

Host impact of monogenean *Lepidotrema bidyana* infection and intensity estimates for onsite monitoring

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ABSTRACT: We developed a rapid effective method for accurate estimation of intensity for the monogenean *Lepidotrema bidyana*, a gill parasite of silver perch *Bidyanus bidyanus*. This parasite requires monitoring because high-intensity infections reduce host growth and can lead to secondary bacterial and fungal infections. The most accurate method for counting *L. bidyana* was visual examination of fresh gills. There was a significant relationship between fish size and parasite intensity; however, there was no significant relationship between fish condition and parasite intensity. Parasite intensity estimates were generated by using the mean intensity of worms on the posterior hemibranch on the first left gill arch, compared to the total mean intensity of worms on all hemibranchs. Estimates were validated by predicting *L. bidyana* intensity from a random sample of silver perch obtained from aquaculture ponds. Parasite intensity estimates correlated strongly to real counts, and this method can be used to accurately predict parasite intensity on an individual host, and thus represents an improvement over previous methods.

KEY WORDS: Monogenea · *Bidyanus bidyanus* · *Lepidotrema bidyana* · Prevalence · Intensity

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INTRODUCTION

In aquaculture, high-intensity infections of monogenean parasites can cause stress, poor feeding response, reduced growth, and anaemia (Paperna 1991) and induce histopathological changes in gills (Hayward et al. 2001, Padrós et al. 2001, Ogawa 2002, Montero et al. 2004, Dezfuli et al. 2007), which can lead to secondary infections (Cone 1995) and ultimately death (Cone 1995, Ogawa 1996). The Australian freshwater fish, silver perch *Bidyanus bidyanus* (Terapontidae), is cultured in ponds in Australia, Israel, China and Taiwan (Rowland & Barlow 1991, Allan et al. 2000, Barki et al. 2000). Diseases have had serious impacts on the development of this aquaculture industry (Rowland et al. 2007), including disease caused by the monogenean *Lepidotrema bidyana* (Diplectanidae) (see Read et al. 2007). *L. bidyana* is a

gill-dwelling monopisthocotylean, with a size range of 500 to 600 µm for adults (70 to 200 µm for juveniles) (Rowland et al. 2007). Detection of *L. bidyana* when viewed under a microscope is aided by the worm's stretch and recoil movement (Read et al. 2007) and 4 well-defined dark eye spots. Adult worms attach to the host fish between the secondary gill lamellae using their haptor, which bears 2 pairs of hamuli. Parasite attachment causes epithelial hyperplasia on the distal half of the gill filaments (Rowland et al. 2006b).

Adult monogeneans often display a preference for a specific habitat on the gills or body surface (reviewed by Kearn 1994), including parts of different gill arches (reviewed by Rohde 1993). This may be influenced by the direction and force of the respiratory current, seasonality, host sex, age and morphology (Wootten 1974, Koskivaara et al. 1992, Rohde 1993) and extrinsic and intrinsic parasite biological func-

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tions. Extrinsic influences include interspecific competition, predation, hyperparasitism and reinforcement of interspecific reproductive barriers (Rohde 1979). The main intrinsic influence is enhancement of mating success (Rohde 1991, 1993, Geets et al. 1997).

In aquaculture, parasite management systems rely on accurate monitoring methods specific to the target species (Grant 1983). When developing specific counting methods, non-random parasite distributions must be considered. Rowland et al. (2006b) monitored *Lepidotrema bidyana* by sampling 5 *Bidyanus bidyanus* fish from each treated pond, removing the anterior-most left gill arch from each fish, examining that arch at 100× magnification, counting the number of parasites in 5 fields of view and reporting the prevalence and mean number of parasites per field of view. Which part or side of the gill should be examined was not specified (Rowland et al. 2006b). Field veterinarians expressed doubt that the method provided accurate intensity estimates. We evaluated this current counting method, and developed a new method to more accurately estimate *L. bidyana* intensity on an individual host. This method will aid onsite monitoring and research on treatments, and provides information on the relationships between intensity and host growth and condition. We also investigated the relationship between parasite intensity and fish size, and the effect that parasite intensity has on the condition of the host.

