Encystment site affects the reproductive strategy of a progenetic trematode in its fish intermediate host: is host spawning an exit for parasite eggs?

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SUMMARY

Each transmission event in complex, multi-host life cycles create obstacles selecting for adaptations by trematodes. One such adaptation is life cycle abbreviation through progenesis, in which the trematode precociously matures and reproduces within the second intermediate host. Progenesis eliminates the need for the definitive host and increases the chance of life cycle completion. However, progenetic individuals face egg-dispersal challenges associated with reproducing within metacercarial cysts in the tissues or body cavity of the second intermediate host. Most progenetic species await host death for their eggs to be released into the environment. The present study investigated temporal variation of progenesis in *Stegodexamene anguillae* in one of its second intermediate fish hosts and the effect of the fish's reproductive cycle on progenesis. The study involved monthly sampling over 13 months at one locality. A greater proportion of individuals became progenetic in the gonads of female fish hosts. Additionally, progenesis of worms in the gonads was correlated with seasonal daylight and temperature changes, major factors controlling fish reproduction. Host spawning events are likely to be an avenue of egg dispersal for this progenetic species, with the adoption of progenesis being conditional on whether or not the parasite can benefit from fish spawning.

Key words: abbreviated life cycle, *Stegodexamene anguillae*, encystment site, reproductive strategies, complex life cycle.

INTRODUCTION

Complex life cycles with multiple hosts and life stages have evolved in numerous lineages of parasites (Choisy *et al*. 2003). Each transmission event to a subsequent host presents an obstacle for completion of the life cycle (Kearn, 1998; Choisy *et al*. 2003). In response to the selective pressures of transmission to the next host, trematodes have evolved various adaptations, including high fecundity, efficient host-finding mechanisms, extended longevity within intermediate hosts, and the ability to alter host behaviour (Combes, 1991; Poulin, 1995; Moore, 2002; Parker *et al*. 2003; Poulin, 2007). Another adaptation is to truncate the life cycle via progenesis, i.e. precocious maturity in the second intermediate host (Poulin, 2001; Poulin and Cribb, 2002). Progenetic trematodes reproduce via self-fertilization and produce eggs within the metacercarial cyst, eliminating the need for transmission to a definitive host. Progenesis has evolved in numerous parasite lineages and can be obligatory or facultative, with individuals in the same population adopting either life cycle strategy, the 'normal' 3-host or the abbreviated cycle (Poulin and Cribb, 2002; Lefebvre and Poulin, 2005). The plasticity of facultative progenesis is likely to be advantageous when the probability of transmission to the definitive host is low (Poulin and Cribb, 2002), allowing for reproduction in unpredictable or unstable environments. Previous studies have dmonstrated that the plasticity of progenesis is driven by a variety of environmental factors, such as cues from the definitive host, age of the current intermediate host, encystment site, intra-host competition and genetic-relatedness of co-infecting parasites (Poulin, 2003; Lagrue and Poulin, 2007, 2008, 2009; Poulin and Lefebvre, 2006; Lagrue *et al*. 2009). This shows that some parasites are capable of perceiving a wide range of cues and accurately adjusting their reproductive strategy according to their transmission opportunities.

For *Stegodexamene anguillae* (Lepocreadiidae), the typical 3-host life cycle begins with ciliated miracidia hatching from eggs and infecting a snail, *Potamopyrgus antipodarum*, as the first intermediate host. Within the snail, asexual reproduction occurs and results in numerous cercariae, which 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. At this point, metacercariae can either await ingestion by a definitive host, *Anguilla dieffenbachia* (New Zealand longfin eel) or *A. australis* (short-finned eel), develop into adults and sexually

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reproduce, or develop progenetically into the adult stage and reproduce by self-fertilization within the tissue of the second intermediate host (Macfarlane, 1951; Holton, 1984). The progenetic strategy allows the trematode to bypass transmission to the eel definitive host.

The major challenge of progenesis is the release of eggs into the environment from inside a second intermediate host (Poulin and Cribb, 2002). For most progenetic species this may entail waiting for the host to die, either naturally followed by decay or by predation. However, *S. anguillae* may have evolved a solution to this problem. Macfarlane (1951) only found progenetic worms in *Gobiomorphus cotidianus* (common bully) 3–4 cm in size, indicating worms are more likely to undergo progenesis in bullies of reproductive age. Additionally, Poulin and Lefebvre (2006) found the gonads of the fish second intermediate host to harbour a greater proportion of progenetic worms than other tissue, and suggested that eggs of *S. anguillae* are released when the intermediate fish host spawns.

The present study examines temporal variation in environmental variables, both biotic and abiotic, related to the probability of transmission and the growth, development and reproduction of *S. anguillae* metacercariae. The main objective was to investigate the possible relationship between the occurrence of progenesis and the reproductive cycle of the second intermediate host, common bully, using water temperature and day length as variables correlated with fish reproduction. This is one of few studies investigating the plasticity of parasite reproductive strategies under natural conditions, aiming specifically to relate peaks in progenesis with seasonal opportunities for the exit of eggs of progenetic worms.

