Journal of FISH BIOLOGY
Published for the Fisheries Society of the British Isles

Journal of Fish Biology (2012) **80**, 816–830
doi:10.1111/j.1095-8649.2012.03225.x, available online at wileyonlinelibrary.com

The missing host hypothesis: do chemical cues from predators induce life cycle truncation of trematodes within their fish host?

K. K. Herrmann*† and R. Poulin‡

**Department of Biological Sciences, Minnesota State University Mankato, Mankato, MN, 56001, U.S.A. and ‡Department of Zoology, University of Otago, P. O. Box 56, Dunedin, New Zealand*

(Received 20 April 2011, Accepted 10 January 2012)

Using controlled experiments, the ability of the trematode parasite *Stegodexamene anguillae*, encysted within its intermediate fish host, the common bully *Gobiomorphus cotidianus*, was tested to indirectly detect the presence of its definitive host by exposing infected *G. cotidianus* to chemical cues from the definitive host, the short-finned eel *Anguilla australis*. The trematode can abbreviate its normal life cycle and achieve precocious maturity in *G. cotidianus*, or adopt the usual strategy consisting in delaying maturity until it reaches an *A. australis*. The results suggest that chemical cues from the definitive *A. australis* host do not affect the frequency of life cycle abbreviation in *S. anguillae*. Other life-history traits, such as parasite body size or the egg output of early-maturing parasites, were also unaffected by chemical cues from *A. australis* or from an alternative predator of *G. cotidianus*, the perch *Perca fluviatilis*, that is not a suitable host for the trematode. Therefore, factors other than *A. australis* host presence or abundance may be the important selective forces for life cycle abbreviation in this fish parasite.

Key words: abbreviated life cycles; conditional strategies; eel; *Stegodexamene anguillae.*

INTRODUCTION

Many prey species have plastic responses to different levels of perceived predation risk (Helfman, 1989), typically based on chemical cues (Chivers & Smith, 1998). In aquatic systems, chemical cues are especially important because visual cues are limited (Smith, 1992). Numerous fish species are known to respond to chemical cues indicative of the presence of predators (Ferrari *et al*., 2010*a*). Typically, these responses include behavioural ones such as increased use of refuges or decreased activity, as well as physiological responses associated with stress (Kagawa & Mugiya, 2000; Lehtiniemi, 2005; Ferrari *et al*., 2010*a*). The indirect consequences of the detection of predator odours are rarely considered, however. For instance, many prey fish species are utilized as intermediate hosts by parasitic helminths that require trophic transmission to a predatory fish for completion of the life cycle. Manipulation

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†Author to whom correspondence should be addressed. Tel.: +1 507 389 1086; email: kherrmann13@gmail.com

of intermediate host behaviour is a common parasite adaptation to facilitate this transmission event (Combes, 1991; Poulin, 1995, 2007; Moore, 2002; Parker *et al.*, 2003). In addition, some species have evolved the ability to shorten the typical three-host life cycle to two hosts or even one host (Lefebvre & Poulin, 2005). In principle, a shorter life cycle should be easier to complete. In this context, parasites inside a prey fish would benefit if they were capable of adjusting their life-history strategy in response to physiological changes in their current host triggered by detection of chemical cues from the next predatory host; however, this hypothesis remains untested.

Many trematodes can simplify their life cycle from three hosts to two by developing precociously in the second intermediate host, a phenomenon known as progenesis. During progenetic development, juvenile stages, *i.e.* metacercariae, mature into adults and produce eggs *via* self-fertilization within the metacercarial cyst, thus eliminating the necessity of reaching a definitive host to complete the life cycle (Poulin & Cribb, 2002). In some species, the truncated life cycle becomes obligatory. In others, it is facultative, with some individuals of a population adopting the three-host cycle and others adopting the abbreviated life cycle (Poulin & Cribb, 2002; Lefebvre & Poulin, 2005).

The two alternative life cycles, the three-host cycle and the two-host one, could be plastic life-history strategies, adopted in response to environmental cues related to the probability of completing that optional transmission event, ingestion by the definitive host. Transmission probability depends upon abundance of the definitive host (Crossan *et al.*, 2007), which should exert strong selective pressure on trematodes. The rare or missing host hypothesis suggests that progenesis is favoured when the definitive host is periodically unavailable (Holton, 1984; Poulin & Cribb, 2002). Parasites with access to information about the external environment of their host, such as the likelihood of predation of their current host and thus probability of transmission to the next host, would have a selective advantage if they were able to adjust their life cycle strategies accordingly (Thomas *et al.*, 2002).

For example, in the progenetic trematode *Coitocaecum parvum*, the frequency of progenesis by worms in the second intermediate amphipod host depends on chemical cues from its definitive fish host under experimental conditions (Poulin, 2003; Lagrue & Poulin, 2007). When cues from the definitive host are absent, more *C. parvum* adopt the progenetic life cycle, whereas those within amphipod hosts exposed to chemical cues of the definitive fish host are more likely to adopt the three-host strategy and await ingestion by a definitive host. Nevertheless, no correlation between definitive host abundance and progenesis in *C. parvum* was observed in the field (Lagrue & Poulin, 2008). The lack of evidence for this relationship under natural conditions was attributed to the complex interactions among several environmental factors affecting progenesis simultaneously, and thus *C. parvum* may be unable to accurately adjust its life cycle strategy (Lagrue & Poulin, 2008).

