

Ecological response of an estuarine atherinid to secondary salinisation in south-western Australia

MAHMOUD RASHNAVADI^{1,2}, ALAN J LYMBERY^{1*}, STEPHEN J BEATTY¹
& DAVID L MORGAN¹

¹ Freshwater Fish Group & Fish Health Unit, Centre for Fish & Fisheries Research, School of Veterinary and Life Sciences, Murdoch University, WA, 6150, Australia

² School of Veterinary Medicine, Ilam University, Ilam, Iran

* ✉ a.lymbery@murdoch.edu.au

Secondary salinisation of rivers is a major threat to aquatic ecosystems, particularly in the south-west of Western Australia. Salinisation has changed the structure of freshwater fish assemblages in the south-west, with many estuarine species now being found well inland and outside of their historic range. The current study aimed to determine whether plasticity in life-history traits, in addition to physiological tolerance, may facilitate competitive advantage of estuarine invaders in secondarily salinised systems. *Leptatherina wallacei* is a teleost endemic to the region and was historically found in the upper reaches of estuaries and several coastal lakes. Several life-history, morphological and ecological traits of *L. wallacei*, in habitats of differing salinities in the Blackwood River were determined. Our study reveals that, complementing its broad physiological tolerance to salinity, the species has flexible life-history traits, such as a protracted spawning period, early maturation, fast growth and a broad diet. Its spawning period and growth rate also differed among populations in different sections of the river, with those in the more salinised upper catchment spawning at a similar time to estuarine fish, whereas in the less salinised lower catchment, spawning was delayed for a season, reproductive activity was reduced and growth was faster. The prevalence of macroparasites was significantly greater in fish populations in the lower catchment than in the upper catchment, and this may have contributed to reduced reproductive activity. We suggest that phenotypic plasticity in life-history traits, along with broad physiological tolerance to elevated salinity and an escape from parasitic infection, has facilitated a competitive advantage to the species that has enabled it to become a dominant fish in secondarily salinised habitats.

KEYWORDS: salinity, Blackwood River, *Leptatherina wallacei*, life-history, diet, parasitism

INTRODUCTION

Secondary salinisation of rivers is a major global threat to aquatic ecosystems (Cañedo-Argüelles *et al.* 2013). It has ecological impacts on multiple scales including the individual, species and community level. Fish assemblages, in particular, are often dramatically affected by the salinisation of rivers (Higgins & Wilde 2005; Hoagstrom 2009). Differences in fish distributions in relation to salinity reflect differences in historical distribution patterns and in the ability of species to respond at the level of the individual (through phenotypic plasticity) or the population (through adaptation) to secondary salinisation. Salinisation imposes stresses on freshwater organisms both directly, for example on physiological functioning, and indirectly, such as through food availability or competitive interactions (Nielsen *et al.* 2003a).

Aquatic ecosystems in Western Australia have been severely impacted by secondary salinisation. From a fish perspective, most of the impacted areas fall within the Southwestern Province (Morgan *et al.* 2003; Beatty *et al.* 2011), although some rivers within the Pilbara Province have also been affected (Morgan & Gill 2004; Unmack 2013). Secondary salinisation within the Southwestern Province is a consequence of wide scale clearing of native

vegetation during the last century and reduced rainfall. As a result, only ~44% of flow in the largest 30 rivers in the region is now fresh (Mayer *et al.* 2005). While the structural changes in fish communities in the Province are now well understood (Morgan *et al.* 2003; Beatty *et al.* 2011), little is known of how secondary salinisation has driven shifts in life-history, morphology and ecology of fishes in this region or indeed elsewhere.

Within the Southwestern Province, secondary salinisation has led to a number of species that are typically found in estuaries now being found well inland and outside of their historic range (Morgan *et al.* 1998, 2003; Beatty *et al.* 2011). One of these species is the western hardyhead (*Leptatherina wallacei*) (Atherinidae), which is endemic to the region and is widely distributed from the Hill River in the north to the east of Esperance (Figure 1), where it is often abundant in rivers, some saline lakes, a few freshwater lakes and most estuaries (Morgan *et al.* 1998; Potter & Hyndes 1999; Morgan & Gill 2004). Within the Blackwood River, the largest river by discharge in the region, *L. wallacei* is the second most abundant fish species in the estuary (Valesini *et al.* 1997) and also contributes to almost 25% of all fish abundance upstream of the estuary; presumably a consequence of secondary salinisation within this catchment, given that records of the species in the riverine sections of the catchment have been relatively recent (Morgan *et al.* 2003). Therefore, *L. wallacei* represents an ideal model

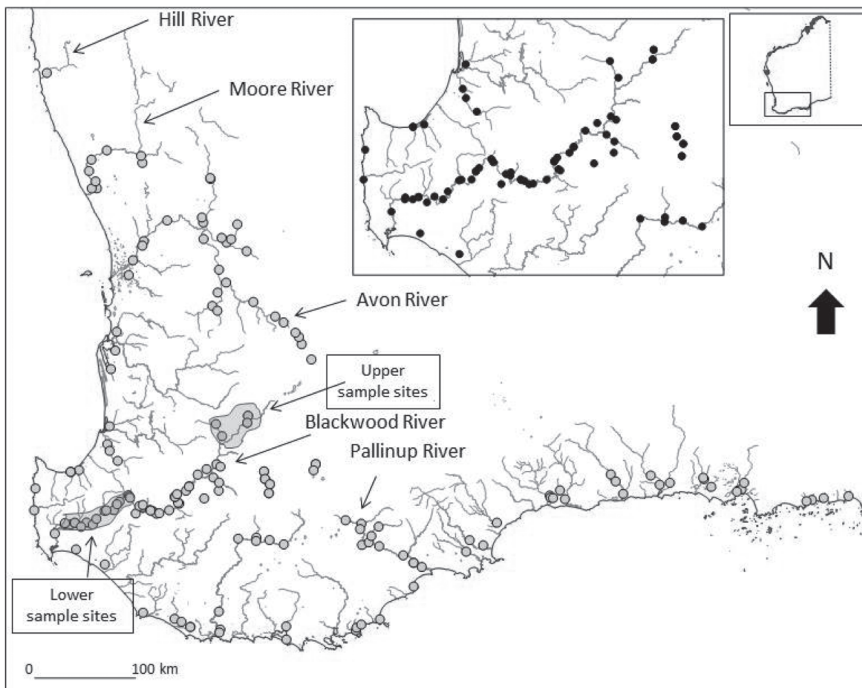


Figure 1 Distribution of *Leptatherina wallacei*, including sample site locations in the upper and lower catchment of the Blackwood River (distributional data from Morgan *et al.* 1998, 2003, 2006).