MATERIALS AND METHODS

Animal collection

In total, 13 monthly samples were collected between September 2008 and September 2009 in Lake Waihola, South Island, New Zealand (46°00′S, 170°06′E). Lake Waihola is a shallow, eutrophic coastal lake (Schallenberg and Burns, 2003). Common bullies have been reported as a dominant fish species in Lake Waihola (Kattel, 1999; Jeppesen *et al.* 2000) and are the main second intermediate host in the lake. In the breeding season, females spawn twice, and some possibly 3 times, from September through March (Stephens, 1982). Collection days occurred within 1 week of the first day of each month from the same site, under similar weather conditions (light or no winds) and at low tide (connected to sea via river) to reduce variation. Relative bully abundance was measured by counting the numbers of fish captured using a seine net to enclose a fixed area (7·0 m^2) and push nets (mesh size 5 mm) to catch the fish. Any remaining fish were captured when retrieving the seine net. Common bullies $\geqslant$4 cm in total length (reproductive age; Stephens, 1982) were kept, while those <4 cm were released. If less than 20 bullies $\geqslant$4 cm in size were captured, an electric fishing machine, a seine net and/or push nets were then used to obtain 20 bullies in total. Water temperature was recorded on each collection date. Fish were transported to the laboratory, euthanized by an overdose of tricaine methanesulfonate (MS-222), and frozen until dissection.

Measures and statistical analyses

Total length, weight and sex of each fish were recorded. Fish body condition was determined by W/L^3, where W and L are the weight and total length (Bolger and Connolly, 1989). Fish were dissected, and all tissues, except the brain and the lumen of the gastrointestinal tract (where metacercariae of *S. anguillae* are never found), were 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 well-developed yolk-producing glands). The body surface of each worm was calculated as a surrogate for body size by using the formula for area 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 (80X). 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 random subsample of 10 eggs from each progenetic worm were measured, and assuming a regular ellipsoid shape, egg volume was calculated as $(\pi LW^2)/6$. The coefficient of variation in egg volume was calculated as the mean egg volume divided by the standard deviation. Variation in the sex ratio of fish hosts among sampling dates was assessed with an ANOVA. Differences in fish length between sexes were assessed with a *t*-test.

Six response variables were assessed using Generalized Linear Mixed Models (GLMM) within an Akaike information criteria (AIC) and model averaging framework (Burnham and Anderson, 2002; see Table 1). First, progenesis in all tissues was the response variable in a GLMM fitted with a binomial error structure. Factors possibly influencing the parasite's developmental strategy i.e. non-progenetic (including worms with vitellaria) versus progenetic, were included in the GLMM to determine the effect of difference in daylight (a surrogate for date that is biologically relevant to fish reproduction) between the collection date and the previous collection date, mid-point of relative bully abundance between the

Table 1. The predictor variables included in the global model of each response variable

Predictor variables	Progenesis	Progenesis in gonads	Worm size	No. of eggs	Egg volume	CV of egg volume
Difference in daylight	√	√	√	√	√	√
Bully abundance	√	√	√	√	√	√
Water temperature	√	√	√	√	√	√
Host body condition	√	√	√	√	√	√
Host length	√	√	√	√	√	√
Host sex	√	√	√	√	√	√
Daylight*host sex	√	√	√	√	√	√
Encystment site	√		√	√	√	√
Abundance of conspecifics	√	√	√	√	√	√
Abundance of *T. opisthorchis*	√	√	√	√	√	√
Abundance of *Apatemon* sp.	√	√	√	√	√	√
Developmental strategy			√			
Worm size				√	√	√
No. of eggs expelled					√	√
Egg volume						√

collection date and the previous collection date, midpoint of water temperature between the collection date and the previous collection date, host body condition, host total length, host sex, encystment site (muscle, head, body cavity, or gonads), mean abundance of conspecifics and mean abundance of two other common species of trematode metacercariae (*Telogaster opisthorchis* and *Apatemon* sp.). The interaction between difference in daylight and host sex was included to assess any change in the effect of host sex over time (Table 1). Interactions between host sex and encystment site and between host sex and temperature were included preliminarily, however inclusion of these interactions resulted in similar model averaging and thus were not retained in order to achieve a simpler global model. Individual fish identity was added as a random factor to account for many *S. anguillae* sharing the same host individual. This set of variables was also used in the following analyses in addition to any other specified (Table 1). Second, another GLMM fitted with a binomial error structure was performed on developmental strategy using only the subset of *S. anguillae* worms found in the gonads, and thus, encystment site was removed from the variable set. Third, worm size data were log-transformed to approach normality and were used as response variable in a GLMM fitted with the Gaussian (normal) distribution. All predictors in the progenesis GLMM were included with the addition of developmental strategy (non-progenetic, vitellaria-present and progenetic). Fourth, those same predictor variables were included in a GLMM, fitted with a quasi-Poisson distribution, on number of eggs expelled per progenetic worm as the response variable with the addition of worm size as an extra factor. Fifth, a GLMM with mean egg volume per progenetic worm as response variable included the additional factors of worm size and number of eggs expelled and was fitted with a Gaussian distribution. Finally, the coefficient of variation in mean egg volume was a response variable in a GLMM, fitted with the Gaussian distribution, and included the additional factors of worm size, number of eggs expelled and mean egg volume.