Similarly, *Stegodexamene anguillae* is another progenetic trematode that may be capable of adjusting its reproductive strategy based on transmission opportunities to its definitive host, either New Zealand longfin eel *Anguilla dieffenbachii* Gray 1842 or the short-finned eel *Anguilla australis* Richardson 1841. In the second intermediate host, small freshwater fishes like *Gobiomorphus* (Eleotridae) and *Galaxias* (Galaxiidae) spp. (Macfarlane, 1951, 1952), *S. anguillae* encysted in gonad tissue preferentially adopt progenesis and reproduce before being expelled into the environment during host spawning events (Poulin & Lefebvre, 2006; Herrmann & Poulin, 2011*a*). Under experimental conditions, the frequency of progenesis in *S. anguillae* increases in hosts exposed to stressful conditions that reduce host life span and thus lower transmission probability (Herrmann & Poulin, 2011*b*). Under the rare or missing host hypothesis, the abundance of the definitive anguillid host would be expected to strongly influence the frequency of progenesis in *S. anguillae*. Although anguillid abundance was not correlated with progenesis of *S. anguillae* in a field study (Herrmann & Poulin, 2012), the effect of anguillid abundance may be detectable under controlled experimental conditions, similar to what has been observed in *C. parvum*.

Trematodes exploit predator–prey relationships for transmission to their definitive host. Chemical cues from predators induce anti-predator behaviour in a variety of prey taxa (Ferrari *et al.*, 2010*a*), which are often used as second intermediate hosts by trematodes. In this study, *S. anguillae* in its second intermediate host, common bully *Gobiomorphus cotidianus* McDowall 1975, is used to test the missing or rare host hypothesis, to determine experimentally whether *S. anguillae* is capable of accessing information about the presence, or even abundance, of its definitive anguillid host. When cues indicating imminent predation are withheld from *G. cotidianus* hosts, *S. anguillae* metacercariae are expected to respond by increasing the frequency of progenesis. Other predators in addition to anguillids also stimulate anti-predator behaviour in *G. cotidianus* (Kristensen & Closs, 2004). The ability of *S. anguillae* to distinguish between host stress induced by its definitive host or by an unsuitable (*i.e.* non-host) predator is also tested. Additionally, other variables related to development and reproduction are taken into account, to quantify any subtle effects on progenetic reproduction by *S. anguillae*.

MATERIALS AND METHODS

ANIMAL COLLECTION AND ACCLIMATION

Gobiomorphus cotidianus 4–6 cm in total length (L_T) (reproductive age) were collected in Lake Waihola (46° 00′ S; 170° 06′ E), South Island, New Zealand, using seines in January 2010. The first intermediate snail host, *Potamopyrgus antipodarum*, both anguillid species, the definitive hosts for *S. anguillae*, and perch *Perca fluviatilis* L. 1758, a non-host predator, are also found in Lake Waihola. Natural infections in *G. cotidianus* of this size and from this site range between four and >100 *S. anguillae* per fish (Poulin & Lefebvre, 2006; Herrmann & Poulin, 2011*a*, 2012). Prior to the acclimation period and again during allocation to experimental treatments, fish were treated for fungal skin infection using Profurin (1 g 100 l^{-1}) for 30 min and given a 5 min saltwater bath to eliminate monogenean ectoparasites due to the problematic nature of these infections while housing *G. cotidianus*. Fish were kept in filtered and aerated aquaria, fed frozen bloodworms *Chironomus sp.* (Aqua One; www.aquaone.co.uk) and live amphipods from Lake Waihola and allowed to acclimate for 1 week. During the acclimation period, water was slowly changed from fresh lake water to a mixture of 67% spring water and 33% sea water to inhibit fungal growth in captivity (the coastal lake of origin is mildly brackish due to tidal influences). The photoperiod was set at 12L:12D.

To obtain water for the scented experimental treatments, two *A. australis*, the definitive host, were purchased from New Zealand Eel Processing Company (Te Kuiti, North Island), and one *P. fluviatilis*, a non-host predator, was collected from Lake Waihola with a seine. *Anguilla australis* were housed together. Water volume was adjusted in the tanks holding *A. australis* and *P. fluviatilis* to maintain a ratio of 5 g fish l^{-1} in an effort to standardize cue concentration. To avoid any other fish odour contaminating these tanks, *P. fluviatilis* was fed earthworms every 2 days, and the two *A. australis* were occasionally fed minced beef.

