International Journal for Parasitology 41 (2011) 697–704

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Life cycle truncation in a trematode: Does higher temperature indicate shorter host longevity?

Kristin K. Herrmann *, Robert Poulin

Department of Zoology, University of Otago, Dunedin, New Zealand

ARTICLE INFO

Article history:
Received 1 November 2010
Received in revised form 3 January 2011
Accepted 20 January 2011
Available online 15 February 2011

Keywords:
Abbreviated life cycle
Conditional strategies
Progenesis
Stegodexamene anguillae
Trematode

ABSTRACT

The typical three-host life cycle of most trematodes creates transmission challenges for which a variety of adaptations have evolved to increase the probability of transmission. Some species can abbreviate their life cycle via progenesis, the precocious maturation of the parasite in the second intermediate host resulting in the production of eggs through self-fertilisation without requiring a definitive host. Adoption of the progenetic life cycle may be a conditional strategy in response to different environmental cues related to low probability of transmission to the definitive host. Using high water temperature and/or limited diet as experimental stressors, we tested the effect of body condition and life span of the fish second intermediate host on facultative truncation of the typical three-host life cycle by progenesis in *Stegodexamene anguillae*. The results suggest that environmental cues, such as temperature and encystment site, may signal transmission opportunities to the parasite so that it may adjust its developmental strategy accordingly. Indeed, a greater proportion of worms became progenetic at higher temperatures, and progenesis was more common among worms encysted in the gonads or body cavity of their fish hosts than among those in other host tissues. These findings highlight the often unrecognised plasticity in parasite developmental and transmission strategies.

1. Introduction

Evolution of complex life cycles with multiple hosts and life stages has occurred in many phylogenetically distant groups of parasites (Poulin, 2007). For example, it is thought that the complex life cycle of trematodes evolved from a single-host life cycle with a vertebrate host to a multi-host life cycle through the addition of intermediate hosts (Choisy et al., 2003; Cribb et al., 2003). Presently, the life cycle of many trematodes involves morphologically different stages in three different hosts (Kearn, 1998). In the first stage of this three-host cycle, eggs usually hatch into free-living larvae that need to find and penetrate an invertebrate, usually a mollusc, as the first intermediate host (Schell, 1970). Secondly, cercariae leave this first host and must find and penetrate a second intermediate host, either an invertebrate or vertebrate (Kearn, 1998). Finally, transmission to the definitive vertebrate host normally requires ingestion of metacercariae through predation upon the second intermediate host, after which worms mature and sexual reproduction occurs.

The selective pressures imposed by a series of improbable transmission events have favoured various adaptations by trematodes to increase the probability of transmission to each of the subsequent hosts (Poulin, 2007). These adaptations include high fecundity in sexually reproducing adults, efficient host-finding mechanisms in larval stages, extended longevity within intermediate hosts, and the ability to alter host behaviour in ways that facilitate transmission (Combes, 1991; Poulin, 1995, 2007; Moore, 2002; Parker et al., 2003a). Another adaptation to the constraints of transmission events is to secondarily shorten the life cycle by eliminating one of those events (Poulin, 2001). Truncation of the life cycle has independently evolved in many parasite lineages and is thought to be favoured when the probability of transmission is low or extremely variable (Poulin and Cribb, 2002; Lefebvre and Poulin, 2005). For some species truncation is obligatory, but often it is facultative, with both life cycle strategies, i.e. the 'normal' and the abbreviated cycles, occurring in the same population (Lefebvre and Poulin, 2005). Typically, facultative truncation of the life cycle in trematodes occurs via progenesis, i.e. precocious development in the second intermediate host resulting in eggs being produced by an adult worm through self-fertilisation within the metacercarial cyst (Poulin, 2001; Poulin and Cribb, 2002). Thus an unlikely transmission event, ingestion by the definitive host, becomes unnecessary.

Progenesis makes completing the life cycle easier but occurs via self-fertilisation, resulting in the most severe level of inbreeding and offspring with reduced genetic heterogeneity (Charlesworth and Charlesworth, 1987; Christen et al., 2002). If the shortened life cycle results in a greater net benefit when transmission

* Corresponding author. Tel.: +64 3 479 5848, fax: +64 3 479 7584.
E-mail addresses: herkr385@student.otago.ac.nz, kherrmann13@gmail.com (K.K. Herrmann).

0020-7519/$36.00
doi:10.1016/j.ijpara.2011.01.009

opportunities are low, then parasites with the ability to perceive environmental cues related to the probability of transmission to the definitive host and adjust their strategy accordingly would be favoured by natural selection (Thomas et al., 2002; Poulin, 2003). Many environmental factors could provide information about transmission probability, such as temperature, host age, reproductive status of the host, host condition, other parasites residing in the host (including whether those parasites are conspecifics), predation risk of the current host by the subsequent host and population density of the current host (Hine, 1978; Holton, 1984; Poulin and Cribb, 2002; Thomas et al., 2002; Poulin, 2003; Lefebvre and Poulin, 2005; Lagrue and Poulin, 2007). Thus, the adoption of the progenetic life cycle may be a conditional strategy in response to different environmental cues indicating low probability of transmission (Poulin and Cribb, 2002; Thomas et al., 2002; Poulin, 2003; Lagrue et al., 2009).

In the typical three-host life cycle of the trematode *Stegodexamene anguillae* (Lepocreadiidae), miracidia hatch from eggs and infect a snail, *Potamopyrgus antipodarum*, as the first intermediate host. Within the snail, numerous cercariae are produced through asexual reproduction, leave this first host and search for the second intermediate host, small freshwater fish, mostly *Gobiomorphus* and *Galaxias* spp. (Macfarlane, 1951, 1952). After penetrating the fish host, cercariae encyst as metacercariae, the infective stage to definitive hosts, *Anguilla dieffenbachia* (New Zealand longfin eel) and *Anguilla australis* (short-finned eel). Transmission to the definitive host occurs when an eel feeds on a fish infected with metacercariae (Macfarlane, 1951, 1952). Development into the adult stage and sexual reproduction occur in the digestive tract of the eels, and trematode eggs are shed into the water with the host's faeces. Alternatively, at the metacercarial stage inside the second intermediate fish host, *S. anguillae* can develop progenetically into the adult stage and reproduce by self-fertilisation (Macfarlane, 1951; Holton, 1984), allowing the trematode to bypass transmission to the eel definitive host.