Global models were fitted using the package lme4 (Bates and Maechler, 2009) in the program R (R Development Core Team, 2009). The global model was then used to generate a set of all possible models, with functions from the R package MuMIn (Bartoñ, 2009). Each model in the set was ranked by AIC_C and model averaging using MuMIn was performed on all models within 2 AIC_C of the best model. There was no difference in the final results if model averaging was done on models within 8 AIC_C of the best model. In the analysis on number of eggs expelled using a quasi-Poisson distribution, $QAIC_C$ was used to rank the models. The predictor variables in the top models are reported with their relative importance weights, model-averaged parameter estimates, unconditional standard error and 95% confidence intervals. When only one top model emerged, that model is reported.

RESULTS

In total, 247 common bullies were collected, from which 4941 *S. anguillae* worms were measured (Table 2). The host sex ratio varied among the monthly samples ($F_{12,242} = 2{\cdot}24$, $P = 0{\cdot}011$; Table 2). Fish length did not differ between sexes ($t = 0{\cdot}86$, D.F. = 240, $P = 0{\cdot}390$). The average total length was $5{\cdot}0 \pm 0{\cdot}09$ cm and $4{\cdot}9 \pm 0{\cdot}06$ cm for females and males, respectively, and total length ranged from 3·9 to 7·4 cm for both sexes. Relative abundance of bullies at the collection site peaked sharply in January (Fig. 1a). Water temperature varied between 4·3 °C and 18·1 °C on the August and February collection dates, respectively (Fig. 1a).

Table 2. Number and sex ratio of common bullies collected, and total number of *Stegodexamene anguillae* measured from each monthly sample

Collection date	Common bully	Host sex ratio (males/females)	*S. anguillae*
Sept. 2008	18	0·80	511
Oct.	20	0·60	1042
Nov.	20	0·46	349
Dec.	20	0·78	419
Jan. 2009	20	6·50	377
Feb.	20	1·11	215
Mar.	19	4·33	494
April	20	1·38	157
May	19	4·00	147
June	20	0·55	302
July	20	0·80	278
Aug.	12	1·50	276
Sept.	19	1·57	374
Total	247	1·18	4941

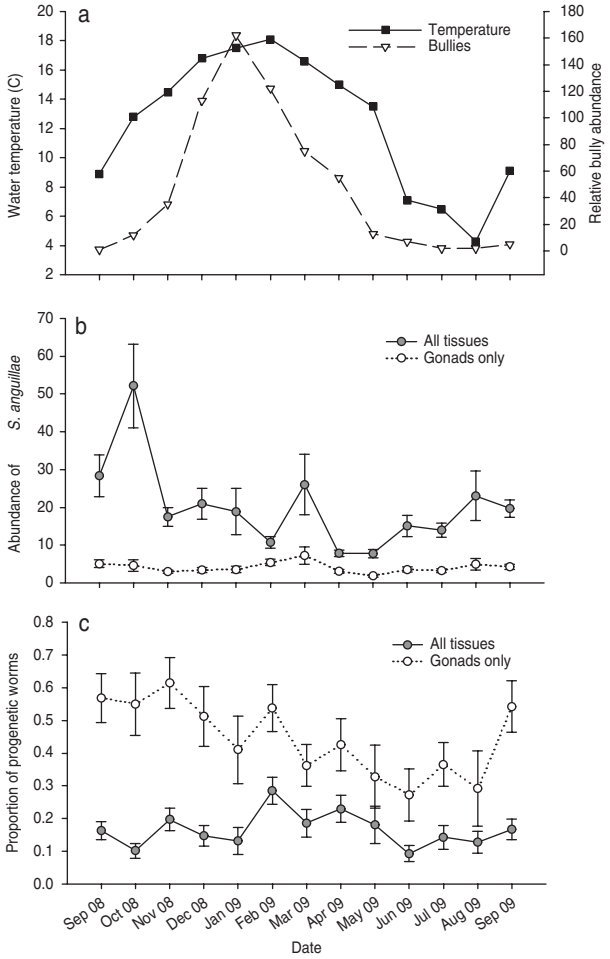

Fig. 1. Annual variation of (a) water temperature (°C) and relative abundance of the second intermediate host, common bully, (b) mean abundance of *Stegodexamene anguillae* in all tissues and in the gonads only, and (c) proportion of progenetic worms in all tissues and in the gonads only. Error bars indicate the standard error of the mean.

