0-2.5)	
	P. caudimaculatus†	43	18.6 (8.4-33.4)	3.3 (2)	1.5 (1.0-2.0)	U = 13.5 (1)
	G. occidentalis	11	0.0 (0.0-28.5)		-	
Serpentine River						
1 (Bush Forever site 368)	*P. olorum*	25	88.0 (68.8-97.5)		4.4 (3.1-6.4)	
	G. occidentalis	31	83.9 (66.3-94.55)	7.5 (2)	4.5 (3.1-7.0)	***H* = 10.0 (2)***
	G. holbrooki†	48	62.5 (47.4-76.1)		2.0 (1.5-2.6)	
2 (Lowlands Gauging Station)	*G. occidentalis*	18	16.7 (3.6-41.4)	0.2 (1)	1.0*	U = 6.0 (1)
	G. holbrooki†	34	11.8 (3.3-27.5)		1.0*	
Collie River	*A. supposititus*	18	83.3 (58.6-96.4)		2.3 (1.5-3.2)	
	N. vittata	16	37.5 (15.2-64.6)		2.2 (1.0-3.3)	
	G. holbrooki†	18	27.8 (9.7-53.5)	**109.5 (4)***	1.6 (1.0-2.2)	H = 3.3 (4)
	L. wallacei	32	21.9 (9.3-40.0)		1.1 (1.0-1.3)	
	T. bostocki	222	2.3 (0.7-5.2)		1.4 (1.0-1.6)	
Margaret River	*N. vittata*	12	100.0 (73.5-100.0)	3.5 (1)	7.1 (5.1-9.2)	U = 11.0 (1)
	G. occidentalis	20	75.0 (50.9-91.4)		1.5 (1.1-2.0)	
	G. occidentalis	14	50.0 (23.0-77.0)		1.9 (1.1-2.3)	
Shannon River	*B. porosa*	25	32.0 (14.9-53.5)	1.5 (2)	4.1 (2.4-6.3)	H = 4.0 (2)
	N. vittata	13	30.8 (9.1-61.4)		1.5 (1.0-1.8)	

†Alien fish species

Table 3. The number of fishes collected, for each species, within each river where 10or more individuals were captured and examined for glochidia of *Westralunio carteri*, the proportion of each species infested by glochidia and the mean intensity (number of glochidia per infested fish) of each species (95% confidence intervals in parentheses). Comparisons were made between rivers within species; differences in prevalence were analysed by Chi-square and differences in intensity by Mann-Whitney or Kruskal-Wallis tests. Test statistics in bold with an asterisk indicate significance at the $P<0.05$ level, with the Bonferroni correction.

Fish Species	River Name (Site no.)	No. fish examined	Mean Prevalence (%)	Prevalence χ^2 (d.f.)	Mean Intensity	Intensity Statistic (d.f.)
B. porosa	Serpentine River 2	17	35.3 (14.2–61.7)	0.1 (2)	2.3 (1.3-3.0)	H = 14.5 (2)
	Shannon River	25	32.0 (14.9-53.5)		4.1 (2.5-6.5)	
	Serpentine River 1	48	64.0 (49.2-77.1)		2.0 (1.6-2.6)	
G. holbrooki†	Collie River	18	27.8 (9.7-53.5)	**24.4 (1)***	1.6 (1.0-2.2)	U = 3.0 (1)
	Serpentine River 2	34	11.8 (3.3-27.5)		1.0*	
G. occidentalis	Bennett Brook	21	100.0 (83.9-100.0)		5.9 (4.9-7.3)	
	Serpentine River 1	31	85.7 (69.7-95.2)		1.9 (3.1-6.4)	
	Margaret River	20	75.0 (50.9-91.4)	**58.2 (4)***	1.5 (1.1-1.9)	***H* = 31.0 (3)***
	Serpentine River 2	18	16.7 (3.6-41.4)		4.4*	
	Canning River	11	0.0 (0.0-28.5)		-	
N. vittata	Margaret River	12	100.0 (73.5-100.0)		7.1 (5.1-9.1)	
	Fly Brook	40	42.5 (27.0-59.1)		2.1 (1.5-3.4)	
	Lefroy Brook	40	42.5 (27.0-59.1)		1.8 (1.2-3.1)	
	Collie River	16	37.5 (15.2-64.6)	**21.5 (6)***	2.2 (1.0-3.3)	***H* = 22.2 (6)***
	Bennett Brook	17	35.3 (14.2-61.7)		2.7 (1.0-5.8)	
	Shannon River	13	30.8 (9.1-61.4)		1.5 (1.0-1.8)	
	Canning River	15	26.7 (7.8-55.1)		1.8 (1.0-2.5)	
P. olorum	Bennett Brook	17	94.1 (71.3-99.9)	0.4 (1)	5.6 (3.7-8.8)	U = 152.0 (1)
	Serpentine River 1	25	88.0 (68.8-97.5)		4.4 (3.1-6.4)	
T. bostocki	Blackwood River	84	27.4 (18.2-38.2)	**46.3 (1)***	4.2 (2.3-8.6)	U = 46.5 (1)
	Collie River	222	2.3 (0.7-5.2)		1.4 (1.0-1.6)	