Here we test experimentally whether this progenetic trematode, *S. anguillae*, is capable of perceiving cues associated with longevity of its second intermediate fish (*Gobiomorphus cotidianus*, common bully) host and whether it has the ability to respond to those signals by adjusting its life cycle strategy in a state-dependent manner. We used temperature and host diet as known stressors affecting longevity of the fish host (Liu and Walford, 1966; Das, 1994) and thus altering the probability of transmission to the eel definitive host. Specifically, we test the prediction that parasites within fish exposed to stressful conditions (high temperatures and/or low ration) will be more likely to adopt progenesis and a truncated life cycle than those in fish more likely to survive longer, when controlling for inter-individual variability among fish with respect to size, sex and infection by other parasites. In addition, we test whether the adoption of progenesis is also followed by adjustments in either the number or size of eggs produced in response to external conditions. Our study is one of the very few that have addressed experimentally whether parasites adjust their transmission mode in a state-dependent fashion.

2. Materials and methods

2.1. Animal collection

Common bullies between 4 and 6 cm (reproductive age) were collected in Lake Waihola, South Island, New Zealand, using seine nets on 3rd December 2008. Natural infections in 4–6 cm bullies from this site range between five and >100 *S. anguillae* per fish (Poulin and Lefebvre, 2006). Prior to their use in experiments, fish were treated for fungal skin infection using Profurin (1 g/100 L) for 30 min and given a 5-min saltwater bath to eliminate monogenean ectoparasites due to the problematic nature of these infections while housing common bullies. Fish were kept in aerated and filtered lake water, fed ad libitum with commercial fish pellets (Reliance Stock Foods, Dunedin, New Zealand), and allowed to acclimate for 1 week. The photoperiod was set at 12 light:12 dark with a 2 h dawn/dusk ramping period.

2.2. Treatment

A factorial design was used with three temperatures (12 °C, 16 °C, 20 °C) and two diet levels (low = 0.005 g/fish/day, high = 0.012 g/fish/day) of commercial fish pellets. Chosen temperatures were based on thermal tolerance and preference of common bullies (Richardson et al., 1994) and were well within the natural water temperature range in Lake Waihola (Hall and Burns, 2002). Diet levels were determined during a prior 2-week trial, in which four diet rations were used and weight change was monitored; the low-diet level was chosen because it led to weight loss, whereas the high-diet level allowed fish to gain weight.

Our experiment monitored changes in the developmental stage of naturally-acquired parasites in response to treatment conditions. This was done instead of using experimental infections because the latter proved impossible, and because very few parasites in fish taken from the wild are at the progenetic stage (7.5%, Poulin and Lefebvre, 2006); therefore, random allocation of fish to treatments will result in equally low initial frequencies of progenetic worms across treatments. At the beginning of the experiment, 20 fish were euthanised by an overdose of tricaine methanesulfonate (MS-222) to obtain a baseline for assessing changes in the development of naturally-acquired parasites during the experiment. Ten fish were placed into each of 12 tanks (30 L) based on their body length to control for variation in size among treatments, with two tanks randomly assigned to each of the six treatment combinations. This resulted in four tanks at each of the three temperatures, and six tanks receiving each of the two diet levels. Fish were tagged using two colours of visible implant elastomer (Northwest Marine Technology, Inc., Washington, USA) in combination with five locations on their body, allowing for 10 unique individual marks. Prior to marking, fish were anesthetized in a solution of MS-222 (10 mg/L). Marking methods followed those of Goldsmith et al. (2003). Fish were again treated with Profurin after tagging and at week three of the experiment. Fish length and the average of three wet weights were recorded for each fish at the start of the experiment. Every 2 days, aquaria were cleaned and 30% of the water was replaced with fresh lake water stored at the same temperature as the treatment.

2.3. Measures and statistical analyses

After 51/2 weeks, the experiment was terminated due to high mortality in the 20 °C-low diet treatment; although the small sample size was not ideal, the low survival confirmed that this treatment did induce stress in fish hosts created by the high temperature-low diet conditions. In the 12 °C-high diet treatment, 10 and nine fish survived, seven and seven in 12 °C-low diet, eight and eight in 16 °C-high diet, eight and six in 16 °C-low diet, six and seven in 20 °C-high diet, and two and seven in 20 °C-low diet, in replicates one and two, respectively. Fish were euthanised by an overdose of MS-222, and total length, weight and sex of each fish were recorded. Fish body condition was determined as W/L^3, where W and L are the weight and total length (Bolger and Connolly, 1989). Fish were dissected and all tissue, except the brain and the lumen of the gastrointestinal tract (where metacercariae of *S. anguillae* are never found), was examined for *S. anguillae* as well as other parasites. All *S. anguillae* worms were individually

removed from their cysts and classified as progenetic (eggs present), non-progenetic (no eggs present) or non-progenetic but with visible vitellaria (no eggs present but yolk-producing glands developed). Body surface of each worm was calculated as a surrogate for body size by using the formula of an ellipsoid, $(\pi LW)/4$, where L and W are the length and width of the parasite; the latter measurements were taken under a dissecting microscope (80×). If the worm was progenetic, all eggs expelled from the worm and free within the cyst were counted. Length (L) and width (W) of a subsample of 10 eggs from each progenetic worm were measured, and assuming a regular ellipsoid shape, egg volume was calculated as $(\pi LW^2)/6$.

ANOVAs were used to compare initial fish length and host condition among the baseline group and treatments, and to compare change in host condition among treatments. Initial fish length difference between sexes was assessed with a t-test. Two developmental stages, non-progenetic and vitellaria-present, were combined into one category of non-progenetic worms based on preliminary tests that showed no difference in the outcome of analysis of progenesis if vitellaria-present worms were removed. Thus, factors influencing the parasite's developmental strategy, non-progenetic versus progenetic, were assessed using a Generalized Linear Mixed Model (GLMM) with a binomial error distribution. This analysis was used to determine the effect of diet, temperature, diet * temperature interaction, initial host length, change in host body condition, host sex, encystment site (muscle, head, body cavity, gonads), host sex * encystment site interaction, abundance of conspecifics and of two other common species of trematode metacercariae (*Telogaster opisthorchis* and *Apatemon* sp.), while individual fish identity and aquaria were added as random factors. The interaction between host sex and temperature was included preliminarily, however inclusion of this interaction resulted in a similar final model and thus was not retained in order to achieve a simpler model.