EXPERIMENTAL TREATMENT

Ten *G. cotidianus* were placed into each of eight tanks (27 l) based on their L_T, resulting in fish of a similar range of L_T in all tanks to avoid any variation in fish size among treatments. Two tanks were randomly assigned to each of four treatments (unscented control, low concentration of *A. australis* scent, high concentration of *A. australis* scent and *P. fluviatilis* scent). At the beginning of the experiment and every 2 days thereafter, 9 l of water was removed from each treatment tank and replaced with one of four types of water depending on treatment. Tanks in the control treatment received 6 l of spring water and 3 l of sea water (unscented). Tanks in the treatment with the low concentration of *A. australis* scent received 2 l of spring water, 1 l sea water and 6 l from the tank housing the two *A. australis*. Tanks in the treatment with high concentration of *A. australis* scent received 9 l of water from the tank housing the two *A. australis*. Tanks in the *P. fluviatilis* scented treatment received 9 l of water from the tank housing the *P. fluviatilis*. The tanks housing *A. australis* and *P. fluviatilis* were drained until 30% of the water had been removed; it was then replaced with the mixture of 67% spring water and 33% sea water.

The experiment monitored changes in the developmental status of naturally acquired parasites in response to treatment conditions. Responses by naturally acquired parasites were used instead of experimental infections because the latter proved impossible and because very few parasites in fish taken from the wild are at the progenetic stage (7·5–17·1%; Poulin & Lefebvre, 2006; Herrmann & Poulin, 2011*a*, 2012); therefore, random allocation of fish to treatments results in equally low initial frequencies of progenetic worms across treatments, and any differential increase in these frequencies can be interpreted as a treatment effect.

MEASUREMENTS AND STATISTICAL ANALYSES

Fish were euthanized by an overdose of tricaine methanesulphonate (MS-222) after 8 weeks, which is sufficient time for worms to grow, develop and begin producing eggs (Macfarlane, 1951). In the control treatment, 10 and 10 fish survived per tank, eight and nine in the low *A. australis* treatment, 10 and 10 in the high *A. australis* treatment, nine and nine in the *P. fluviatilis* scented treatment. The L_T, mass (M) and sex of each fish were recorded. Fish body condition (K) was determined $K = ML_T^{-3}$ (Bolger & 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 yolk-producing glands developed). Body surface area of each worm was calculated as a surrogate for body size by using the formula of an ellipsoid, $0{\cdot}25\ (\pi LW)$, where L and W are the length and width of the parasite; these 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. The L and W of a subsample of 10 eggs from each progenetic worm were measured, and assuming a regular ellipsoid shape, egg volume was calculated as $0{\cdot}16\ (\pi LW^2)$.

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 the determinants of progenesis if vitellaria-present worms were excluded. Thus, factors influencing the parasite's developmental strategy, non-progenetic *v.* progenetic, were assessed using a generalized linear mixed model (GLMM) with a binomial error distribution. This analysis was used to determine the effect of treatment (unscented, *A. australis* scented at low concentration, *A. australis* scented at high concentration and *P. fluviatilis* scented), host L_T, host K, host sex, encystment site (muscle, head, body cavity or gonads), abundance of conspecifics and of two other common species of trematode metacercariae (*Telogaster opisthorchis* and *Apatemon* sp.), while individual fish identity and aquarium were added as random factors. The interaction between host sex and encystment site 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 additional ones specified for each case. First, worm size data were $\log_{10}$ 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) as an additional factor. Second, number of eggs expelled per progenetic worm was used as response variable in a GLMM with the quasipoisson distribution and the addition of worm size as an extra factor. Third, a GLMM with mean egg volume per progenetic worm as response variable and the Gaussian (normal) distribution included the additional factors of worm size and number of eggs expelled. Finally, a GLMM with coefficient of variation (C.V.) in mean egg volume per progenetic worm as a response variable and the Gaussian (normal) distribution included additional factors of worm size, number of eggs expelled and mean egg volume. All GLMMs were fitted using the package lme4 (Bates & Maechler, 2009) in the programme R (R Development Core Team; www.r_project.org).

RESULTS

Out of the 75 surviving experimental fish, 1799 metacercariae of *S. anguillae* were recovered, with numbers ranging from three to 90 per fish and mean ± S.E. abundance of 24·0 ± 2·3 per fish. Across all *S. anguillae* in all treatments, 16·9% were progenetic and an additional 8·2% had developed vitellaria. Differences among individual worms in development strategy were explained only by encystment site (Table I). Of the 304 progenetic worms, more were found in the body cavity (74 of 270) and gonads (196 of 437) than in the muscle (18 of 674) or the head [16 of 418; Fig. 1(a)]. Although a slightly lower proportion of worms became progenetic in the high eel abundance treatment [Fig. 1(a)], there was no significant treatment effect (Table I).

Mean ± S.E. size of worms was 0·40 ± 0·01 mm^2. Worm size was affected by encystment site and developmental stage, but not by treatment (Table I). Mean ± S.E. size of all worms was slightly larger in the head (0·22 ± 0·01 mm^2) and markedly larger in the body cavity (0·53 ± 0·03 mm^2) and gonads (0·81 ± 0·04 mm^2) than in muscle tissue [0·21 ± 0·01 mm^2; Fig. 1(b)]. Progenetic worms were significantly larger than non-progenetic worms, with those worms having well-developed vitellaria but no eggs falling in between [Fig. 1(b)].

The mean ± S.E. number of eggs released per progenetic worm was 235·9 ± 16·9 and ranged from zero to 1380. Number of eggs expelled by progenetic individuals was not affected by treatment or any of the variables measured and tested (Table I).