†Alien fish species

Table 4. Comparison of median total lengths (TL) of fishes infested and uninfested with glochidia of *Westralunio carteri*. Differences are significant at the $P<0.05$ level and trends appear at the $P<0.10$ level, given in bold. Species are listed in alphabetical order.

Species	TL infested (mm)	TL uninfested (mm)	*U*-test	*P*-value
A. suppositus	57.0	61.0	30.0	0.84
B. porosa	**92.0**	**80.0**	**373.5**	**0.04**
C. auratus†	120.0	75.0	4.5	0.50
G. occidentalis	64.5	75.0	1914.5	0.52
G. holbrooki†	**40.0**	**35.0**	**1229.0**	**0.09**
G. brasiliensis†	---	69.0	---	---
L. wallacei	54.0	51.0	73.5	0.54
N. vittata	43.0	43.0	4356.0	0.63
P. caudimaculatus†	37.5	31.0	99.5	0.21
P. olorum	37.0	42.0	64.5	0.21
T. bostocki	**272.0**	**145.0**	**1799.0**	**<0.001**

†Alien fish species

Table 5. The number of fish with glochidia in each infestation site within each species. Species and infestation sites are significantly related (χ^2 = 235.728, d.f. = 54, $P < 0.001$). *Carassius auratus, L. wallacei* and *P. caudimaculatus* were excluded from statistical analysis due to a low number of infested individuals ($n < 10$), but shown here for comparative purposes.

		Infestation site											
Species	No. of infested individuals	eye	mouth	opercula	gills	body	pectoral fins	pelvic fins	anal fins	dorso-caudal fin	1st dorsal fin	2nd dorsal fin	caudal fin
A. suppositus	15	-	-	-	-	-	6	6	4	1	-	-	5
B. porosa	19	-	-	-	-	-	14	7	4	5	3	X	10
C. auratus†	1	-	-	-	-	-	-	-	-	-	-	X	1
G. occidentalis	80	-	6	4	-	-	50	26	22	X	36	X	19
G. holbrooki†	45	1	-	-	-	-	2	3	14	X	13	X	28
L. wallacei	7	-	-	-	-	-	4	2	2	X	-	X	-
N. vittata	89	1	2	4	-	2	25	19	32	X	43	16	29
P. olorum	40	-	-	2	-	-	32	14	10	X	11	13	11
P. caudimaculatus†	8	-	-	-	1	-	1	3	1	3	2	X	X
*T. bostocki**	28	-	-	1	1	-	1	-	1	22	2	X	X

†Alien fish species; *A sub-sample of five *T. bostocki* was examined for glochidia on all tissues in the laboratory, but 23 fishes were examined for glochidia on the fins only in the field; X = anatomical feature not present.

Table 6. Metamorphosis from the glochidia to the juvenile stage of *Westralunio carteri* exposed to potential host fish species under controlled laboratory conditions. Rows are arranged alphabetically by fish species. Dashes indicate metamorphosis was not observed.

Fish species	No. individuals exposed	Time to metamorphosis (d)
A. suppositus ‡	17	20
C. auratus †	26	--
G. holbrooki †	15	21
G. brasiliensis †	5	--
N. vittata ⌂	20	21-27
P. olorum ‡	10	26
T. bostocki ⌂	18	21

†Alien species; ‡Native euryhaline species; ⌂Native freshwater species.