species upon which to conduct a field-based investigation of how flexibility in life-history, morphology, and ecological traits may help facilitate the exploitation of secondarily salinised systems.

Although the diet, reproductive period and longevity of *L. wallacei* have been elucidated within an estuarine environment (the Swan River Estuary; see Prince *et al.* 1982; Prince & Potter 1983), there have been no ecological studies within non-estuarine environments for this species. The aim of the current study was to quantify variability in the life-history, morphology and ecology of *L. wallacei* in habitats of varying salinity in the Blackwood River. We hypothesised that there would be considerable differences in traits between these populations, that may help explain the successful inland colonisation by *L. wallacei* of secondarily salinised systems.

MATERIALS AND METHODS

Sampling protocol

The catchment of the Blackwood River covers an area of more than 21,900 km² in the Southwestern Province. Increasing dryland salinity in the catchment has led to salinisation of the Blackwood River. Water salinity varies from >30 ppt in the upper catchment to approximately 1.5–4 ppt in the lower reaches of the main channel, which receives low salinity groundwater, and less than 0.5 ppt in forested tributaries which feed into the lower main channel (Morgan *et al.* 2003; Mayer *et al.* 2005; Beatty *et al.* 2011).

Four sites were selected in each of the highly salinised main channel of the upper catchment and the less salinised main channel of the lower catchment of the Blackwood River, and three sites were selected in freshwater tributaries in the lower catchment to assess

the ecology of *L. wallacei* (Figure 1). Sites were sampled at approximately monthly intervals over a 12 month period in 2006. As the target species was not collected from the tributary sites, for a comparison of the biology of *L. wallacei*, sites were grouped into upper catchment or lower catchment (main channel). For some comparisons, sampling dates were grouped into wet season (June to October) or dry season (November to April).

Juvenile and adult fishes were captured at each locality using seine nets (10 or 15 m long, fishing to a depth of 2 m) or fyke nets (11.2 m wide with two 5 m wings and a 1.2 m wide mouth fishing to a depth of 0.8 m, and a 5 m pocket with two internal funnels); all nets being of 2 or 3 mm woven mesh. Collected fishes were identified to species in the field using Allen *et al.* (2002), counted and all native non-target species returned immediately to the water. Larval, juvenile or adult fish were euthanised in an ice slurry and preserved in 100% ethanol for subsequent determination of life-history traits.

Size, morphology, age and reproductive status

Preserved fish were weighed to the nearest 1 mg and the total length (TL) and standard length (SL) measured to the nearest 1 mm. Fish were then dissected, sexed and the gonads removed and weighed to the nearest 1 mg. The gonadosomatic index (GSI) (= gonad weight/body weight × 100) was calculated for a subsample of fish from both upper and lower catchment sites. Sample sizes for GSI studies from the upper catchment sites across months were n = 42 (October), n = 46 (November), n = 19 (February) and n = 33 (March); and for the lower catchment sites were n = 22 (October), n = 10 (November), n = 4 (December), n = 22 (February) and n = 43 (March). At the same time, gonads were assigned, on the basis of their morphological appearance, to one of the following seven maturity stages (Laevastu 1965): I-virgin; II-maturing virgin or recovering spent; III-developing; IV-

developed; V-mature or gravid; VI-spawning; or VII-spent.

Sagittal otoliths are widely used to provide age estimates of various fish species, including atherinids (Campana 2001). The highly seasonal Mediterranean climatic regime in south-western Australia has resulted in most of the regions' freshwater fish species having otoliths with clearly defined annuli that are laid down once each year (Pen & Potter 1991; Morgan *et al.* 1995, 2000; Morgan 2003). Therefore, although previous age analysis for *L. wallacei* was based on length-frequency distributions (Prince & Potter 1983), we have provisionally assumed that otoliths are appropriate for ageing the fish in this study, although we note that the formation of annuli on the otoliths has not yet been validated for the species. The sagittal otoliths of each fish were removed, cleaned and stored in gelatin capsules. To count the number of translucent zones, they were later placed in methyl salicylate in a black dish and examined under a dissecting microscope at 40x magnification using reflected light. The approximate age of fish was calculated by using a birth date of 1st October for fish from the upper catchment and a birth date of 1st January assigned for fish from the lower catchment (see results for rationale). von Bertalanffy growth curves were then fitted to each of the fish at an estimated age from each of the upper and lower reaches of the catchment. These curves were fitted with SigmaPlot using the growth equation $L_t = L_\infty [1 - e^{-K(t-t_0)}]$, where L_t is the length at age t (years), L_∞ is the asymptotic length, K is the growth coefficient, and t_0 is the hypothetical age at which the fish would have zero length. A likelihood ratio test (Cerrato 1990) was employed to determine whether the growth curves of *L. wallacei* differed between the upper and lower catchment.

Differences in sex ratio of fish populations between catchments were compared by Fisher exact test. The effect of sex and catchment region on fish TL was tested by a two-factor analysis of variance, while the effect of these variables on fish weight was tested by a two factor analysis of covariance, with TL as a covariate. All statistical tests were conducted in JMP v4 (SAS Institute, Cary, NC) and for parametric tests, variables were tested for normality and homogeneity of variances prior to analysis.