The mean ± S.E. egg volume per progenetic worm was $8{\cdot}35^{-05} \pm 1{\cdot}03^{-06}$ mm^3 ($n = 910$) and ranged from $4{\cdot}36^{-05}$ to $1{\cdot}21^{-04}$ mm^3. Egg volume was not affected by treatment, but it varied as a function of worm size and host K (Table I), with larger eggs being produced by larger worms [Fig. 2(a)] and in hosts with better K [Fig. 2(b)]. The relationship between host K and egg size, however, was driven by one fish with a high K value index [the outlier to the far left on Fig. 2(a)]. The effect of host K disappeared once this outlier was removed.

Finally, the C.V. in egg volume was also unaffected by treatment conditions but decreased with increasing mean egg volume and increasing worm size [Table I and Fig. 3(a)]. The C.V. in egg volume also decreased as host K decreased [Fig. 3(b)] but increased as abundance of *Apatemon* sp. increased [Fig. 3(c)]. Worm size, host

TABLE I. Results from the five generalized linear mixed models on the response variables (progenetic v. non-progenetic, worm size, number of eggs expelled, mean egg volume per progenetic worm and coefficient of variation (c.v.) of egg volume) in relation to the predictor variables. Variables ± s.e. estimate and estimate divided by s.e. are reported. The significant main effects are in bold

Response	Predictor variable	Estimate ± S.E.	Estimate divided by S.E.
Progenesis	Intercept	−0·744 ± 2·972	−0·25
	Treatment: low *Anguillia australis*	0·925 ± 0·492	1·88
	Treatment: high *A. australis*	−0·095 ± 0·489	−0·19
	Treatment: *Perca fluviatilis*	0·251 ± 0·465	0·54
	Host length	−0·445 ± 0·467	−0·95
	Host sex	0·210 ± 0·372	−0·94
	Host body condition	−177·000 ± 188·900	0·56
	Site: head	0·321 ± 0·411	0·78
	Site: body cavity	**2·737 ± 0·335**	**8·16*****
	Site: gonads	**3·421 ± 0·320**	**10·71*****
	Number of *Telogaster opisthorchis*	0·002 ± 0·002	0·99
	Number of *Apatemon* sp.	0·003 ± 0·003	0·91
	Number of *Stegodexamene anguillae*	−0·007 ± 0·010	−0·72
Worm size	Intercept	−1·540 ± 0·850	−1·81
	Treatment: low *A. australis*	−0·014 ± 0·144	−0·10
	Treatment: high *A. australis*	−0·021 ± 0·138	−0·15
	Treatment: *P. fluviatilis*	0·059 ± 0·130	0·45
	Host length	−0·010 ± 0·133	−0·07
	Host sex	−0·093 ± 0·105	−0·86
	Host body condition	−41·990 ± 51·600	−0·81
	Site: head	**−0·102 ± 0·048**	**−2·11***
	Site: body cavity	**0·254 ± 0·058**	**4·35*****
	Site: gonads	**0·134 ± 0·005**	**2·45***
	Stage: vitellaria	**1·376 ± 0·069**	**20·09*****
	Stage: progenetic	**1·857 ± 0·060**	**30·76*****
	Number of *T. opisthorchis*	−0·001 ± 0·001	−1·05
	Number of *Apatemon* sp.	0·000 ± 0·001	0·27
	Number of *S. anguillae*	0·003 ± 0·003	0·98
Number of eggs	Intercept	4·4e+00 ± 2·0e+02	0·02
	Treatment: low *A. australis*	2·6e-02 ± 3·5e+01	0·00
	Treatment: high *A. australis*	−5·7e-01 ± 3·5e+01	−0·02
	Treatment: *P. fluviatilis*	−1·3e-01 ± 3·5e+01	−0·00
	Host length	−8·6e-01 ± 3·4e+01	−0·03
	Host sex	−6·7e-01 ± 2·8e+01	−0·02
	Host body condition	3·3e+02 ± 1·2e+04	0·03
	Site: head	−6·5e-01 ± 2·0e+00	−0·32
	Site: body cavity	−2·9e-01 ± 1·6e+00	−0·19
	Site: gonads	2·1e-01 ± 1·5e+00	0·14
	Worm size	5·0e-01 ± 5·3e-01	0·95
	Number of *T. opisthorchis*	−5·3e-03 ± 1·7e-01	−0·03
	Number of *Apatemon* sp.	−2·4e-03 ± 2·0e-01	−0·01
	Number of *S. anguillae*	4·4e-02 ± 7·4e-01	0·06