Prevalence was 100%, except for the January collection when prevalence was 95·0%. The mean abundance (± S.E.) of *S. anguillae* varied from the lowest in May at 7·7 ± 1·11 per fish to highest in October at 52·2 ± 11·05 (Fig 1b.). On average, progenetic worms comprised 13·9% of all *S. anguillae* worms, ranging from 10·2 ± 2·27 to 28·5 ± 0·04% in October and February, respectively (Fig. 1c). When all worms were considered, model analysis on progenesis resulted in 16 top models within 2 AIC_C of the best model (Table 3). With the exception of the difference in daylight and host sex interaction, all other explanatory variables considered in the global model were included in at least 1 model in the top model set, with encystment site as the only predictor variable with a 95% confidence interval bounded away from zero (Tables 3 and 4). There was a greater proportion of progenetic worms in the body cavity and the gonads than in the muscle, which was no different than the head (Fig. 2a).

Analysis of the subset of worms in the gonads showed a mean abundance (± S.E.) of *S. anguillae* being highest in March (7·3 ± 2·27) and decreasing in May (1·9 ± 0·33; Fig. 1b). The percentage of progenetic worms in the gonads varied from 27·2 ± 7·96 to 61·5 ± 7·77% in June and November, respectively (Fig. 1c). The top models included 12 models (Table 3), again retaining all predictor variables from the global model except for the interaction between difference in daylight and host sex (Table 4). Host sex emerged as the most robust variable affecting progenesis in worms in the gonads indicated by both a relative importance weight of 1·00 and a 95% confidence interval bounded away from zero (Table 4). Worms in the gonads of female fish were more likely to be progenetic than those in the gonads of a male (Fig. 3a). The confidence intervals of 2 other variables, difference in daylight and water temperature, did not include zero but had lower relative importance weights. Progenesis in the gonads increased when the difference in daylight since the previous collection date also increased (Fig. 3b) and with increasing water temperature (Fig. 3c).

Mean worm size (± S.E.) was 0·41 ± 0·006 mm^2 for all worms but varied widely from 0·01 to 3·50 mm^2. Only one top model emerged (Table 3), with a difference of over 19 AIC_C to the next closest ranking model. Developmental stage and encystment site were the only predictors in this top model explaining worm size (Table 4). Worms increased in size as they developed from non-progenetic to progenetic; those worms with well-developed vitellaria were of intermediate size (Fig. 2b). Worms encysted in the body cavity and gonads grew to larger sizes than those in the muscle (Fig. 2b).

The mean (± S.E.) number of eggs expelled by progenetic worms was 229·8 ± 10·81 and ranged from zero (from those worms with eggs *in utero* only) to

Table 3. The top-ranked candidate models for each response variable

(If more than one model within 2 AIC_C of the top model emerged, then models were sorted by corrected Akaike information criteria (AIC_C or $QAIC_C$), with model deviance, AIC_C (or $QAIC_C$), difference in AIC_C from the best model (ΔAIC_C) and weight (AIC_W) values given for each model.)