Dietary analysis

In order to determine the diet of *L. wallacei*, which lacks a stomach, the contents of the anterior half of the intestine were examined under a dissecting microscope. All matter was identified to the lowest possible taxonomic level using Davis & Christides (1997). Gut fullness was also estimated on a scale from 0 to 10, with 0 being an empty intestine, 8 being full and 10 being a fully distended intestine. Contents were analysed using the percentage frequency of occurrence and the percentage of volumetric contribution of each dietary item. The percentage frequency of occurrence is the proportion of the total number of guts that contain a particular prey type, while the percentage of volumetric contribution is the proportion of each prey type to the overall gut contents of each fish, calculated using the points method (Hynes 1950; Hyslop 1980).

Similarities in diet among individual fish were estimated from percentage volumetric data using the Bray-Curtis similarity coefficient (Bray & Curtis 1975). Percentage volumetric data were square root transformed prior to analysis to downweight the influence of dominant species. The significance of differences in diet among fish from different catchment areas or seasons was tested by a pseudo two-way analysis of variance procedure applied to the pairwise similarity matrix (PERMANOVA, implemented using the computer packages PRIMER 6.0 and PERMANOVA+; Clarke & Gorley 2006; Anderson *et al.* 2008). The contribution of individual prey items to the similarity within groups or the dissimilarity among groups was assessed by averaging the Bray-Curtis similarity (or dissimilarity) term for each species over all pairwise fish combinations, using the SIMPER procedure in PRIMER 6.0 (Clarke & Gorley 2006).

Fluctuating asymmetry

Fluctuating asymmetry (FA) is often used as an indicator of environmental stress during development (see Allenbach *et al.* 1999; Almeida *et al.* 2008). FA values were calculated from paired measurements of the number of fin rays on the right and left pectoral and pelvic fins, and length, width, perimeter and area of the right and left sagittal otoliths for each fish. Initially, directional asymmetry was tested by the skewness and anti-symmetry by the kurtosis of signed differences of right and left variable distributions. If the signed variable distributions were not significantly skewed, bimodal or platykurtic, FA was calculated as the unsigned difference between right and left measurements (this corresponds to the FA1 measure of Palmer (1994)).

Relationships between FA and character size were tested with Pearson product-moment correlations and differences in FA values between catchments, seasons, fish age classes and rates of parasitism were tested by analyses of variance. All statistical tests were conducted in JMP v4 (SAS Institute, Cary, NC). All variables were tested for normality and homogeneity of variances prior to analysis. Tests of differences in FA using different morphological variables were regarded as multiple tests of the same hypothesis, and therefore a Bonferroni correction was applied to ensure an experiment-wide error rate of 5%.

Parasitism

Parasitic organisms have recently been shown to be highly sensitive indicators of environmental stress and examining how parasite populations vary across environmental gradients may provide valuable information on the ecological impact of pollutants (Marcogliese 2005; Lafferty 2008). During morphological measurements and dissection, each fish was examined for macroparasites (helminths and arthropods) using methods described in Lymbery *et al.* (2010). All detected parasites were removed, photographed and then preserved in 70% ethanol. If necessary, specimens were cleared in lactophenol and prepared as whole mounts for microscopic identification. Parasite specimens were identified to the lowest taxon possible using standard taxonomic keys.