The following analyses used the same factors with the specified additional factors. First, worm size data were log-transformed to approach normality and were used as a response variable in a GLMM with the Gaussian (normal) distribution including developmental strategy (non-progenetic, vitellaria-present and progenetic) and the quadratic function of change in host body condition as additional factors. Second, the number of eggs expelled per progenetic worm was used as a response variable in a GLMM with the quasipoisson distribution and the addition of worm size and the quadratic function of change in host body condition as extra factors. Third, a GLMM with mean egg volume per progenetic worm as a response variable and the Gaussian (normal) distribution included the additional factors of worm size, the quadratic function of change in host body condition and the number of eggs expelled. Finally, a GLMM with the coefficient of variation in mean egg volume and the Gaussian (normal) distribution as a response variable included the additional factors of worm size, the quadratic function of change in host body condition, the number of eggs expelled and egg volume.

3. Results

Overall, 507 metacercariae of *S. anguillae* were recovered from the 20 baseline fish, with numbers ranging from two to 88 *S. anguillae* per fish. In baseline fish, progenetic worms comprised 17.6% of all *S. anguillae* and another 6.5% had developed vitellaria. The mean size (±S.E.) of worms was 0.48 ± 0.061 mm^2 in the baseline group.

Out of the 85 surviving experimental fish, 2622 metacercariae of *S. anguillae* were recovered, with numbers ranging from four to 168 per fish and mean abundance (±S.E.) of 54.9 ± 0.8. Of all *S. anguillae* in experimental fish, 14.8% were progenetic and 5.2% had developed vitellaria. No difference in either initial host length ($F_{6,116} = 0.090$, $P = 0.997$) or initial host body condition ($F_{6,116} = 1.045$, $P = 0.400$) was found among the baseline group and treatments. The change in host body condition for the duration of the experiment was not different among treatments ($F_{5,80} = 1.574$, $P = 0.177$); overall, fish increased by approximately 6.1% of their initial length.

Differences among individual worms in development strategy were explained by temperature, encystment site and the number of *T. opisthorchis* (Table 1). There were significantly more progenetic worms in fish at 16 °C and 20 °C than at 12 °C (Table 1, Fig. 1). Of the 389 progenetic worms, more were found in the body cavity (76 of 366) and gonads (286 of 645) than in the muscle (14 of 909) or the head (13 of 702) (Fig. 2A). Lastly, the abundance of the trematode *T. opisthorchis* in a fish was negatively correlated with the proportion of progenetic *S. anguillae* in the same fish (Fig. 3A).

Worm size was affected by developmental stage, encystment site, host sex and the interaction between encystment site and host sex (Table 1). Mean size (±S.E.) of worms was 0.38 ± 0.009 mm^2 in the experimental fish. Mean size of worms was larger in the body cavity and gonads than in the muscle or head and larger in female hosts than males in all tissues except the body cavity (Fig. 2B). Progenetic worms were significantly larger (1.32 ± 0.027 mm^2) than non-progenetic worms (0.19 ± 0.004 mm^2), with those worms with well-developed vitellaria but no eggs falling in between (0.72 ± 0.024 mm^2; Fig. 4).

The mean number (±S.E.) of eggs released per progenetic worm was 224.9 ± 13.2 and ranged from zero to 1125. The number of eggs expelled by progenetic individuals was not affected by any of the parameters measured and tested, however worm size was close to significant with larger worms producing more eggs (Table 1, Fig. 5A).

The mean (±S.E.) volume of eggs released by progenetic worms was $1.02^{-4} \pm 3.05^{-6}$ mm^3 and ranged from 5.76^{-5} to 9.37^{-4} mm^3. Egg volume was only affected by worm size (Table 1), with larger eggs being produced by larger worms (Fig. 5B). Additionally, abundance of *T. opisthorchis* was on the edge of significance with larger eggs produced in hosts with high abundance of *T. opisthorchis* (Fig. 3B). Finally, the coefficient of variation in egg volume decreased with increasing worm size (Fig. 5C) and increased with abundance of *T. opisthorchis* (Fig. 3C).

4. Discussion

The improbable transmission events involved in the complex life cycles of parasites promote the evolution of alternative life cycle strategies such as progenesis (Poulin and Cribb, 2002). Shortening the life cycle makes completing it easier, however progenesis is accompanied by the cost of producing offspring of lower genetic quality due to self-fertilisation (Charlesworth and Charlesworth, 1987; Christen et al., 2002). Consequently, it would be advantageous for parasites to use environmental cues related to the probability of transmission to make adaptive adjustments to their development as a state-dependent response (Poulin and Cribb, 2002; Thomas et al., 2002; Poulin, 2003). Thus trematodes with the ability to perceive signals related to their transmission opportunities and adjust their life history strategy accordingly should adopt progenesis when presented with environmental cues indicating low probability of transmission to the usual definitive host.

The present results suggest that progenesis in *S. anguillae* may be directly or indirectly cued by temperature. This trematode preferentially adopts the progenetic strategy at higher temperature. Additionally, the baseline fish harboured a slightly greater proportion of progenetic worms than the experimental fish as a whole and similar to fish at 12 °C, suggesting that worms at low

Table 1
Results from the five Generalized Linear Mixed Models on the response variables (proportion of progenetic worms, mean worm size, number of eggs expelled, mean egg volume per progenetic worm and coefficient of variation of egg volume) in relation to the predictor variables. The significant main effects are in bold.