Response	Model	Deviance	AIC_C	ΔAIC_C	AIC_W
Progenesis	Body condition + sex + site + *Apatemon* + *T. opisthorchis*	1757·85	1775·88	0·00	0·11
	Body condition + sex + site + fish length + *T. opisthorchis*	1757·86	1775·90	0·02	0·11
	Body condition + sex + site + *S. anguillae* + *T. opisthorchis*	1758·14	1776·18	0·30	0·09
	Body condition + sex + site + *Apatemon* + *S. anguillae* + *T. opisthorchis*	1756·45	1176·49	0·61	0·08
	Body condition + sex + site + fish length + *S. anguillae* + *T. opisthorchis*	1756·74	1776·79	0·90	0·07
	Body condition + sex + site + *Apatemon* + fish length + *T. opisthorchis*	1756·82	1776·86	0·98	0·07
	Body condition + sex + site + *T. opisthorchis*	1760·99	1777·02	1·13	0·06
	Body condition + daylight difference + sex + site + fish length + *T. opisthorchis*	1757·33	1777·37	1·49	0·05
	Body condition + daylight difference + sex + site + *Apatemon* + *T. opisthorchis*	1757·38	1777·42	1·54	0·05
	Body condition + bully abundance + sex + site + fish length + *T. opisthorchis*	1757·41	1777·45	1·57	0·05
	Body condition + bully abundance + sex + site + *Apatemon* + *T. opisthorchis*	1757·47	1777·52	1·63	0·05
	Body condition + sex + site + fish length + *T. opisthorchis* + temperature	1757·55	1777·59	1·71	0·05
	Body condition + sex + site + *Apatemon* + *T. opisthorchis* + temperature	1757·64	1777·68	1·80	0·04
	Body condition + daylight difference + sex + site + *S. anguillae* + *T. opisthorchis*	1757·66	1777·71	1·82	0·04
	Body condition + bully abundance + sex + site + *S. anguillae* + *T. opisthorchis*	1757·75	1777·80	1·91	0·04
	Body condition + sex + site + *S. anguillae* + *T. opisthorchis* + temperature	1757·80	1777·84	1·96	0·04
Progenesis in gonads	Body condition + daylight difference + sex + *Apatemon* + *T. opisthorchis* + temperature	966·11	982·25	0·00	0·16
	Body condition + bully abundance + daylight difference + sex + *Apatemon* + *T. opisthorchis*	967·53	982·68	0·43	0·13
	Body condition + daylight difference + sex + fish length + *T. opisthorchis* + temperature	967·03	983·17	0·92	0·10
	Body condition + daylight difference + sex + *Apatemon* + *S. anguillae* + *T. opisthorchis* + temperature	965·34	983·52	1·27	0·08
	Body condition + daylight difference + sex + *Apatemon* + fish length + *T. opisthorchis* + temperature	965·40	983·58	1·33	0·08
	Body condition + daylight difference + sex + *S. anguillae* + *T. opisthorchis* + temperature	967·59	983·74	1·49	0·07
	Body condition + bully abundance + daylight difference + sex + fish length + *T. opisthorchis*	967·60	983·75	1·49	0·07
	Body condition + bully abundance + sex + *Apatemon* + *T. opisthorchis*	969·79	983·90	1·65	0·07
	Body condition + bully abundance + daylight difference + sex + *Apatemon* + fish length + *T. opisthorchis*	965·93	984·12	1·86	0·06
	Body condition + bully abundance + daylight difference + sex + *Apatemon* + *S. anguillae* + *T. opisthorchis*	965·98	984·17	1·91	0·06
	Body condition + bully abundance + daylight difference + sex + *Apatemon* + *T. opisthorchis* + temperature	966·02	984·20	1·95	0·06
	Body condition + daylight difference + sex + fish length + *S. anguillae* + *T. opisthorchis* + temperature	966·04	984·22	1·97	0·06
Worm size	Development + site	−12955·00	−12889·00	–	1·00
No. of eggs	Body condition + worm size	57730·28	120·48	0·00	0·42
	Body condition + worm size + sex	57404·60	121·95	1·46	0·20
	Body condition + worm size + *Apatemon*	57459·03	122·05	1·56	0·19
	Body condition + worm size + *T. opisthorchis*	57503·18	122·13	1·65	0·18
Egg volume	Intercept only	−9502·00	−9470·00	–	1·00
CV egg volume	Egg volume	−1733·00	−1726·00	–	1·00

Table 4. Predictor variables from top models for each response variable

(Relative importance weights ($w_+(i)$), coefficient estimates, their unconditional standard error (S.E.) and 95% confidence interval (CI) after model averaging. Values from top model reported for response variables with only one top model. The significant main effects are given in bold.)

Response	Predictor variable	$w_+(i)$	Estimate	S.E.	95% CI
Progenesis	Intercept	–	−4·730	1·130	−6·950 to −2·500
	Site: muscle v head	1·00	0·218	0·371	−0·508 to 0·945
	Site: muscle v body cavity	**"**	**2·820**	**0·311**	**2·210 to 3·430**
	Site: muscle v gonads	**"**	**4·010**	**0·298**	**3·430 to 4·590**
	Host body condition	1·00	−8·920	116·000	−237·000 to 219·000
	Host sex	1·00	−0·099	0·174	−0·441 to 0·243
	No. of *T. opisthorchis*	1·00	−0·002	0·003	−0·007 to 0·003
	No. of *Apatemon* sp.	0·40	0·002	0·001	−0·001 to 0·004
	Host length	0·39	0·224	0·149	−0·068 to 0·517
	No. of *S. anguillae*	0·37	0·006	0·004	−0·002 to 0·015
	Difference in daylight	0·14	0·001	0·001	−0·001 to 0·003
	Relative bully abundance	0·14	0·001	0·002	−0·002 to 0·005
	Temperature	0·13	0·011	0·020	−0·028 to 0·049
Progenesis in gonads	Intercept	–	−1·190	1·480	−4·100 to 1·720
	Host sex	**1·00**	**−0·442**	**0·220**	**−0·873 to −0·011**
	Host body condition	1·00	9·830	155·000	−295·000 to 314·000
	No. of *T. opisthorchis*	1·00	−0·007	0·004	−0·014 to 0·0004
	Difference in daylight	**0·93**	**0·004**	**0·002**	**0·0001 to 0·007**
	No. of *Apatemon* sp.	0·69	0·003	0·002	−0·0004 to 0·007
	Temperature	**0·61**	**0·063**	**0·032**	**0·001 to 0·126**
	Relative bully abundance	0·45	0·004	0·003	−0·001 to 0·010
	Host length	0·37	0·260	0·208	−0·148 to 0·669
	No. of *S. anguillae*	0·28	0·006	0·006	−0·005 to 0·018
Worm size	Intercept	–	−0·018	0·003	−0·024 to −0·012
	Developmental stage	–	**0·104**	**0·002**	**0·101 to 0·107**
	Site: muscle v head	–	0·002	0·002	−0·002 to 0·005
	Site: muscle v body cavity	–	**0·021**	**0·003**	**0·014 to 0·027**
	Site: muscle v gonads	–	**0·044**	**0·003**	**0·038 to 0·050**
No. of eggs	Intercept	–	16·709	290·065	−551·819 to 585·237
	Worm size	1·00	0·541	0·422	−0·285 to 1·368
	Host body condition	1·00	−1·4e+03	3·2e+06	−6·2e+06 to 6·2e+06
	Host sex	0·20	−0·692	3·868	−8·273 to 6·888
	No. of *Apatemon* sp.	0·19	0·004	0·011	−0·019 to 0·026
	No. of *T. opisthorchis*	0·18	0·013	0·034	−0·054 to 0·080
Egg volume	Intercept	–	8·3e–05	8·7e–07	8·1e−05 to 8·5e−05
CV of egg volume	Intercept	–	0·201	0·010	0·181 to 0·221
	Egg volume	–	**−998·143**	**119·277**	**−1236·697 to −759·588**