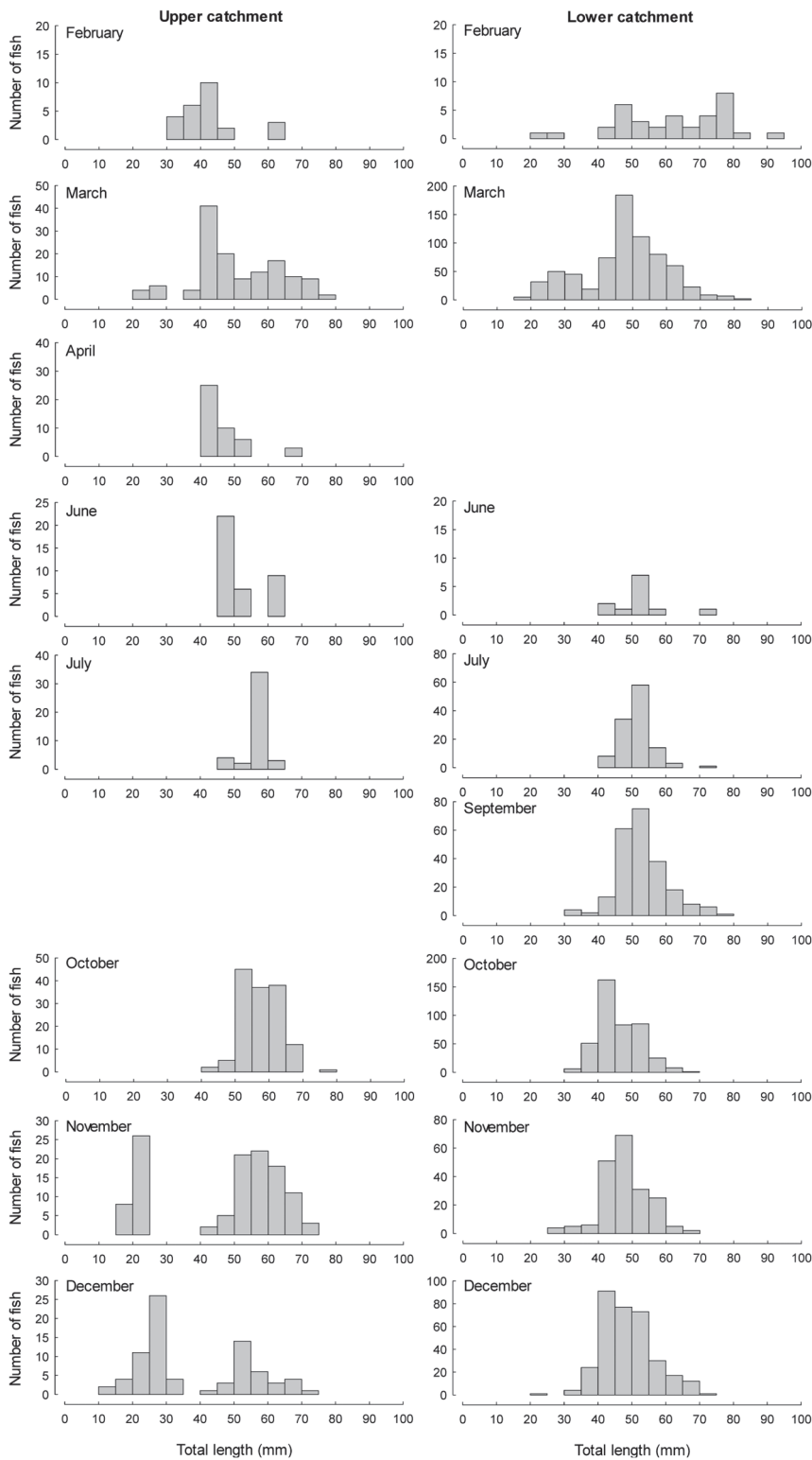


Figure 2 Length-frequency histograms of *Leptatherina wallacei* captured during this study from the upper (left) and lower (right) catchment of the Blackwood River.

From all fish sampled and for fish from different parts of the catchment (i.e. upper or lower) or seasons, the overall prevalence of parasitic infection (i.e. the proportion of fish infected with any species of parasite) as well as prevalence of each parasite species, with 95% confidence intervals calculated assuming a binomial distribution, were determined using the software Quantitative Parasitology 3.0 (Rózsa *et al.* 2000). Mean

intensity (i.e. the number of parasites per infected fish), with 95% bootstrap confidence intervals, was also calculated for each parasite species using Quantitative Parasitology 3.0 (Rózsa *et al.* 2000). Differences in parasite prevalence among groups were investigated using Fisher exact tests and differences in mean intensity using the bootstrap.

RESULTS

Fish distribution

Leptatherina wallacei was found throughout the year in both main channel upper catchment and main channel lower catchment sites, but was never recorded from tributary sites. Over all sampling sites and times, *L. wallacei* was the second most abundant fish species found in the upper catchment, making up 14% of the total fish catch (total $n = 11,798$), while in the lower catchment it was the third most abundant species, making up 22% of the total fish catch (total $n = 14,931$). In addition to *L. wallacei*, three other fish species (the native estuarine species *Pseudogobius olorum*, the native freshwater *Galaxias occidentalis* and the alien *Gambusia holbrooki*) were found in upper catchment sites, while in addition to *P. olorum*, *G. occidentalis* and *G. holbrooki*, a number of other fish species (the native estuarine *Afurcagobius suppositus*, and the native freshwater *Bostockia porosa*, *Nannoperca vittata*, *Nannatherina balstoni* and *Tandanus bostocki*) were found in lower catchment sites.

Size, age and growth rates

Based on the number of translucent zones on the otoliths, *L. wallacei* of three different age classes (0+, 1+ or 2+) were found in the study; the largest fish being 90 mm TL and in its third year of life. From the von Bertalanffy growth equations (where $L_{\infty} = 75.12$ and 83.83 for fish from the upper and lower catchments, respectively, $t_0 = -0.6785$ and 0.1463 , respectively and $K = 0.9659$ and 1.8335 , respectively) on average, fish in the upper catchment attained ~ 60 mm TL at the end of their first year of life and ~ 69.5 mm TL at the end of their second year of life, while fish in the lower catchment attained ~ 66 and 81 mm TL at these respective ages. These growth curves were found to differ significantly ($p > 0.05$) between fish in the upper and lower catchments.

Over all sampling sites and dates, there was a strongly female-biased sex ratio (1.63 female: 1 male, $n = 776$). This was more pronounced in lower catchment sites than in upper catchment sites (73% versus 56% female; significantly different by Fisher exact test, $p = 0.01$). There was a strong positive relationship between TL and weight for both female and male fish. Fish TL was significantly affected by sex ($F_{1,772} = 16.33$, $p < 0.0001$), but not by position in the catchment ($F_{1,772} = 2.55$, $p = 0.11$) or the interaction of sex and catchment ($F_{1,772} = 0.34$, $p = 0.56$). Female fish (mean TL = 60.2 ± 0.6 mm) were, on average, longer than male fish (mean TL = 54.5 ± 1.2 mm). Fish weight, with length as a covariate, was significantly affected by position in the catchment ($F_{1,772} = 6.62$, $P = 0.01$), but not by sex or the interaction of sex and position in the catchment. Fish from lower catchment sites (mean weight = 1.11 ± 0.03 g) were significantly heavier than fish from upper catchment sites (mean weight = 1.03 ± 0.01 g).

The length-frequency distributions of *L. wallacei* showed different trends in upper and lower catchment sites (Figure 2). In the upper catchment, new recruits (15–24 mm TL) first appeared in late spring (November) and grew rapidly over summer to reach 40–44 mm TL by autumn (March). Large fish (>50 mm TL) persisted throughout the year but appeared to decline in number