Response	Predictor variable	Estimate	S.E.	Effect size
Progenesis	Intercept	−2.574	1.407	−1.83
	Diet	0.724	0.651	1.11
	Temperature: 12–16 °C	**1.160**	**0.579**	**2.00**[a]
	Temperature: 12–20 °C	**2.670**	**1.063**	**2.51**[a]
	Initial host length	−0.177	0.317	−0.56
	Host sex	−0.997	0.715	−1.39
	Change in host body condition	−0.518	0.310	−1.67
	Site: Muscle v head	0.120	0.481	0.25
	Site: Muscle v body Cavity	**2.462**	**0.589**	**4.18**[c]
	Site: Muscle v gonads	**3.422**	**0.803**	**4.26**$_c$
	No. of *Telogaster opisthorchis*	**−0.006**	**0.003**	**−2.33**[a]
	No. of *Apatemon* sp.	−0.001	0.002	−0.52
	No. of *Stegodexamene anguillae*	−0.009	0.006	−1.69
	Diet * temperature	−0.377	0.322	−1.17
	Host sex * site	0.102	0.191	0.54
Worm size	Intercept	−1.248	0.420	−2.97
	Diet	0.290	0.195	1.48
	Temperature: 12–16 °C	0.158	0.178	0.89
	Temperature: 12–20 °C	0.503	0.325	1.55
	Initial host length	−0.114	0.096	−1.19
	Host sex	**−0.515**	**0.112**	**−4.61**[c]
	Change in host body condition	0.046	0.191	0.24
	(Change in host body condition)^2	−0.015	0.021	−0.70
	Site: Muscle v head	0.004	0.099	0.04
	Site: Muscle v body cavity	**0.146**	**0.059**	**2.46**[a]
	Site: Muscle v gonads	**0.287**	**0.132**	**2.18**[a]
	Stage: Non-progenetic-vitellaria	**1.476**	**0.075**	**19.78**[c]
	Stage: Non-progenetic-progenetic	**1.905**	**0.057**	**33.26**[c]
	No. of *T. opisthorchis*	−0.001	0.001	−1.37
	No. of *Apatemon* sp.	0.000	0.000	0.98
	No. of *S. anguillae*	−0.003	0.002	−1.73
	Diet * temperature	−0.127	0.098	−1.29
	Host sex * site	**0.117**	**0.031**	**3.84**[c]
No. of eggs	Intercept	3.719	71.333	0.05
	Diet	0.576	34.894	0.02
	Temperature: 12–16 °C	0.525	30.161	0.02
	Temperature: 12–20 °C	1.099	57.861	0.02
	Initial host length	−0.050	16.814	0.00
	Host sex	−0.182	16.294	−0.01
	Change in host body condition	−1.260	34.715	−0.04
	(Change in host body condition)^2	−5.174	86.746	−0.06
	Site: Muscle v head	0.960	2.320	0.41
	Site: Muscle v body cavity	1.155	2.617	0.44
	Site: Muscle v gonads	1.208	3.488	0.35
	Worm size	0.769	0.400	1.92
	No. of *T. opisthorchis*	−0.003	0.146	−0.02
	No. of *Apatemon* sp.	0.001	0.080	0.01
	No. of *S. anguillae*	−0.003	0.299	−0.01
	Diet * temperature	−0.254	17.044	−0.02
	Host sex * site	−0.092	0.776	−0.12
Egg size	Intercept	7.9e−05	1.8e−05	4.51
	Diet	−7.9e−06	1.2e−05	−0.69
	Temperature: 12–16 °C	−4.5e−06	9.8e−06	−0.46
	Temperature: 12–20 °C	−8.2e−06	1.8e−05	−0.46
	Initial host length	−5.1e−07	3.9e−06	−0.13
	Host sex	1.8e−05	1.2e−05	1.44
	Change in host body condition	1.9e−06	7.9e−06	0.24
	(Change in host body condition)^2	−2.1e−05	2.8e−05	−0.76
	Site: Muscle v head	9.0e−06	8.5e−06	1.06
	Site: Muscle v body cavity	7.8e−06	9.1e−06	0.89
	Site: Muscle v gonads	1.9e−05	1.3e−05	1.49
	Worm size	**1.1e−05**	**2.0e−06**	**5.84**[c]
	No. of eggs per worm	−9.4e−10	3.8e−09	−0.25
	No. of *T. opisthorchis*	6.9e−08	3.5e−08	1.97
	No. of *Apatemon* sp.	−1.4e−09	2.0e−08	−0.07
	No. of *S. anguillae*	9.3e−08	9.0e−08	1.03
	Diet * temperature	3.0e−06	5.5e−06	0.55
	Host sex * site	−4.5e−06	3.2e−06	−1.43
Egg Size CV	Intercept	1.3e−01	4.01e−02	3.14
	Diet	−1.4e−03	1.8e−02	−0.08
	Temperature: 12–16 °C	−8.8e−03	1.5e−02	−0.59
	Temperature: 12–20 °C	−3.1e−02	2.8e−02	−1.12
	Initial host length	2.9e−03	8.9e−03	0.32

Table 1 (*continued*)

Response	Predictor variable	Estimate	S.E.	Effect size
	Host sex	2.2e−02	3.1e−02	0.71
	Change in host body condition	−2.5e−02	1.9e−02	−1.32
	(Change in host body condition)^2	5.8e−02	6.7e−02	0.87
	Site: Muscle v head	−1.2e−02	2.2e−02	−0.56
	Site: Muscle v body cavity	−1.7e−03	2.3e−02	−0.07
	Site: Muscle v gonads	1.5e−03	3.3e−02	0.05
	Worm size	**−1.9e−02**	**5.3e−03**	**−3.60**[c]
	No. of eggs per worm	−2.1e−06	9.8e−06	−0.22
	Egg volume	2.9e+01	1.8e+02	0.12
	No. of *T. opisthorchis*	**1.9e−04**	**8.6e−05**	**2.20**[a]
	No. of *Apatemon* sp.	−9.0e−06	4.7e−05	−0.19
	No. of *S. anguillae*	−3.1e−04	2.0e−04	−1.54
	Diet * temperature	1.9e−03	8.4e−03	0.23
	Host sex * site	−4.6e−03	8.1e−03	−0.57

Level of significance indicated by:
[a] $P = 0.05$.
[b] $P = 0.001$.