MATERIALS AND METHODS

Source of fish and parasites

Silver perch (n = 660) infected with *Lepidotrema bidyana* from Pioneer Fish Farm (PFF) (Gloucester, NSW) were maintained in a 10 000 l recirculation tank at Flinders University, South Australia (FUSA). Infection was maintained by co-habitation of infected fish (Hirazawa et al. 2004) for 4 mo. To examine *L. bidyana* infections, fish were euthanised with an overdose (a 40 ml 1000 l⁻¹ bath) of Aqui-S[®], weighed, measured and examined for parasites. To determine parasite distribution, the branchial baskets were removed and the right (R) and left (L) gill arches were separated. The worms on each gill arch (1 through 4) were counted individually using a dissecting microscope (35× magnification), with the number of worms on the posterior (p) and anterior (a) hemibranchs recorded.

Terminology for parasite infections follows Bush et al. (1997), except when describing and discussing

the development of a new counting method where 1 out of the 25 randomly selected fish investigated was uninfected, but for continuity the word 'intensity' was used instead of 'abundance'.

Evaluation of current monitoring method

Five fish were randomly selected from the population maintained at FUSA and euthanised, and the number of *Lepidotrema bidyana* was counted as outlined by Rowland et al. (2006b). Actual parasite intensity was then determined using the method detailed in the previous subsection and compared to the current monitoring method.

Development of a new monitoring method

Thirty fish were randomly selected from the population maintained at FUSA over 2 d. Hemibranch L1p had the highest mean parasite abundance and was therefore used to predict total parasite intensity. The formula for the estimate is as follows:

$$\frac{\text{Mean of hemibranch L1p}}{\text{Mean of all hemibranchs}} = \text{APC} \quad (1)$$

where APC is the average proportional contribution. Then:

$$\frac{\text{Target fish's L1p hemibranch burden}}{\text{APC}} = \text{Estimate of intensity for that fish} \quad (2)$$

Validation of the new monitoring method

Twenty-five fish were randomly selected, over 2 d, from 5 different ponds at PFF. For each fish, the parasite intensity was determined, and an estimate was made of parasite intensity using the APC obtained from the FUSA fish. The accuracy of the estimates was evaluated by comparing the estimated parasite intensity to the actual parasite intensity for each fish.

Impact of parasite intensity on host

The mass, length and condition of the fish sampled during the development and validation of the new monitoring method were recorded and then compared to the actual parasite intensity on each individual fish.

Statistical analysis

Data were analysed using SPSS 17.0 statistical software. The counting method of Rowland et al. (2006b) and the accuracy of the newly developed monitoring method were evaluated through linear regressions of the mean number of parasites per 5 fields of view and the estimated parasite intensity, respectively, and compared to the actual parasite intensity for each fish.

For each fish, Fulton's condition index K was calculated:

$$K = 100 \left(\frac{W}{L^3} \right) \quad (3)$$

where W is the mass (g) and L is the total length (cm) of the fish. Generalised linear models (GLMs) using a negative binomial function with log link (Venables & Ripley 1994) were used to compare the intensity of parasites per fish to the mass, length and K of the fish (Cone 1989, Springer & Murphy 1990). For all tests, statistical significance was judged at the alpha level of 0.05.

RESULTS

Evaluation of current counting method

The mean number of parasites per 5 fields of view was 0.44 ± 0.12 SE (range: 0.2 to 0.8). Prevalence of *Lepidotrema bidyana* was 100%, and mean intensity was 131 ± 24.1 SE (range: 84 to 211). There was no significant correlation between the mean number of parasites per 5 fields of view and parasite intensity ($r^2 = 0.159$, 1-way ANOVA, $F = 0.569$, $p = 0.505$).

Development of new counting method

The mean mass (g) of the fish ($n = 30$) was 15.7 ± 13.8 SD (range: 2.1 to 68.6) and mean length (cm) was 12.7 ± 3.3 SD (range: 7.1 to 24.2). Average K was 0.63 ± 0.1 SD (range: 0.46 to 0.85). A total of 1863 *Lepidotrema bidyana* were counted. Prevalence of *L. bidyana* was 96.7% (95% CI: 83 to 100%) and mean intensity was $64.2 \pm$ SE 21.8 (range: 1 to 612). The APC developed from the test population and used in estimating parasite intensity was 0.13.

Validation of new monitoring method

The mean mass (g) of the fish ($n = 25$) was 198.2 ± 113.2 SD (range: 16.1 to 357) and mean length (cm) was

23.8 ± 4.7 SD (range: 14.4 to 28.9). Average K was 1.3 ± 0.3 SD (range: 0.11 to 1.53). A total of 24 459 *Lepidotrema bidyana* were counted. Prevalence of *L. bidyana* was 100% (95% CI: 86 to 100%) and mean intensity was 978.4 ± 122.7 SE (range: 124 to 2465). Estimated parasite intensity and counted parasite intensity for each farmed fish were strongly correlated ($r^2 = 0.888$, 1-way ANOVA, $F = 182.73$, $p < 0.001$) (Fig. 1).