TABLE I. Continued

Response	Predictor variable	Estimate ± S.E.	Estimate divided by S.E.
Egg size	Intercept	1·6e-05 ± 2·8e-05	0·95
	Treatment: low *A. australis*	2·1e-06 ± 6·3e-06	0·34
	Treatment: high *A. australis*	−2·1e-06 ± 6·4e-06	−0·33
	Treatment: *P. fluviatilis*	−4·2e-06 ± 6·3e-06	−0·67
	Host length	8·5e-07 ± 4·4e-06	0·19
	Host sex	1·3e-06 ± 3·7e-06	0·36
	Host body condition	**4·2e-03 ± 1·5e-03**	**2·84***
	Site: head	−8·4e-06 ± 1·1e-05	−0·79
	Site: body cavity	2·2e-06 ± 8·5e-06	0·26
	Site: gonads	−2·9e-06 ± 8·3e-06	−0·35
	Worm size	**1·5e-05 ± 2·2e-06**	**6·72*****
	Number of eggs per worm	8·6e-09 ± 4·6e-09	1·88
	Number of *T. opisthorchis*	−1·4e-08 ± 2·0e-08	−0·71
	Number of *Apatemon* sp.	2·7e-08 ± 2·1e-08	1·31
	Number of *S. anguillae*	−1·4e-08 ± 8·2e-08	−0·17
Egg size C.V.	Intercept	3·5e-01 ± 7·7e-02	4·63
	Treatment: low *A. australis*	6·7e-04 ± 1·0e-02	0·07
	Treatment: high *A. australis*	−8·0e-03 ± 1·1e-02	−0·76
	Treatment: *P. fluviatilis*	2·2e-03 ± 1·0e-02	0·21
	Host length	−1·7e-02 ± 1·2e-02	−1·43
	Host sex	−4·6e-03 ± 7·9e-03	−0·58
	Host body condition	**−8·5e+00 ± 4·0e+00**	**−2·10***
	Site: head	−4·7e-02 ± 3·3e-02	−1·43
	Site: body cavity	6·3e-03 ± 2·7e-02	0·24
	Site: gonads	−7·3e-03 ± 2·6e-02	−0·28
	Worm size	**−1·6e-02 ± 7·7e-03**	**−2·09***
	Number of eggs per worm	1·3e-05 ± 1·4e-05	0·91
	Egg volume	**−8·5e+02 ± 2·8e+02**	**−3·05****
	Number of *T. opisthorchis*	−4·8e-06 ± 5·1e-05	−0·09
	Number of *Apatemon* sp.	**1·3e-04 ± 4·8e-05**	**2·70***
	Number of *S. anguillae*	−1·3e-04 ± 2·0e-04	−0·65

The effects of the control treatment, male host sex, muscle encystment site and non-progenetic stage are included in the intercept of the appropriate model. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

K and abundance of *Apatemon* sp no longer had an effect once the individual worm with the highest C.V. in egg volume [the outlier at the top of Figs 3(a), (b), (c)] was removed.

DISCUSSION

Encystment site affected progenesis in this study, a pattern consistent with previous studies (Poulin & Lefebvre, 2006; Herrmann & Poulin, 2011*a*, *b*, 2012). Here, the results show a higher frequency of progenesis in worms encysted in the gonads, regardless of experimental treatment. Chemical cues from a predator (host