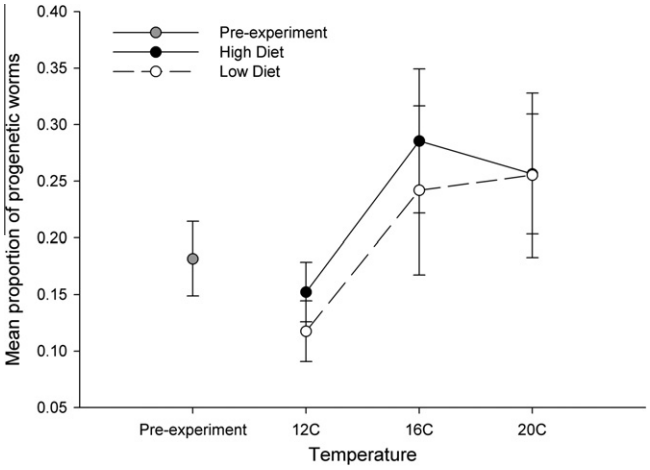

Fig. 1. Proportion of progenetic *Stegodexamene anguillae* in the baseline group and in all experimental groups at different temperature or diet treatments. Error bars indicate the S.E.M.

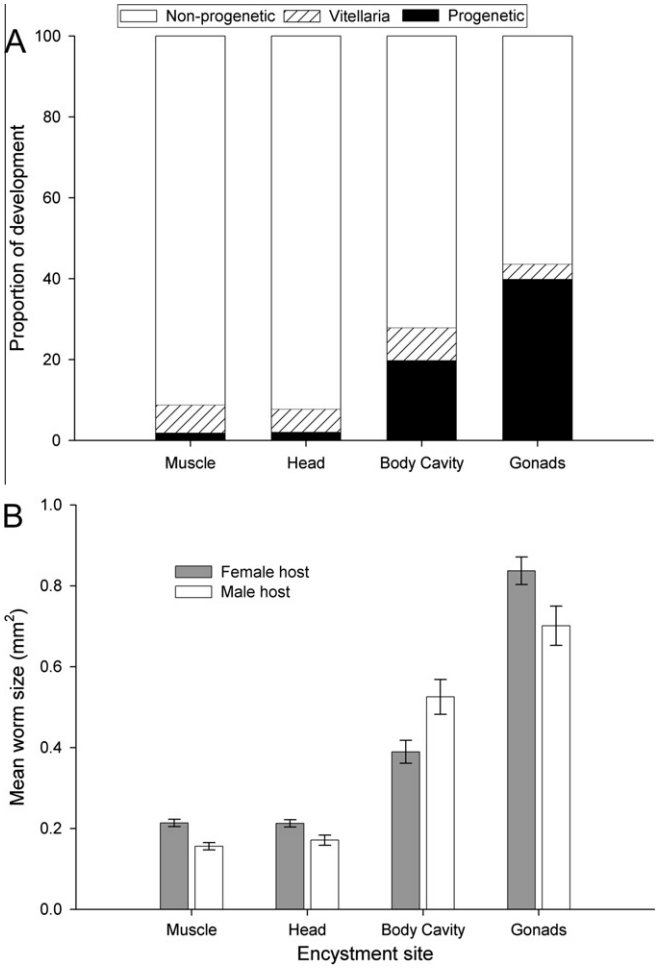

Fig. 2. Relationship between (A) the relative occurrence, expressed as percentage, of developmental stages (non-progenetic, vitellaria-present, progenetic) and (B) worm size (mm^2) of the trematode *Stegodexamene anguillae* in each fish host sex as functions of encystment site for all experimental groups. Error bars indicate the S.E.M.

temperatures did not develop throughout the duration of the experiment. If temperature directly affects growth, and thus development, we would have expected temperature to affect worm size, but that was not the case. Warmer temperatures may be related to increased predation rates since eels are relatively inactive during winter (McDowall, 1990) and thus increased transmission rate, although we found a greater frequency of progenesis at the highest temperature in this experiment. It may be that the increase in abundance of bullies that occurs during warmer months (Stephens, 1982) counteracts the increased foraging of eels and results in similar or even lower predation and transmission rates. Alternatively, temperature could be an indicator of host longevity and the remaining window of opportunity for transfer to a definitive host. Higher temperature lowers fish life span due to increased metabolic rate (Liu and Walford, 1966; Das, 1994). Fish with reduced longevity will be less likely to be preyed upon and the parasites within these fish will be less likely to be transmitted to an eel definitive host. Fish under stressful conditions undergo physiological changes (Barton and Iwama, 1991), and endoparasites access information about host health and stress through the physiology of the host (Thomas et al., 2002; Escobedo et al., 2005). Thus worms may be using temperature directly, or changes in host physiology indirectly, as a cue in a state-dependent response in development and reproduction as the window of transmission narrows with increasing temperature.

Host diet did not seem to have an effect on progenesis, although this could not be thoroughly assessed due to the high level of mortality of fish in the low quantity diet treatment. Even so, the factorial design consisting of three treatments for each diet ration and two replicates of the experiment resulted in a large sample size