Impact of parasite intensity on host

Hemibranchs L1p and R1a had the highest mean abundances of *Lepidotrema bidyana* on each side (Fig. 2). For the FUSA fish, there was a significant relationship between parasite intensity and mass (GLM, likelihood ratio, $p = 0.001$) and length (GLM, likelihood ratio, $p = 0.003$), but no significant relationship between parasite intensity and Fulton's condition index (GLM, likelihood ratio, $p = 0.276$) (Fig. 3).

For the PFF fish, there was a significant relationship between parasite intensity and length (GLM, likelihood ratio, $p = 0.037$) (Fig. 3). There were positive non-significant relationships between parasite intensity and mass (GLM, likelihood ratio, $p = 0.051$) of the PFF fish and Fulton's condition index (GLM, likelihood ratio, $p = 0.06$) (Fig. 3).

DISCUSSION

A rapid, accurate method to estimate intensity of *Lepidotrema bidyana* infection on an individual host

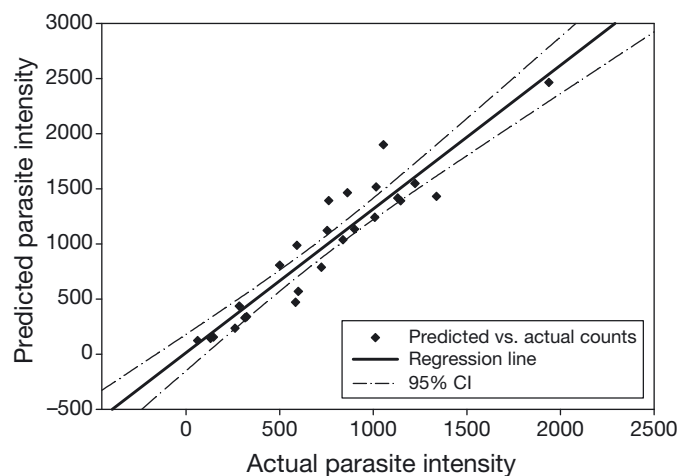


Fig. 1. *Lepidotrema bidyana* infecting *Bidyanus bidyanus*. Relationship between estimated and actual parasite intensity for 25 fish sampled from Pioneer Fish Farm. Estimations were made using the average proportional contribution (APC) of 0.13 from a test sample of 30 fish

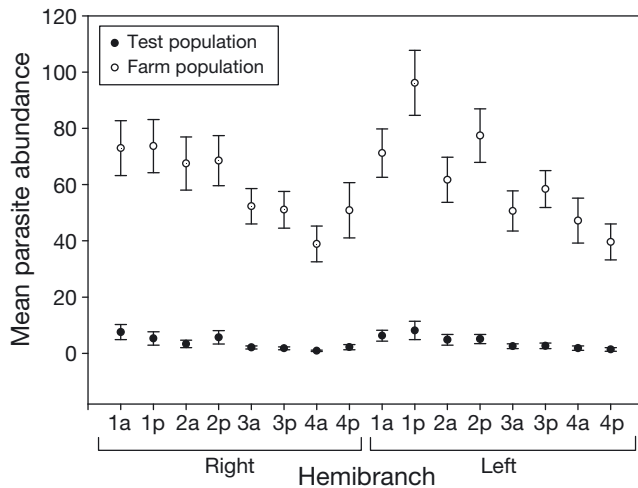


Fig. 2. *Lepidotrema bidyana* infecting *Bidyanus bidyanus*. Mean abundance of *L. bidyana* on each hemibranch of *B. bidyanus*. Error bars represent standard error of the mean. Test population: *L. bidyana* from 30 fish of 660 held at Flinders University; farm population: *L. bidyana* from 25 fish from 5 ponds at Pioneer Fish Farm; 1 to 4: gill arches 1 to 4 respectively; a and p: anterior and posterior hemibranches, respectively

is required for on-farm parasite management and to facilitate research on parasite epidemiology, the effects of this parasite on host growth and condition and efficacy of treatments.