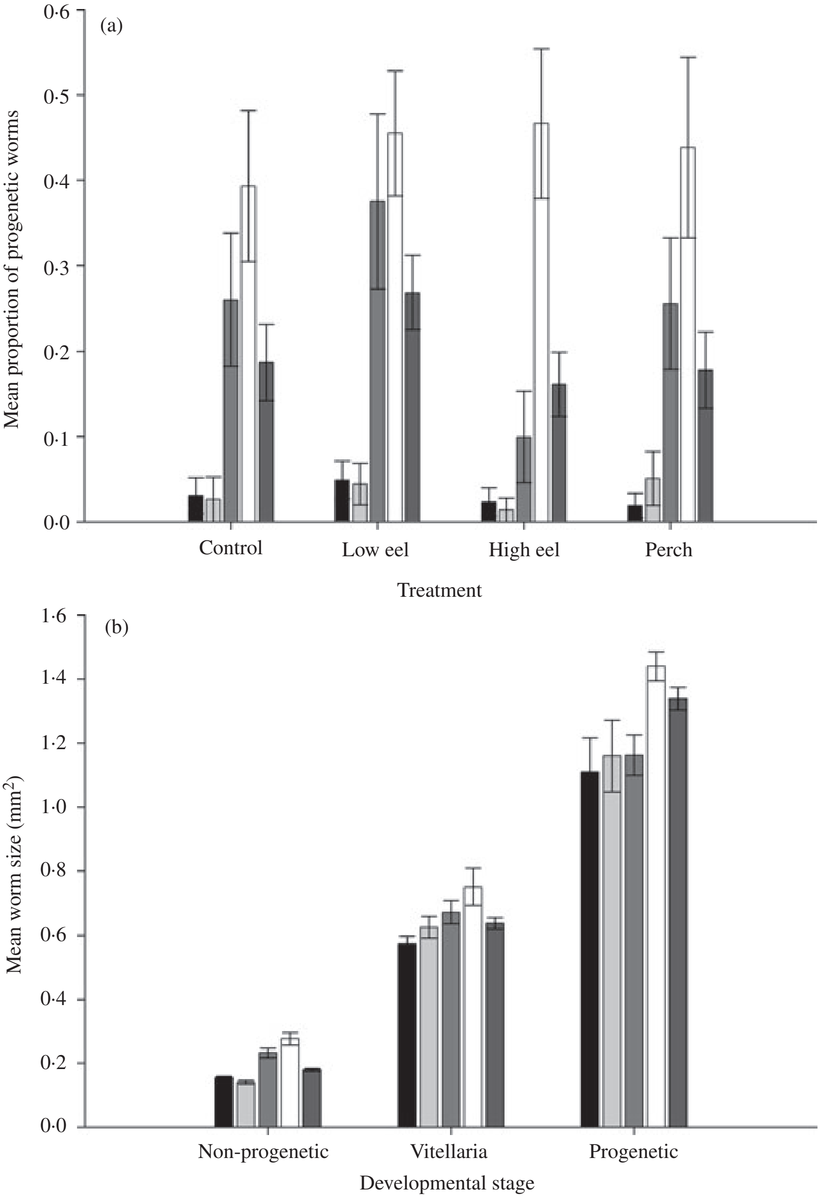

FIG. 1. The relationship between mean ± S.E. (a) proportion of progenetic worms and encystment site as a function of treatment and (b) worm size and encystment site by developmental stage (■, muscle; ░, head; ▒, body cavity; □, gonads; ▓, all tissues).

or non-host), however, did not have an effect on development and reproduction of *S. anguillae*, supporting previous field data suggesting that definitive host abundance does not impact progenesis of this species (Herrmann & Poulin, 2012). In fact, the proportion of progenetic individuals in this study was comparable to that of field data from Lake Waihola at the same time of year (Herrmann & Poulin, 2011*a*),

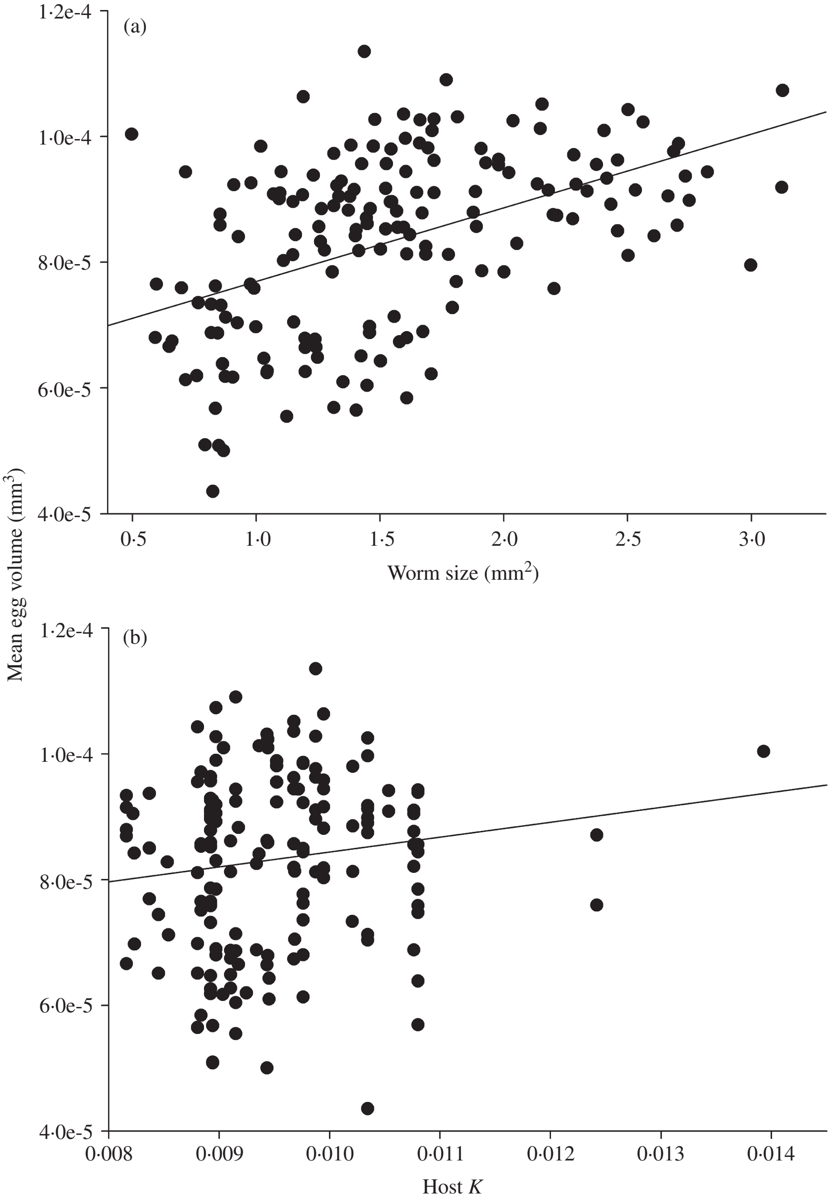

FIG. 2. Mean egg volume per progenetic worm as a function of (a) worm size (mm^2) and b) host body condition (K).

indicating either that abundance of the anguillid host has no effect on the frequency of progenesis or that this experiment did not properly simulate differences in host abundance among treatments.