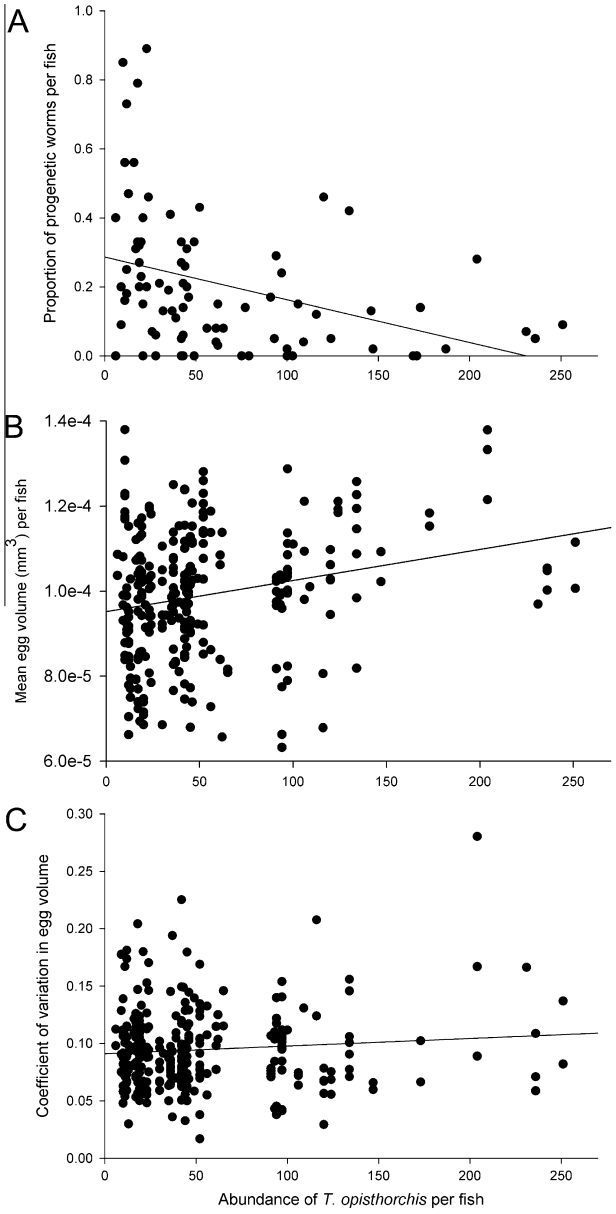

Fig. 3. Relationship between (A) the proportion of progenetic *Stegodexamene anguillae*, (B) mean egg volume per worm per fish host and (C) coefficient of variation in egg volume and the abundance of *Telogaster opisthorchis* metacercariae among fish hosts used in all experimental groups combined.

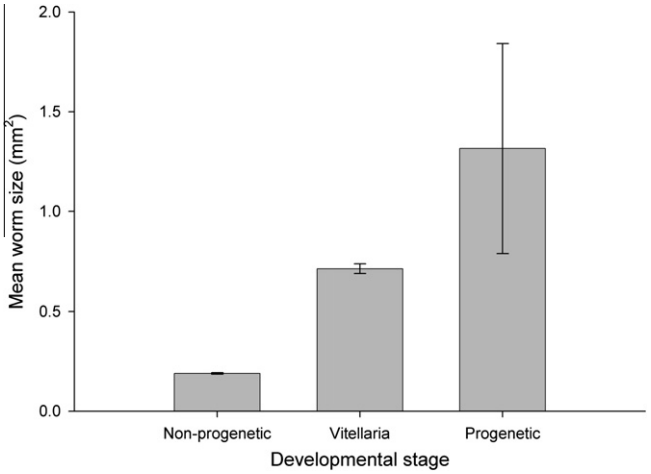

Fig. 4. Mean worm size by development stage for all experimental groups combined. Error bars indicate the S.E.M.

of *S. anguillae* in fish on each diet ration. Although the change in body condition during the experiment was not different among treatments, this was most likely due to fish with lower body condition from the high stress treatments dying during the experiment.

Worms that encyst in the body cavity and gonads were more likely to become progenetic. These tissues are resource-rich, ultimately providing a high level of nutrients to be absorbed by worms for development and egg production. Poulin and Lefebvre (2006) also found a greater proportion of worms in the gonads to be progenetic. The cysts of progenetic worms are of comparable size and shape to fish eggs and are thought to be released during spawning (Lefebvre and Poulin, 2005; Poulin and Lefebvre, 2006). This exit for eggs of progenetic worms encysted in the ovaries is beneficial, since egg dispersal is the major reproductive challenge of progenetic species and is usually possible only via host decomposition or predation for worms encysted elsewhere (Poulin and Cribb, 2002; McLaughlin et al., 2006). The progenetic strategy is highly advantageous for worms encysted in the ovaries because those worms that do not become progenetic would also be expelled during spawning and die without reproducing.

However, this exit strategy may constrain worms in the gonads of male fish to a smaller size because the vas deferens is narrower than the oviduct of female fish (Ross, 1984). Worms in female fish achieved greater sizes than those in male fish when encysted in all tissues except for the body cavity, contrary to the trend of parasites growing larger in male hosts than in females due to steroid hormones (Poulin, 1996). Parasites are capable of perceiving a wide range of signals relating information about their host's biology (Thomas et al., 2002), and *S. anguillae* worms could be using differences in host sex hormones as a signal for growth. Overall, worms in the body cavity and gonads grew to larger sizes than those encysted elsewhere no matter the sex of the host. The better resources provided by the tissues in the body cavity and gonads would not only supply nutrients for parasite development but for extra growth as well. In our experiment, encystment site also affected development, and development affected worm size. Worms grew larger as they proceeded through development into the progenetic stage, reaching a size similar to adults found in eels (Macfarlane, 1951). Progenetic individuals in other trematode species also reach sizes comparable to those of adults in definitive hosts (Lagrue and Poulin, 2007).

Worm size is commonly associated with egg production (Fredensborg and Poulin, 2005; Lagrue and Poulin, 2007). Larger progenetic *S. anguillae* produced more eggs of larger and consistent size, with smaller worms producing fewer, smaller eggs of varying sizes. Variation in egg size is often related to environmental variability (Poulin and Hamilton, 2000), and egg production is likely influenced by resource availability, which should be related to encystment site (Poulin, 1997). It is possible that muscle tissue, where the majority of smaller worms are encysted, is a more heterogeneous environment compared with other tissues. Further, cysts found in the muscle were enveloped in an additional thick capsule of host origin not observed in other tissues (Herrmann, personal observation). This could possibly limit uptake of particular nutrients needed for egg production or block chemical signals normally used by the parasite, making the environment seem less predictable and resulting in varying amounts of investment into eggs to ensure survival of some.