1413. Four top models emerged within 2 $QAIC_C$ of the top model (Table 3). Worm size and host body condition were included in all 4 models; however, neither variable had a 95% confidence interval bounded away from zero (Table 4). Host sex, abundance of *Apatemon* sp. and abundance of *T. opisthorchis* were each included in one model in the top model set, but all included zero in the 95% confidence interval (Tables 3 and 4).

The mean (± S.E.) egg volume was 8·3e-05 ± 7·21e-07 mm^3 (n=4877). The intercept-only model emerged as the top model with the next closest model differing by over 23 AIC_C (Table 3). None of the variables measured explain the variation in egg size. However, the coefficient of variation in egg volume is explained by egg volume as the only predictor in the one top model (Tables 3 and 4). The next closest model differed by over 8 AIC_C. As mean egg size increased the coefficient of variation tended to decrease (Fig. 4).

DISCUSSION

Parasites showing plasticity in developmental strategies increase the probability of completing their life cycle. Facultative life-cycle abbreviation enables

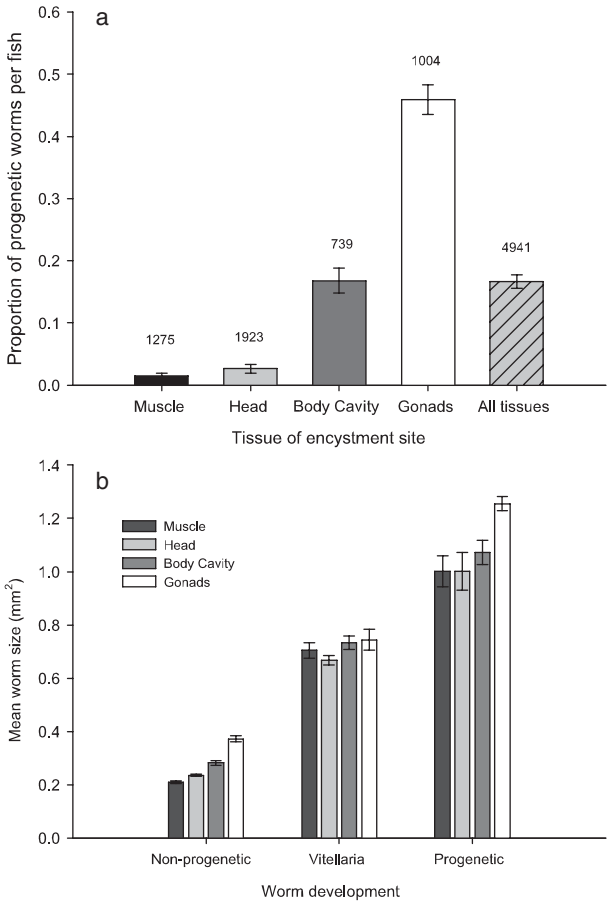

Fig. 2. (a) Proportion of progenetic *Stegodexamene anguillae* within each tissue of encystment with number of *S. anguillae* in each tissue shown above bars and (b) mean worm size (mm^2) as a function of developmental stage and tissue of encystment in the second intermediate host, common bully. Error bars indicate the standard error of the mean.