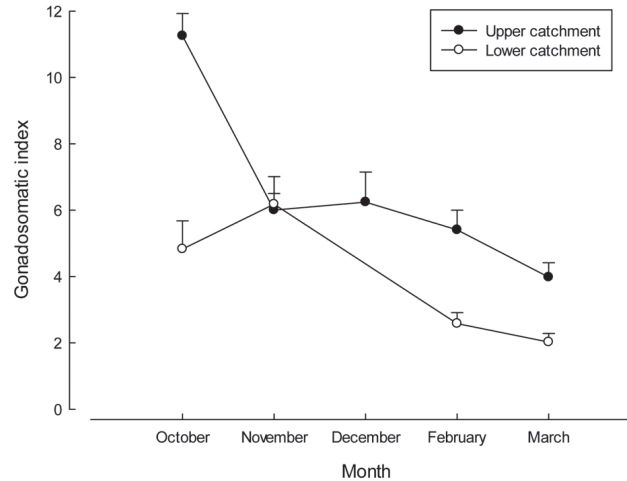


Figure 3 Gonadosomatic index (95% CI) of female *Leptatherina wallacei* from upper (black circles) and lower (white circles) catchment of the Blackwood River between mid-spring and early-autumn.

post spawning. By contrast, in lower catchment sites new recruits (<20 mm TL) were not detected until autumn (March) and appeared to grow slowly through winter and spring, with numbers in the 40–44 mm TL class peaking in October. There was no dramatic reduction in the numbers of large fish (>50 mm TL) after the appearance of these recruits.

Gonadal development

There was a marked difference in the timing and extent of gonadal development between female fish in the upper and lower catchments. In upper catchment sites mean GSI's of female *L. wallacei* peaked in mid-spring when they reached 11.25, before declining to ~ 6.0 in both November and December, 5.4 in February, and 4.0 in March (Figure 3). In lower catchment sites mean GSI's peaked in November at 6.18, before declining to less than 3.0 in February and March (Figure 3). In comparing gonadal development stage between fish in mid-spring (October), those in the upper catchment were mature (stage V), spawning (stage VI) or spent (stage VII), while those in the lower catchment were developing or maturing, with less than 15% having attained maturity (stage V), and none had spawned or were spent (Figure 4). Spawning and spent fish were first recorded in the lower catchment in late summer and at this time fish in the upper catchment exhibited a full range of gonadal development stages, indicating that the spawning period is more protracted for fish in the upper catchment (Figure 4). The smallest total length at which a fish with stage VI or VII gonads was found, was 47 mm TL in the upper catchment and 48 mm TL in the lower catchment.

Fish diets

Leptatherina wallacei was found to be carnivorous, with a wide range of invertebrate taxa consumed (Table 1). The most common prey were of the insect orders Diptera and Trichoptera and the crustacean orders Amphipoda, Copepoda and Ostracoda. There was a significant effect of catchment ($F_{1,251} = 22.80$, $p = 0.001$), season ($F_{1,251} = 8.65$,

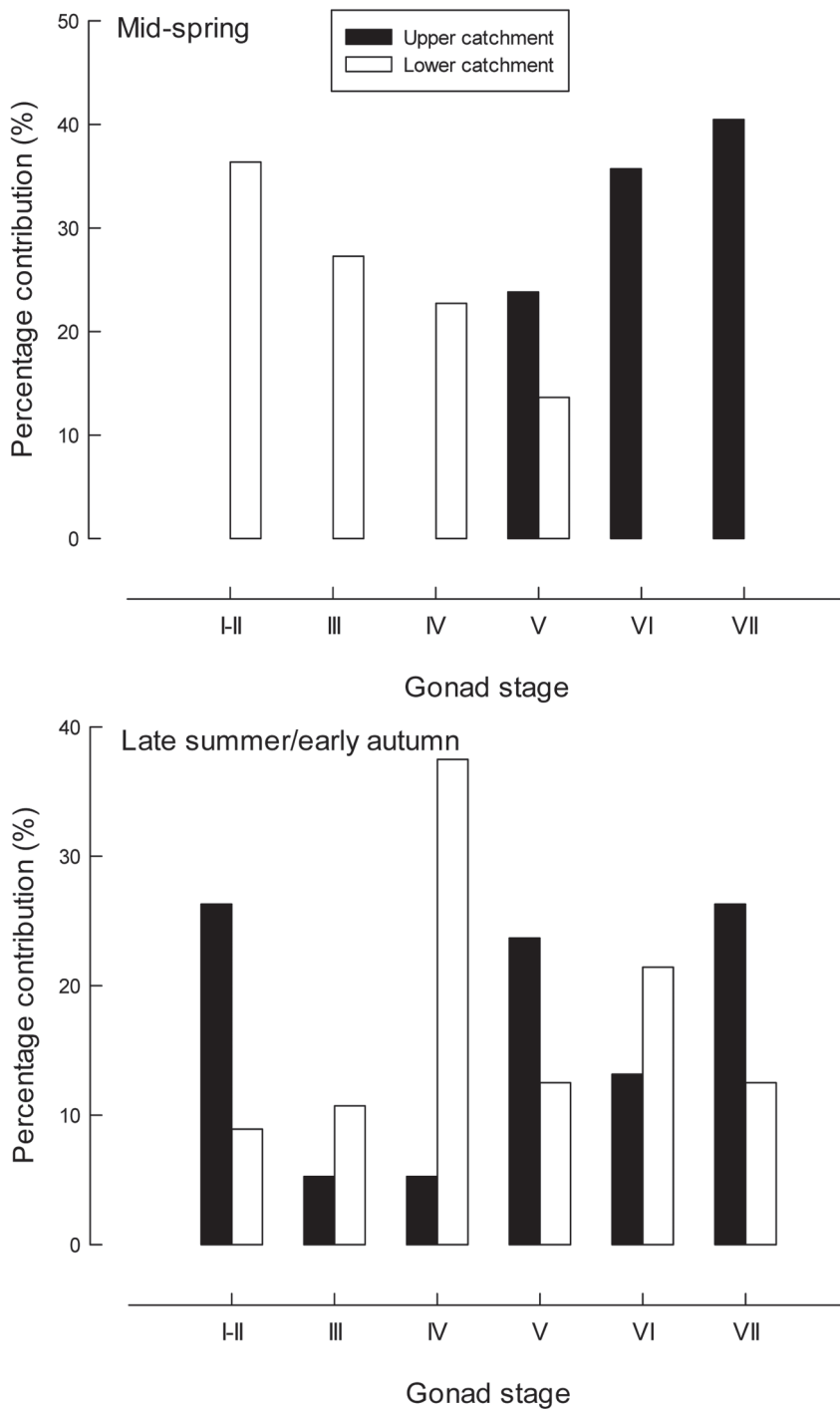


Figure 4 Percentage contributions of different gonadal stages for female *Leptatherina wallacei* from the upper (black bars) and lower (white bars) catchment of the Blackwood River in mid-spring and late summer/early autumn.