The counting method of Rowland et al. (2006b) for *Lepidotrema bidyana* is inaccurate; it is unclear if it was intended to estimate intensity or prevalence, and how its results should be used. It is difficult to standardise from which part of the gill the '5 fields' are counted, and those 5 fields cover a fixed area although fish of dissimilar sizes have gills of differing area. Non-random distribution of *L. bidyana* also contributes to the inaccuracy of this method. Obtaining the mean number of parasites per field of view does not, therefore, provide an accurate prediction of parasite intensity on a host. We found that counting all the worms on hemibranch L1p provides an accurate basis for estimating intensity over a wide size range of silver perch and is strongly correlated with parasite intensity for individual farmed fish.

Lepidotrema bidyana are currently managed on silver perch farms by applying chemical treatments when counts made using Rowland et al.'s (2006b) method exceed 30 *L. bidyana* individuals (M. Landos pers. comm.). An accurate counting method is a key initial component in developing an understanding of the effects and management of *L. bidyana* in aquaculture. The initial benefit to industry will be reducing the chance of overestimation of intensity that can result in premature use of treatments leading to in-

creased cost and increased stress to fish (Grant 2002), and likelihood of emergence of resistance (Umeda et al. 2006), or of underestimation of intensity, causing an increased likelihood of the farmed fish being negatively affected by their *L. bidyana* load.

Monogeneans often show seasonal fluctuations in prevalence and abundance (Gonzalez-Lanza et al. 1991, Kim et al. 2001, Rubio-Godoy & Tinsley 2008, Antonelli et al. 2010). Temperature has been identified as having a major influence on monogenean life cycles (Cecchini et al. 1998), with higher water temperatures facilitating greater egg production rate, shorter hatching time and quicker development to sexual maturity (Kearn 1986, Buchmann 1988, Ogasawa 1998, Yoshinaga et al. 2000, Tubbs et al. 2005). Seasonal fluctuations of *Lepidotrema bidyana* were observed during monitoring at Grafton Aquaculture Centre (GAC), with 'occurrence' greatest during the winter and spring (Rowland et al. 2007). Gonzalez-Lanza et al. (1991) also reported a higher prevalence and intensity during winter for other diplectanids infecting sea bass *Dicentrarchus labrax* (Moronidae). It is unclear how these fluctuations relate to temperature, but this suggests that monitoring should be targeted during winter and spring when the prevalence and intensity of *L. bidyana* are greatest and most management is required.

The silver perch sampled from PFF had greater intensities of *Lepidotrema bidyana* than the FUSA population. Commercial silver perch aquaculture uses stocking densities of up to 43 000 fish ha⁻¹ in aerated earthen ponds (Rowland 1995) and 200 fish m⁻³ in cages (Rowland et al. 2006a). Higher stocking density facilitates monogenean transmission (Hirazawa et al. 2004), which may explain the higher intensities recorded. Both samples of fish showed a high prevalence, which is typical of monogeneans in closed and semi-closed systems (Thoney & Hargis 1991).

There were strong relationships between *Lepidotrema bidyana* intensity and host length and mass in the fish sampled from the FUSA fish. In the PFF group, there was a significant relationship between *L. bidyana* intensity and host length, and the relationship between intensity and host mass was almost within statistical significance. This suggests that there is a positive relationship between host size and parasite intensity, as also described for other monogenean infections (Frankland 1955, Paperna et al. 1984, Buchmann 1989, Kim et al. 2001). Fish size influences intensity either because greater host surface area leaves larger hosts more susceptible to invasion or because hosts that have lived longer have had more time to accrue parasites.