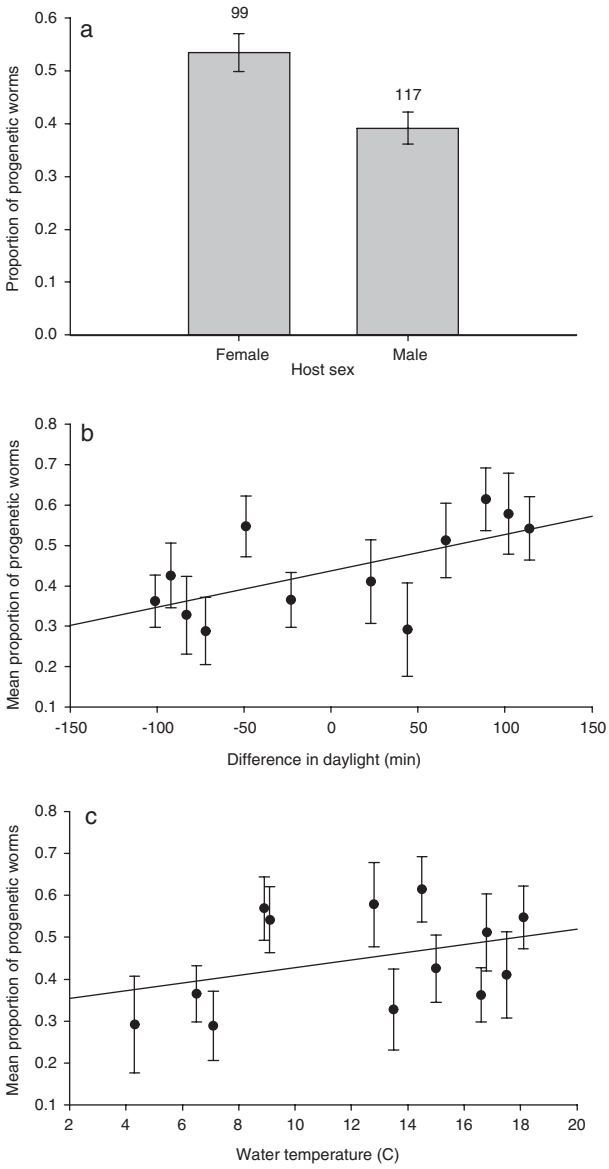

Fig. 3. For the subset of *Stegodexamene anguillae* in the gonads only, the relationship between proportion of progenetic worms and (a) fish host sex with number of males and females shown above bars, (b) difference in daylight time (min) since the last collection date and (c) water temperature (°C). Error bars indicate the standard error of the mean.

progenetic species to respond to environmental variability and reproduce when the probability of transmission to a definitive host is low (Lefebvre and Poulin, 2005; Lagrue and Poulin, 2007). Poulin and Lefebvre (2006) suggested that progenetic *S. anguillae* in fish gonads would be expelled during spawning. Thus *S. anguillae* should benefit by adjusting its reproductive strategy according to encystment site and relative to the reproductive season of its second intermediate fish host.

When investigating progenesis in all worms throughout the fish's body, only the encystment site accounted for developmental strategy. Worms in the body cavity and gonads were far more likely to adopt the progenetic strategy, supporting findings by Poulin and Lefebvre (2006) of a greater proportion of progenetic worms in the gonads. These tissues may provide better nutritive resources for absorption by worms for growth, development and reproduction than muscle tissue (Poulin, 1997). Indeed, worm size was also affected by encystment site, with worms in the body cavity and gonads growing to larger sizes than those in the muscle independently of their developmental strategy, which affected worm size as well. Worms grew larger as they matured from non-progenetic to developing vitellaria to producing eggs, with those reproducing reaching sizes 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).

In addition to supplying ample resources, the gonads also provide an exit for eggs of progenetic worms. Progenetic cysts are similar in size and shape to fish eggs, and those encysting in the gonads would be released during spawning (Lefebvre and Poulin,

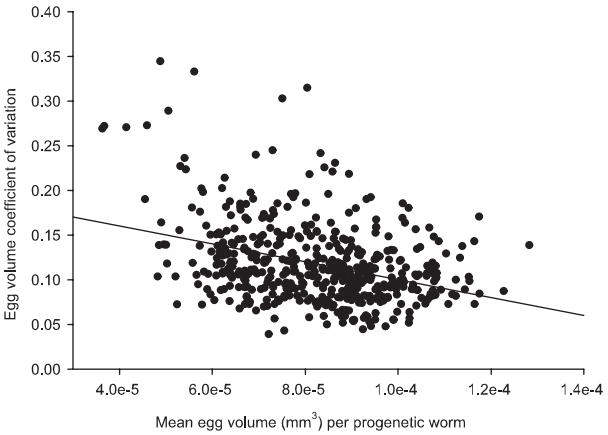

Fig. 4. The relationship between coefficient of variation of egg volume and mean egg volume (mm^3) of eggs produced by progenetic *Stegodexamene anguillae* in the second intermediate host, common bully.