$p = 0.001$) and their interaction ($F_{1,251} = 5.22, p = 0.001$) on prey consumed. SIMPER analysis found that in the dry season, 75% of differences in diet between fish in different parts of the catchment were explained by a relatively greater abundance of trichopteran larvae and hymenopterans in the diets of fish from lower catchment sites, and a relatively greater abundance of dipteran larvae, amphipods and ostracods in the diets of fish from upper catchment sites (Table 2). In the wet season, almost 90% of the differences between catchments were explained by a greater abundance of trichopteran adults

and larvae, and dipteran larvae in the diets of fish from lower catchment sites, and a relatively greater abundance of amphipods and copepods in the diets of fish from upper catchment sites (Tables 1, 2).

Fluctuating asymmetry

All fin and otolith traits were variable, with ranges of 11–13 for pectoral fin ray number, 5–6 for pelvic fin ray number, 1.6–2.2 mm for otolith length, 1.4–2 mm for otolith width, 2.5–4.1 mm² for otolith perimeter and 2–2.8 mm for otolith area. The distributions of all right-left

Table 1 Proportional contribution (by volume) of different prey taxa in the diet of *Leptatherina wallacei* from the upper and lower catchment of the Blackwood River in wet and dry seasons.

Prey type	Upper catchment		Lower catchment	
	Dry season (n = 128)	Wet season (n = 73)	Dry season (n = 47)	Wet season (n = 23)
Amphipoda	0.21	0.26	–	–
Ostracoda	0.21	0.05	–	–
Copepoda	0.06	0.24	–	0.06
Cladocera	0.06	0.01	–	0.02
Diptera larvae	0.35	0.39	0.11	0.41
Diptera adults	0.06	0.03	0.06	0.04
Hymenoptera	–	–	0.12	–
Coleoptera larvae	–	0.01	0.04	–
Coleoptera adults	0.02	–	0.09	–
Odonata	0.01	–	–	–
Trichoptera larvae	0.02	–	0.54	0.26
Trichoptera adults	–	–	–	0.19
Ephemeroptera	–	–	–	0.01
Arachnida	–	–	0.02	–
Decapoda	0.06	–	–	–
Fish larvae	0.02	–	–	–
Plant material	–	–	0.02	–

Table 2 Mean abundance of different prey taxa in the diet of *Leptatherina wallacei* from upper and lower catchment sites in each season, and cumulative percentage of dissimilarity between these site explained by these taxa (calculated from one-way SIMPER analyses, because of significant interaction between catchment and season). Taxa are arranged in order of decreasing contribution to dissimilarity, and taxa which contributed less than 1% to the cumulative percentage of dissimilarity have been omitted.

Prey type	Mean abundance		Cumulative % dissimilarity
	Upper catchment	Lower catchment	
Dry season			
Trichoptera larvae	0.02	0.54	28.9
Diptera larvae	0.35	0.11	49.2
Amphipoda	0.21	–	60.3
Ostracoda	0.17	–	69.1
Hymenoptera	–	0.12	75.3
Diptera adults	0.06	0.02	81.3
Coleoptera adults	0.06	–	86.9
Cladocera	0.02	0.01	90.3
Wet season			
Diptera larvae	0.39	0.41	29.0
Amphipoda	0.26	–	45.2
Copepoda	0.24	0.06	61.4
Trichoptera larvae	–	0.26	77.4
Trichoptera adults	–	0.19	89.2
Diptera adults	0.03	0.04	93.6
Ostracoda	0.05	–	96.6
Cladocera	0.01	0.02	98.5

difference values for fins and otolith traits were not significantly skewed and were all slightly leptokurtic, indicating that they were not directionally symmetric or anti-symmetric. FA values were therefore calculated for all traits and in no case were FA values significantly correlated with trait size. There were no significant differences in FA for any trait between sexes, between adult and juvenile fish, between fish infected or not infected with parasites (see below), between fish sampled in wet and dry seasons or between fish from upper catchment and lower catchment sites.

Parasite fauna

Four species of endoparasitic helminths (three nematodes and a trematode) were found in *L. wallacei*, with an overall prevalence of parasitic infection (i.e. infection with any species of parasite) of 0.06 (n = 333). All of the helminth parasites were larvae. Of the nematodes, a species of *Eustrongylides* (possibly *Eustrongylides gadopsis*) was found in the abdominal cavity, a species of *Contracaecum* was found in the abdominal cavity, liver and ovaries, and a third, unidentified species of nematode was found in the gastrointestinal tract. Metacercariae of *Diplostomum* sp. (possibly *Diplostomum galaxiae*) were found in the skin and internal organs.

Significantly more fish were infected with parasites in lower catchment sites than in upper catchment sites (overall prevalence of infection of 0.14 compared to 0.03, Fisher exact test, $p < 0.001$) (Table 3). There were no significant differences in mean intensity of parasites between the upper and lower catchment for any species, but prevalences of *Contracaecum* sp. and the unknown species of nematode were significantly greater in fish from lower catchment sites than in fish from upper catchment sites (Table 3; Fisher exact test, $p < 0.01$ for both parasite species).

DISCUSSION

Distribution

Leptatherina wallacei is widely distributed in the south of Western Australia (Morgan *et al.* 1998). It is absent from marine environments and has historically been most often associated with upper estuaries, extending into

Table 3 Prevalence and mean intensities of infection (with 95% confidence intervals in parentheses) of parasite species found in *Leptatherina wallacei* sampled from sites in the upper catchment and lower catchment of the Blackwood River.