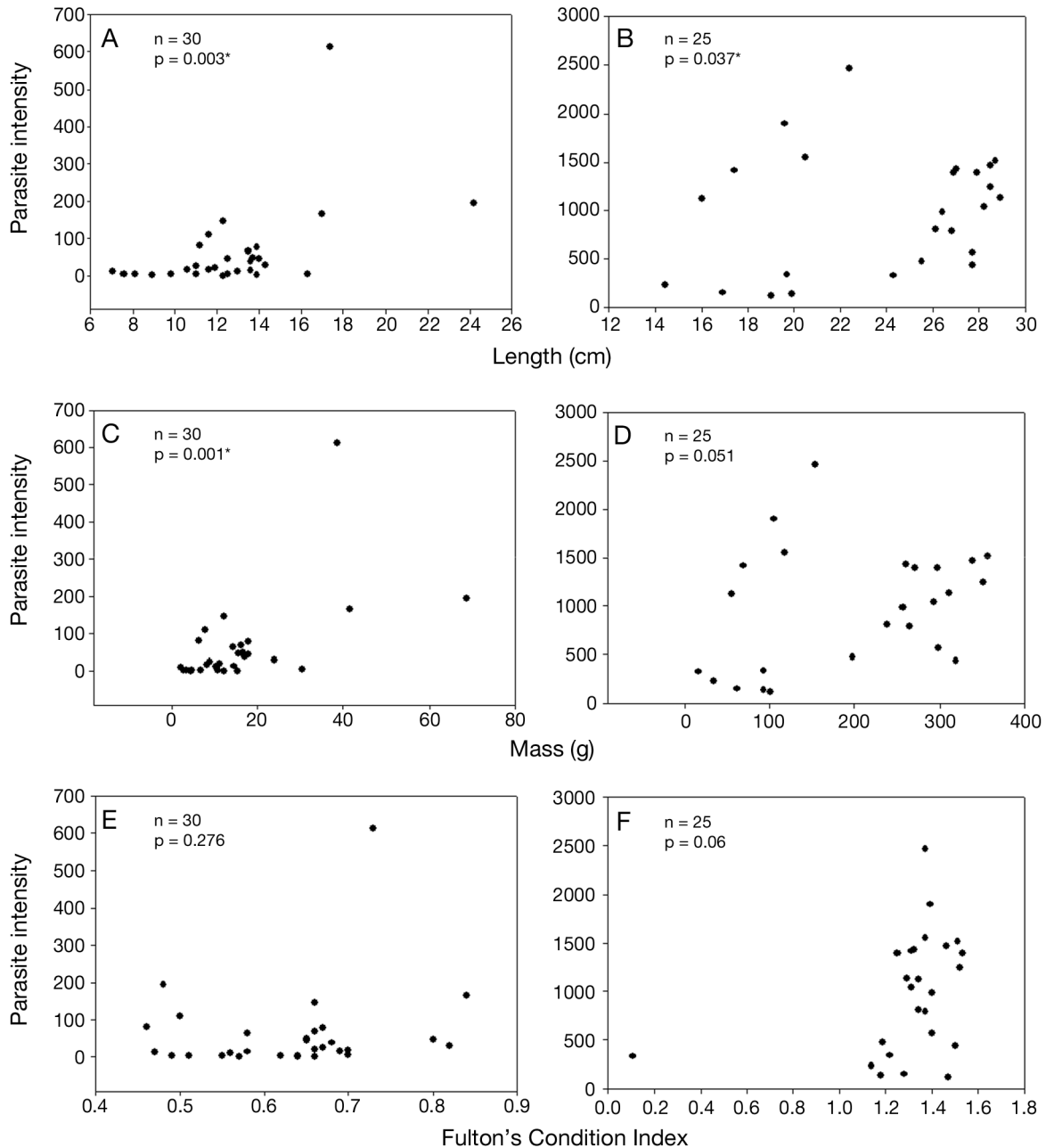


Fig. 3. *Lepidotrema bidyana* infecting *Bidyanus bidyanus*. Scatter plots of relationship between *L. bidyana* intensity and (A,B) length (C,D) mass and (E,F) condition of *B. bidyanus* from Flinders University (A,C,E) and Pioneer Fish Farm (B,D,F). *Significant relationship ($p < 0.05$) assessed by generalised linear models using a negative binomial function with log link

The parasite intensities observed in the present study were insufficient to cause a significant effect on condition. The intensity of *Lepidotrema bidyana* in the PFF fish was higher than that in the FUSA fish and was more strongly correlated with the host's condition index, although this correlation was not significant. It does, however, suggest that higher-intensity *L. bidyana* infections may be associated with re-

duced host condition. A comparison of growth and condition index of silver perch infected with, and silver perch free of, *L. bidyana* is required to clarify this. Few studies have examined the effects of gill-dwelling monogenean intensity on host growth. Hirayama et al. (2009) reported significantly slower growth in *Seriola dumerili* (Carangidae) when $>0.285 \text{ cm}^{-2}$ of the fish's skin was infected with

Neobenedenia girellae (Capsalidae). Understanding this relationship will be important for developing criteria for treatment based on parasite intensity and geared to minimising lost production and the deleterious economic and biological effects of treatment.

Using the APC generated from the sample population provides an accurate intensity estimate for *Lepidotrema bidyana* on an individual host. This technique can be used by farmers to obtain more accurate counts to better inform management decisions. This information, combined with data on the effect of *L. bidyana* on the condition of the host over time, egg-laying rates and the time to sexual maturity of *L. bidyana* at varying temperatures, will facilitate the development of a monitoring programme and defined criteria for treatment of *L. bidyana* infections.

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