2005; Poulin and Lefebvre, 2006). In contrast, progenetic worms found elsewhere have to wait for the bully host to die, either naturally followed by decay or through predation, to be released into the environment (Poulin and Cribb, 2002; McLaughlin *et al*. 2006). However, fish spawning should not equally affect all worms throughout the host but only those found in the gonads. In fact, model analysis on the subset of worms in the gonads showed that host sex, water temperature and daylight time affect progenesis in those worms encysted in the gonads. As water temperature and day length increased, so did the proportion of progenetic worms in the gonads. Both water temperature and day length are important factors correlated with the reproductive cycle of fish (e.g. Bullough, 1939; Siefert, 1968; Billard and Breton, 1978; Huber and Bengston, 1999; Davies and Bromage, 2002), suggesting that *S. anguillae* worms encysted in the gonads are developing in response to cues related to their host's reproductive cycle. The frequency of progenesis has been shown to increase with increasing temperature under experimental conditions (Herrmann and Poulin, 2011). However, this effect is assumed to operate indirectly via fish host stress because temperature is a significant factor in fish longevity (Herrmann and Poulin, 2011). Further, since temperature did not explain progenesis in worms encysted in other tissues besides the gonads, it is unlikely that temperature directly affects progenesis. Alternatively, parasites are known to respond in growth, differentiation and reproduction to a variety of physiological cues within their host (Thomas *et al*. 2002; Escobedo *et al*. 2005), and *S. anguillae* may be using the changes in hormones during the bully reproductive cycle as a signal for development.

Further, worms were more likely to become progenetic if encysted in female ovaries rather than in male testes. Female gonads provide a definite exit for progenetic worms and their eggs, whereas worms encysted in male gonads may be unlikely to pass through the vas deferens and exit the fish because of their size. The progenetic strategy is highly advantageous for those worms encysted in female ovaries because worms that do not develop would also be expelled during a spawning event and die without reproducing. Common bullies spawn twice during the reproductive season, with females first spawning around September and a second spawning from October through March (Stephens, 1982). A decrease in mean abundance of *S. anguillae* in gonads was not observed during the bully host reproductive season, most likely due to new infections acquired at the same time. However, progenesis in worms in the gonads showed a decreasing trend through the bully reproductive season and into winter followed by an increase prior to the next reproductive season. This suggests that *S. anguillae* worms within the gonads are exploiting cues related to the likelihood of being expelled during host spawning and reproducing prior to the time when the probability of being released from the host is high.

Individuals of progenetic species are typically encysted in muscle tissue or inside the body cavity of the second intermediate host and must await host death in order for their eggs to be released into the environment (Macy and Basch, 1972; Poulin and Cribb, 2002; Lefebvre and Poulin, 2005; McLaughlin *et al*. 2006). *Stegodexamene anguillae* has solved this problem, and similarly, a few other progenetic species have evolved other mechanisms to overcome this obstacle. For instance, high virulence in *Aphalloides coelomicola*, and its association with a tissue-liquefying myxozoan, allows acceleration of host death and thus release of the parasite's eggs (Pampoulie *et al*. 1999, 2000). Metacercariae of *Alloglossidium macrobdellensis* migrate to the intestinal lumen of the second intermediate host and then begin progenetic reproduction, allowing eggs to be passed into the environment along with host faeces (Corkum and Berkerdite, 1975). The progenetic cysts of *Coitocaecum anaspidis* easily burst within its crustacean host, and eggs disperse throughout the body via the haemolymph, causing blockage and resulting in deterioration of appendages thereby releasing the eggs into the water (Hickman, 1934). There may be many more strategies for egg dispersal in progenetic species that have yet to be discovered.

None of the variables measured in this study affected the number of eggs produced or the size of those eggs. However, the number of eggs produced by progenetic *S. anguillae* was unexpected. Progenetic worms are thought to be less fecund, limited to 100–200 eggs, than worms reproducing in the definitive host (Poulin and Cribb, 2002; Lagrue and Poulin, 2008). For *S. anguillae*, the mean number of eggs produced by progenetic worms was greater than 200 with one worm producing over 1400 eggs. Still, this may be less than the thousands of eggs an adult

within an eel may produce. Moreover, worms that produced larger eggs produced eggs of consistent size; those that produced smaller eggs produced eggs of varying size. Progenetic *S. anguillae* producing large eggs may be better at exploiting host resources consistently and produce eggs of similar size, whereas those producing smaller eggs of varying sizes may be investing differentially among eggs due to variability in resource availability within the host (Poulin and Hamilton, 2000). An alternative possibility is that variation in egg size and mean egg size are only spuriously correlated, since one is derived from the other, although there is no solid statistical reason to dismiss a biological explanation on that basis alone (Prairie and Bird, 1989).

In conclusion, being expelled from the host during spawning exerts strong selective pressure for those worms encysting in female ovaries. It is highly advantageous for *S. anguillae* to adjust its reproductive strategy according to its probability of being expelled from the second intermediate host before being transmitted to the definitive host. Here, the results show that *S. anguillae* is capable of accurate adjustments in its reproductive strategy based on encystment site, as well as environmental factors related to the reproductive cycle of its second intermediate host. For progenetic species reproducing within the tissues of a second intermediate host, egg dispersal is the major reproductive challenge (Poulin and Cribb, 2002). However, progenetic *S. anguillae* may be exploiting their host's reproduction as an exit strategy for their eggs. These results demonstrate the adaptive plasticity of reproductive strategies in *S. anguillae* depending on its host's reproductive cycle.

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