Metacercariae of *T. opisthorchis*, a locally common trematode, had a negative effect on the likelihood of progenesis in *S. anguillae*. Lagrue and Poulin (2008), based on studies of another trematode,

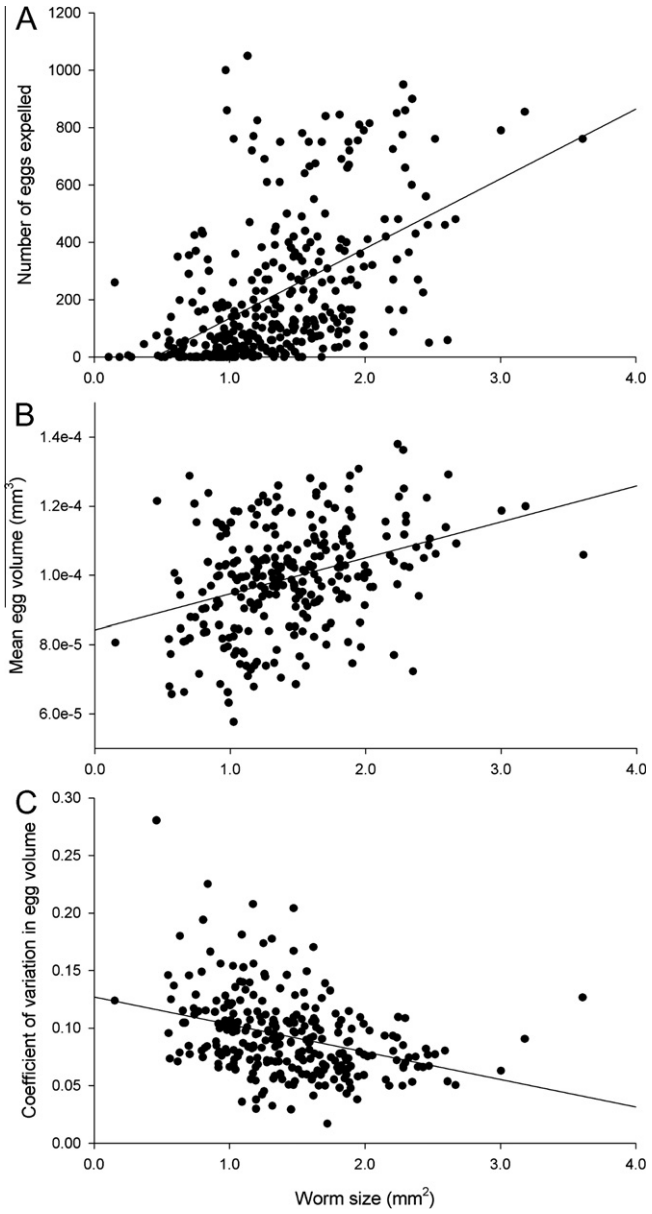

Fig. 5. Relationship between (A) number of eggs expelled per worm, (B) mean egg volume (mm^3) per worm and (C) coefficient of variation in egg volume and worm size for progenetic *Stegodexamene anguillae* only, in fish from all experimental groups combined.

Coitocaecum parvum, suggested that progenetic species may adjust their life cycle strategy according to whether they share their current host with other parasite species and the identity of the latter's definitive host. *Stegodexamene anguillae* and *T. opisthorchis* share a similar life cycle with the same hosts, and both may benefit by reducing exploitation of the current host, ensuring their survival and increasing the probability of transmission to the definitive eel host (Parker et al., 2003b). However, competition with other parasites within a host is commonly due to limited space and energy (Parker et al., 2003b). This is particularly a constraint for progenetic species that absorb nutrients for growth, development and egg production (Brown et al., 2003; Fredensborg and Poulin, 2005). The overcrowding effect of strong competition from other highly abundant parasite species would be expected to negatively impact a progenetic species, as seen here by the lowered proportion of progenesis of *S. anguillae* and investment into varying egg sizes that were overall larger in fish also harbouring many *T. opisthorchis*.

The facultative nature of the two life cycle strategies may indicate the plasticity of an adaptive phenotypic response to variable environmental conditions (Poulin and Cribb, 2002; Thomas et al., 2002; Poulin, 2003), similar to phenotypic variation found in free-living species (Gotthard and Nylin, 1995; Van Buskirk and Schmidt, 2000; Seigel and Ford, 2001). For instance, phenotypic variation in specific morphological traits of tadpoles is associated with local levels of predation (Van Buskirk and McCollum, 1999); likewise, variation in life history traits of trematodes may be associated with local levels of transmission. Our results suggest that progenesis in *S. anguillae* may be a conditional response to certain environmental factors, such as temperature and encystment site, signalling opportunities of transmission. These findings provide further evidence that parasite development is characterised by much greater adaptive plasticity than previously thought.

Acknowledgements

We thank K. Garrett and B. Presswell for laboratory assistance and members of the Evolutionary and Ecological Parasitology Research Group at the University of Otago, New Zealand for providing feedback on earlier versions of this paper. Handling and treatment of animals in this study was approved by the University of Otago's Animal Ethics Committee (Application No. 15/08). K.K.H. was funded by an University of Otago Postgraduate Scholarship.