It is possible that the experimental design was flawed based on three main factors. First, cues may have been given too infrequently and at too low of a concentration. Prey adjust their anti-predator responses based on cue concentration (Helfman, 1989;

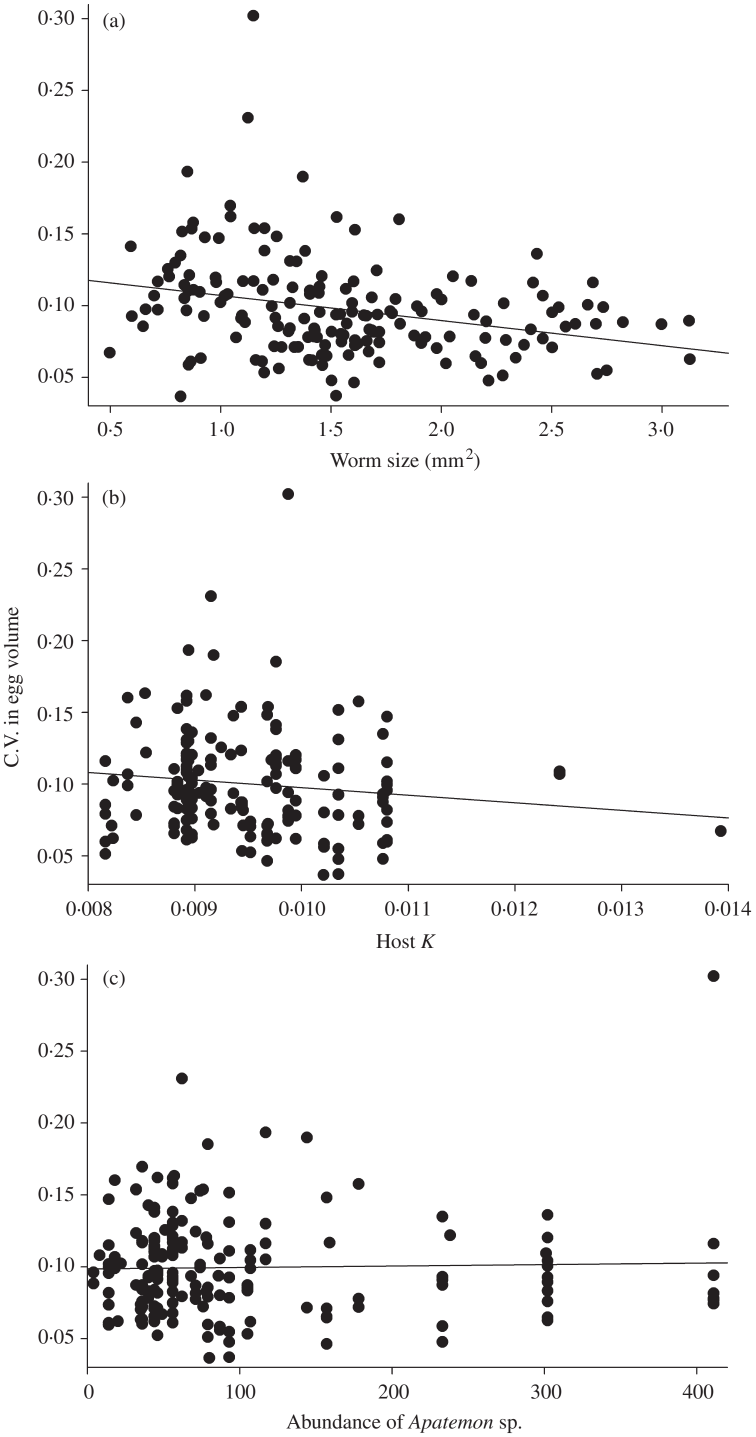

FIG. 3. The coefficient of variation (c.v.) in egg volume as a function of (a) mean egg volume per progenetic worm, (b) host body condition (K) and (c) abundance of *Apatemon* sp.

Ferrari *et al.*, 2006, 2010*a*), and behavioural studies with mixed results are often explained by differences in cue concentration (Stabell & Lwin, 1997). Additionally, chemical alarm cues are known to decay in laboratory settings in 3–6 h (Wisenden *et al.*, 2009), and this may apply to predator odour cues as well. In this study, fresh scented water was added every 48 h to aquaria with filters. Thus, cues from predators may have been too low or infrequent in all treatments of the experiment to induce a response in the fish.

Second, fish used in the experiment may have been aware that predators were present when given water with predator odour, but it is the alarm cues from the skin of injured conspecifics and the digestion cues from faeces of predators that label a predator as threatening (Mathis & Smith, 1993; Chivers & Mirza, 2001; Chivers *et al.*, 2001). Without the alarm or digestion cues that are only provided by an actively feeding predator, fish in the experiment may not have had a strong response to a predator that is present but not actively foraging, and thus non-threatening (Ferrari *et al.*, 2010*a*).

Most predator–prey studies have not differentiated between alarm cues and predator-only cues, because predators used for these studies have been fed conspecifics of the prey species being tested (Ferrari *et al.*, 2010*a*). For those studies that have distinguished between the two sources of cues, phenotypic change in the prey species was only induced when both predator odour and conspecific alarm cues were given (Harvell, 1984, 1986; Appleton & Palmer, 1988; Stabell *et al.*, 2003; Schoeppner & Relyea, 2005; Fässler & Kaiser, 2008). For example, anti-predator behaviour in tadpoles *Hyla versicolor* was induced when they were given cues of crushed conspecifics, but morphological change was only induced when they were presented with cues of predators consuming prey (Schoeppner & Relyea, 2005). Similarly, *Daphnia galeata* did not show any phenotypic change when given cues from a predator fed on earthworms but did respond when the cue was from a predator fed conspecifics (Stabell *et al.*, 2003). Alarm cues may need digestive enzymes to be activated, and perhaps only the predictable metabolites of alarm cues actually trigger phenotypic change in prey species (Stabell *et al.*, 2003). This trend may extend to phenotypic change in parasites within those prey.