Parasite species	Upper catchment		Lower catchment	
	Prevalence	Intensity	Prevalence	Intensity
<i>Eustrongylides</i>	0.013 (0.004-0.038)	1	0.020 (0.004-0.073)	1
<i>Contracaecum</i>	0.009 (0.002-0.031)	1	0.070 (0.033-0.139)	3.71 (1-9.00)
Unknown nematode sp.	0.004 (0.001-0.025)	1	0.050 (0.020-0.113)	3 (1.4-5.8)
<i>Diplostomum</i>	–	–	0.030 (0.008-0.084)	1

the lower reaches of associated rivers (Prince *et al.* 1982; Prince & Potter 1983; Valesini *et al.* 1997; Morgan *et al.* 1998). The current study found that *L. wallacei* was most abundant throughout the upper catchment of the Blackwood River, occurred at lower abundance in the main channel of the lower catchment, and was absent from lower catchment tributaries. This supports the distribution and abundance of the species previously reported in the Blackwood River and other salinised rivers in the south-west of Western Australia (Morgan *et al.* 1998, 2003; Beatty *et al.* 2008). It has been hypothesised that the reversal of the typical longitudinal salinity gradient in many rivers in the south-west (i.e. increasing salinity with increasing distance from the ocean) has facilitated the upstream movement of estuarine fishes, such as *L. wallacei*, where they replace salt-sensitive freshwater fishes (Morgan *et al.* 2003; Beatty *et al.* 2010). Although *L. wallacei* is sympatric with six native and two introduced freshwater fish species in the lower catchment of the Blackwood River, the only other species found in the upper catchment are the native freshwater species *G. occidentalis*, the native estuarine/occasional freshwater species *P. olorum* and the alien freshwater species *G. holbrooki*.

Whether the greater relative abundance of *L. wallacei* in the upper catchment of the Blackwood River is a direct consequence of increased salinity *per se*, or of other abiotic or biotic factors, is not something that can be definitively determined from our study. One of the problems with inferring the effects of salinisation on freshwater organisms from field observations is that salinity is likely to covary with other environmental factors, thereby making it difficult to attribute salinity as the direct cause of distributional changes. There is a need for experimental studies, such as determining the salinity tolerance of *L. wallacei*, so that the effects of salinity can be separated from other factors. Although the precise salinity tolerance of *L. wallacei* has not been investigated, there is some information on upper and lower tolerance levels. Thompson & Withers (1992) found that *L. wallacei* could survive in salinities of up to 85 ppt for at least seven days in the laboratory, while in the field Morgan *et al.* (1998, 2003) have found *L. wallacei* at salinities from <1 ppt up to 38 ppt and Beatty *et al.* (2008) reported spawning at salinities as low as 1–2 ppt.

Life-cycle

The life-cycle of *L. wallacei* in the Blackwood River could be most clearly determined in the upper catchment. It appears that in the upper catchment, *L. wallacei* typically survives for just over one year, with spawning occurring in spring followed by some mortality of adults. The presence of some fish with significantly greater lengths than 0+ cohorts, and with one or two translucent zones on their otoliths, indicates that these individuals can survive well into their second or even third year of life, although this interpretation should be treated cautiously until the counting of otolith annuli is validated for determining age in this species. An annual life-cycle with spring spawning of *L. wallacei* in the upper catchment of the Blackwood River is consistent with that reported for an estuarine population in the Swan-Avon river system by Prince & Potter (1983). The life-cycles of three other

species of Atherinidae (*Atherinosoma presbyteroides*, *Atherinosoma elongata* and *Allanetta (Craterocephalus) mugiloides*) in the Swan-Avon River system were also investigated by Prince & Potter (1983) and they found that all of these species typically have a one year life-cycle, as is also common for many Northern Hemisphere atherinids (e.g. Nelson 1968; Davis & Louder 1969; Henderson & Bamber 1987; Huber & Bengtson 1999).

In the lower catchment of the Blackwood River, *L. wallacei* appeared to have a delayed and shorter spawning period and a much reduced reproductive output compared to the upper catchment. Peak GSI values were markedly less in fish from the lower catchment, and these fish were also significantly heavier than fish from upper catchment sites and attained larger sizes at the end of their first and second year of life; this is consistent with reduced reproductive effort because energy goes first into reproduction in fishes with short life-cycles (Henderson & Bamber 1987). Fish appeared to spawn in early summer in the lower catchment, and the post-spawning mortality of adults was not as marked as in the upper catchment. Although there have been no previous comparative studies of different populations of *L. wallacei*, other atherinids have been shown to have phenotypically plastic reproductive strategies, presumably as an adaptation to the variable environmental conditions experienced in estuaries (Henderson & Bamber 1987; Huber & Bengtson 1999).

The reduced reproductive activity and atypical life-cycle of *L. wallacei* in the lower catchment of the Blackwood River, compared to upper catchment and estuarine populations (Prince & Potter 1983), may be due to a number of causes. First, the lower salinities in this part of the river may place the fish under physiological stress. Fluctuating asymmetry is often used as a measure of developmental instability in fishes and other organisms, reflecting the inability of individuals to maintain homeostasis when faced with environmental stresses during development (Somarakis *et al.* 1997; Panfili *et al.* 2005; Almeida *et al.* 2008). Although there was no evidence of fluctuating asymmetry in *L. wallacei* from the lower catchment, we do not know the developmental history of these fish. Second, even if fish are not under physiological stress, there may be differences in environmental requirements for spawning between upper and lower catchment populations. For example (in addition to salinity) water temperatures, dietary items and rates of parasitism also differ between upper and lower catchments and these differences may impact on gonadal development rates (Pollard 1974; Morgan 2003).