References

Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Ann. Rev. Fish Dis. 1, 3–26.
Bolger, T., Connolly, P.L., 1989. The selection of suitable indices for the measurement and analysis of fish condition. J. Fish Biol. 34, 171–182.
Brown, S.P., De Lorgeril, J., Joly, C., Thomas, F., 2003. Field evidence for density-dependent effects in the trematode *Microphallus papillorobustus* in its manipulated host, *Gammarus insensibilis*. J. Parasitol. 89, 668–672.
Charlesworth, D., Charlesworth, B., 1987. Inbreeding depression and its evolutionary consequences. Annu. Rev. Ecol. Syst. 18, 237–268.
Choisy, M., Brown, S.P., Lafferty, K.D., Thomas, F., 2003. Evolution of trophic transmission in parasites: why add intermediate hosts? Am. Nat. 162, 172–181.
Christen, M., Kurtz, J., Milinski, M., 2002. Outcrossing increases infection success and competitive ability: experimental evidence from a hermaphrodite parasite. Evolution 56, 2243–2251.
Combes, C., 1991. Ethological aspects of parasite transmission. Am. Nat. 138, 866–880.
Cribb, T.H., Bray, R.A., Olson, P.D., Littlewood, D.T.J., 2003. Life cycle evolution in the Digenea: a new perspective from phylogeny. Adv. Parasitol. 54, 197–254.
Das, M., 1994. Age determination and longevity in fishes. Gerontology 40, 70–96.
Escobedo, G., Roberts, C.W., Carrero, J.C., Morales-Montor, J., 2005. Parasite regulation by host hormones: an old mechanism of host exploitation? Trends Parasitol. 21, 588–593.
Fredensborg, B.L., Poulin, R., 2005. In vitro cultivation of *Maritrema novaezealandensis* (Microphallidae): the effect of culture medium on excystation, survival and egg production. Parasitol. Res. 95, 310–313.
Goldsmith, R.J., Closs, G.P., Steen, H., 2003. Evaluation of visible implant elastomer for individual marking of small perch and common bully. J. Fish Biol. 63, 631–636.
Gotthard, K., Nylin, S., 1995. Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. Oikos 74, 3–17.
Hall, C.J., Burns, C.W., 2002. Mortality and growth responses of *Daphnia carinata* to increases in temperature and salinity. Freshwater Biol. 47, 451–458.
Hine, P.M., 1978. Distribution of some parasites of freshwater eels in New Zealand. New Zeal. J. Mar. Fresh 12, 179–187.
Holton, A.L., 1984. Progenesis as a means of abbreviating life histories in two New Zealand trematodes, *Coitocaecum parvum* Crowcroft, 1945 and *Stegodexamene anguillae* MacFarlane, 1951. Mauri. Ora 11, 63–70.
Kearn, G.C., 1998. Parasitism and the Platyhelminths, first ed. Chapman & Hall, London, New York.
Lagrue, C., Poulin, R., 2007. Life cycle abbreviation in the trematode *Coitocaecum parvum*: can parasites adjust to variable conditions? J. Evol. Biol. 20, 1189–1195.
Lagrue, C., Poulin, R., 2008. Intra- and interspecific competition among helminth parasites: effects on *Coitocaecum parvum* life history strategy, size and fecundity. Int. J. Parasitol. 38, 1435–1444.

Lagrue, C., Poulin, R., Keeney, D.B., 2009. Effects of clonality in multiple infections on the life-history strategy of the trematode *Coitocaecum parvum* in its amphipod intermediate host. Evolution 63, 1417–1426.

Lefebvre, F., Poulin, R., 2005. Progenesis in digenean trematodes: a taxonomic and synthetic overview of species reproducing in their second intermediate hosts. Parasitology 130, 587–605.

Liu, R.K., Walford, R.L., 1966. Increased growth and life-span with lowered ambient temperature in the annual fish, *Cynolebias adloffi*. Nature 212, 1277.

Macfarlane, W.V., 1951. The life cycle of *Stegodexamene anguillae* n.g., n. sp., an allocreadiid trematode from New Zealand. Parasitology 41, 1–10.

Macfarlane, W.V., 1952. Bionomics of two trematode parasites of New Zealand eels. J. Parasitol. 38, 391–397.

McDowall, R.M., 1990. New Zealand freshwater fishes: a natural history and guide. MAF Publishing Group, Wellington, New Zealand.

McLaughlin, J.D., Marcogliese, D.J., Kelly, J., 2006. Morphological, developmental and ecological evidence for a progenetic life cycle in *Neochasmus* (Digenea). Folia Parasit. 53, 44–52.

Moore, J., 2002. Parasites and the Behavior of Animals. Oxford Press, Oxford, UK.

Parker, G.A., Chubb, J.C., Ball, M.A., Roberts, G.N., 2003a. Evolution of complex life cycles in helminth parasites. Nature 425, 480–484.

Parker, G.A., Chubb, J.C., Roberts, G.N., Michaud, M., Milinski, M., 2003b. Optimal growth strategies of larval helminths in their intermediate hosts. J. Evol. Biol. 16, 47–54.

Poulin, R., 1995. "Adaptive" changes in the behaviour of parasitised animals: a critical review. Int. J. Parasitol. 25, 1371–1383.

Poulin, R., 1996. Helminth growth in vertebrate hosts: does host sex matter? Int. J. Parasitol. 26, 1311–1315.

Poulin, R., 1997. Egg production in adult trematodes: adaptation or constraint? Parasitology 114, 195–204.

Poulin, R., 2001. Progenesis and reduced virulence as an alternative transmission strategy in a parasitic trematode. Parasitology 123, 623–630.

Poulin, R., 2003. Information about transmission opportunities triggers a life-history switch in a parasite. Evolution 57, 2899–2903.

Poulin, R., 2007. Evolutionay Ecology of Parasites, second ed. Princeton University Press, Princeton, NJ, USA.

Poulin, R., Cribb, T.H., 2002. Trematode life cycles: short is sweet? Trends Parasitol. 18, 176–183.

Poulin, R., Hamilton, W.J., 2000. Egg size variation as a function of environmental variability in parasitic trematodes. Can. J. Zool. 78, 564–569.

Poulin, R., Lefebvre, F., 2006. Alternative life-history and transmission strategies in a parasite: first come, first served? Parasitology 132, 135–141.

Richardson, J., Boubee, J.A.T., West, D.W., 1994. Thermal tolerance and preference of some native New Zealand freshwater fish. New Zeal. J. Mar. Fresh 28, 399–407.

Ross, R.M., 1984. Anatomical changes associated with sex reversal in the fish *Thalassoma duperrey* (Teleostei: Labridae). Copeia 1984, 245–248.

Schell, S.C., 1970. How to know the trematodes. Wm. C. Brown Company Publishers, Iowa, USA.

Seigel, R.A., Ford, N.B., 2001. Phenotypic plasticity in reproductive traits: geographical variation in plasticity in a viviparous snake. Funct. Ecol. 15, 36–42.

Stephens, R.T.T., 1982. Reproduction, growth and mortality of the common bully, *Gobiomorphus cotidianus* McDowall, in a eutrophic New Zealand lake. J. Fish Biol. 20, 259–270.

Thomas, F., Brown, S.P., Sukhdeo, M., Renaud, F., 2002. Understanding parasite strategies: a state-dependent approach? Trends Parasitol. 18, 387–390.

Van Buskirk, J., McCollum, S.A., 1999. Plasticity and selection explain variation in tadpole phenotype between ponds with different predator composition. Oikos 85, 31–39.

Van Buskirk, J., Schmidt, B.R., 2000. Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. Ecology 81, 3009–3028.