Without cues indicating a foraging predator, *G. cotidianus* may begin to disassociate the predator cue with danger even though these fish have naturally experienced both anguillid and *P. fluviatilis* predators in Lake Waihola and thus should have responded with anti-predator behaviours (Kristensen & Closs, 2004). While this experiment lasted 56 days, an experiment with juvenile rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) showed that recognition of the predator can be lost after only 21 days when predator odour is not paired with alarm cues (Brown & Smith, 1998). Further, for the expression of anti-predator responses in fathead minnows *Pimephales promelas* Rafinesque 1820, recent cue information weighs more than old information (Ferrrari & Chivers, 2006). Losing predator recognition may be an adaptation to avoid expensive responses in changing conditions that become non-threatening, such as prey growing too large to be consumed by a particular predator species (Ferrari *et al.*, 2010*a*, *b*) or predictable temporal patterns in foraging habits of predators (Wisenden *et al.*, 2009). Anguillids do not actively forage during the day or during winter (McDowall, 1990), so *G. cotidianus* may not maintain an anti-predator response to predator odour without accompanying cues indicating active foraging by eels.

Finally, a third factor is that no visual stimulus was given to fish in this experiment. Kagawa & Mugiya (2000) showed that plasma cortisol levels only increased in goldfish *Carassius auratus* (L. 1758) when visual cues of a predator were given, with or without a chemical cue, but no increase was detected when only chemical cues were provided. Parasites have evolved structures similar to hormone receptors found in their vertebrate hosts, and it is through the host's endocrine system and hormonal fluctuations that information about host health, stress and reproductive cycle is communicated to endoparasites (Thomas *et al*., 2002; Escobedo *et al*., 2005). Many studies have shown parasites to exploit the host endocrine system to favour their establishment, growth and reproduction (Morales-Montor *et al*., 2001; Remoue *et al*., 2002; Escobedo *et al*., 2004, 2005). In the case of *S. anguillae*, plasma cortisol levels of the *G. cotidianus* host may indicate future probability of transmission to an anguillid host, with increased levels of plasma cortisol induced by actively foraging predators within sight. If the predator odour cue failed to increase plasma cortisol levels in fish in this experiment, then *S. anguillae* within those fish may not have perceived a differential transmission probability among the treatments.

Not only did host/predator odour not affect frequency of progenesis in *S. anguillae* but it also had no effect on growth, egg production or egg size. On the other hand, encystment site and developmental stage did affect worm size, with worms growing larger as they developed to more advanced stages, and those encysted in the body cavity and gonads achieving larger sizes than those in the muscle, a pattern consistent with previous studies of *S. anguillae* (Herrmann & Poulin, 2011*a*, *b*, 2012). Worm size significantly affected egg size, with larger worms producing larger eggs on average. Additionally, there was a positive relationship between the C.V. in egg volume and mean egg volume. Worms producing large eggs of consistent size may be able to better exploit host resources consistently, and those producing smaller eggs of varying sizes may invest in eggs differentially due to variability in resource availability within the host (Poulin & Hamilton, 2000). Alternatively, the correlation between variation in egg size and mean egg size may be spurious, because one is derived from the other, although there is no solid statistical reason to dismiss a biological explanation on that basis alone (Prairie & Bird, 1989). Once outliers in egg size and variation in egg size were removed, these results were similar to previous studies on *S. anguillae* (Herrmann & Poulin, 2011*a*, *b*, 2012).

The frequency of progenesis in this experiment was similar to levels found in nature at the same time of year (Herrmann & Poulin, 2011*a*). Additionally, abundance of the anguillid definitive host was not found to affect progenesis in a field survey (Herrmann & Poulin, 2012). Anguillid abundance may be more stable than hypothesized and thus may not be an important selection force for progenesis in *S. anguillae*. Other selective factors may be the drivers of facultative progenesis in *S. anguillae*, such as encystment site and *G. cotidianus* reproductive cycle (Poulin & Lefebvre, 2006; Herrmann & Poulin, 2011*a*) and *G. cotidianus* host longevity (Herrmann & Poulin, 2011*b*), if *S. anguillae* is perceiving transmission opportunities *via* host reproductive hormones and hormones associated with longevity. To assess the role of alarm cues and plasma cortisol levels, it would be interesting to repeat this experiment with cues from predator odour alone, alarm cues alone (from the skin of conspecifics) and cues from a host predator fed conspecifics, using a shared filtration system so that *G. cotidianus* are constantly exposed to cue treatment. This could be paired with a visual stimulus, and by measuring plasma cortisol levels, hormonal changes within the host could be monitored and related to the reproductive response of *S. anguillae*.

We thank K. Garrett, A. Setiawan and R. Paterson for field and laboratory assistance. We are grateful to B. Wisenden for commenting on an earlier version. 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.

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