Diet

This study found *L. wallacei* to heavily rely on a range of aquatic invertebrates or certain life-history stages of terrestrial insects, with only a very small portion of plant material consumed. This result is similar to previous research on the diet of many different atherinid species, including *L. wallacei*, in the south-west of Western Australia, showing that they are opportunistic feeders that consume benthic crustaceans, polychaetes, planktonic crustaceans and small amounts of detritus and plant material (Prince *et al.* 1982; Humphries 1993). Regardless of the season and location, dipteran larvae

made important contributions in terms of number and volume to the diet. Despite the preponderance of dipteran larvae in the diet of all populations of fish in the Blackwood River, there were also important differences in diet between fish from upper catchment and lower catchment sites. Trichopteran larvae and adults were major contributors to the diets of fish from the lower catchment, but not to the diets of fish from the upper catchment. By contrast, fish from the upper catchment had a much greater proportion of crustaceans, particularly amphipods, ostracods and copepods, in their diet than did fish from the lower catchment. These differences most likely reflect differences in the invertebrate composition in different parts of the river, because of differences among invertebrate groups in salinity tolerance. Trichopterans, for example, are very salt-sensitive (Hart *et al.* 1991), and Bunn & Davies (1992) recorded a shift from insect dominated to crustacean dominated aquatic fauna in the river systems of south-western Australia as salinity increased. Hart *et al.* (1991) described crustaceans as the most salt-tolerant of the Australian aquatic macroinvertebrates.

Along with changes in salinity directly impacting invertebrate prey availability due to the relative salinity tolerances of those taxa, secondary salinisation may also indirectly impact invertebrate communities. While there is little information about the response of Australian freshwater algae or plant species toward environmental degradation, the majority of taxa do not appear to be tolerant of increasing salinity, with adverse effects on growth or reproductive ability appearing at salinities above 1 ppt (Nielsen *et al.* 2003b). Algae and aquatic plants do not form a major proportion of the diet of freshwater fishes in south-west of Western Australia (Morgan *et al.* 1998), so their elimination by rising salinity is unlikely to directly affect the growth rate of native fishes. However, there may be indirect effects of their loss by reducing food availability of herbivorous micro- and macroinvertebrates.

Parasites

In this study three species of parasitic nematode (*Contracaecum* sp., *Eustrongylides* sp. and an unidentified species) and one species of trematode (*Diplostomum* sp.) were found in *L. wallacei* in the Blackwood River. All of these parasites were present as larval stages, and use fishes as intermediate hosts in their life-cycle. The most prevalent parasite was *Contracaecum* sp. Species of *Contracaecum* have been previously reported in both estuarine and freshwater fishes in the south-west of Western Australia (Lymbery *et al.* 2002; Chapman *et al.* 2006). In the current study, many internal organs were infected with *Contracaecum*, principally the liver, but also the ovaries, which may cause infertility in the host.

Infections with *Contracaecum* and with the unidentified species of nematode were much more prevalent in *L. wallacei* from the lower catchment than in fish from the upper catchment. We hypothesise that this may be due to differences in salinity between these sites. Parasite populations are often adversely affected by human-induced environmental changes (Lafferty 1997; Tinsley 2005; Lymbery *et al.* 2010), and the present study appears to reinforce this finding. Most previous studies of environmental impacts on the parasites of fishes have

been concerned with the effects of industrial pollution, eutrophication and acidification, and the effects of secondary salinisation have rarely been studied (although see Rogowski & Stockwell 2006). The reduced prevalence of parasites at higher salinities may reflect differences in the survival of free-living parasite stages, differences in the abundance of hosts or differences in transmission efficiency to hosts in different parts of the river. Without knowing the details of the life-cycle of the parasites, it is difficult to distinguish between these possibilities. Species of *Contracaecum* typically use invertebrates as a first intermediate host, fishes as a second intermediate host and piscivorous birds as a definitive host (Lymbery *et al.* 2002). Previous studies have found that piscivorous birds are less abundant in salinised rivers (Halse *et al.* 2003), and this may account for reduced rates of parasitism in the upper catchment sites. However, it may also be likely that the difference found for *L. wallacei* reflects a component of transmission efficiency that is peculiar to this species, such as a reduced consumption of the intermediate host(s) by *L. wallacei* populations in the upper catchment.

Regardless of the reason for the difference in parasite prevalence between populations of *L. wallacei* in the upper and lower catchments, it may have an impact on the relative ecological success of the species in these two areas. It has been suggested that reduced parasite load improves the demographic performance and competitive ability of alien species invading a new area (Torchin *et al.* 2002, 2003), although empirical data to support this are rare. Of particular interest in the current study is the greater prevalence in the lower catchment of *Contracaecum* sp., found in the internal organs, including the ovaries of infected fish. Whether this is a contributing factor to the apparent reduction in reproductive activity in this population is a question that deserves further study.

CONCLUSIONS

Secondary salinisation is a growing problem in the Southwestern Province and an increasing number of studies suggest that it, along with climate change (Beatty *et al.* 2014), is the greatest threat for the endemic freshwater fish species in this area (Morgan *et al.* 2003; Beatty *et al.* 2008, 2010, 2011). Not all fish species may be adversely affected by increasing salinity, however; in the case of *L. wallacei*, which is typically found within estuarine environments and a few coastal lakes, increasing salinity in the headwaters of the Blackwood River may have facilitated further inland penetration. The results of the study supports our hypothesis that there would be plasticity in key ecological traits of *L. wallacei* that may help explain why it is now the second most abundant fish in the upper catchment of the Blackwood River. It appears that increasing salinisation may improve the prospects for survival and spread of *L. wallacei* in the Blackwood River, because the life-cycle and diet of upper catchment populations are more similar to those of estuarine populations than they are to those of lower catchment populations. In addition, populations of *L. wallacei* in the upper catchment have reduced rates of parasitism, possibly because of the effects of salinisation on other parasite hosts.

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