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Genetic life history effects on juvenile survival in bluegill

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Abstract

Foraging behaviour under the risk of predation has important consequences on an individual's survivorship and fitness. In bluegill (*Lepomis macrochirus*), we have recently shown that offspring sired by males of alternative life histories differ in their foraging behaviour. Specifically, offspring sired by 'cuckolder' males take fewer risks during foraging than do offspring sired by 'parental' males. This behavioural difference can have important consequences on the fitness of the two life histories and thus the underlying evolutionary mechanism. Here, we examine the consequences of this behavioural variation on growth rate, condition and survivorship during early development of juveniles. We used split *in vitro* fertilization to generate maternal half-sibs that differed in sire life history. The resulting 18 455 larvae from 50 families were released into a microcosm with safe and risky foraging areas for approximately 2 months. A total of 262 juveniles (1.4%) survived of which parentage was unambiguously determined using microsatellite genetic markers for 254 (97%). Although we found significant dam effects, there was no difference in apparent growth rate or condition of juveniles sired by males of the two life histories. Of the 25 paired half-sib families, 15 had higher survivorship when sired by a cuckolder male, seven had higher survivorship when sired by a parental male and three had no surviving offspring from either sire. Thus, although growth was similar between the two offspring types, survivorship was not. Combining the differential survivorship estimate with paternity data from natural nests and the frequency of males adopting each life history, we calculated that the cuckolder life history has 1.87 times higher fitness than the parental life history. As such, the life histories likely are not governed by a genetic polymorphism.

Introduction

Many species in the animal kingdom are characterized by alternative life histories within a sex (Gross, 1996). Two mechanisms that may underlie such alternative life histories include a conditional strategy and alternative strategies (Gross, 1996). In the case of a conditional strategy, the life history adopted by an individual is governed by an aspect of its phenotype, whereas for alternative strategies, different gene variants give rise to the life histories. Consequently, alternative strategies require equal fitnesses of the two life histories and negatively frequency dependent selection – when the fitness of a strategy is negatively related to its frequency in the population – to be evolutionarily stable (Maynard Smith, 1982; Gross, 1996). Equal fitnesses are not expected for a conditional strategy, but the adopted life history, given an individual's condition, is expected to have higher fitness than the alternative life history for that individual (see Box 3 in Gross, 1996).

Studies have provided support for the existence of both conditional strategies and alternative strategies. Conditional strategies are widespread in the animal kingdom (reviewed by Gross, 1996). For example, male dung beetles (*Onthophagus taurus*) are dimorphic with some males developing horns and other males remaining hornless (Hunt & Simmons, 1997). Research has shown that larval food quantity predictably determines the development of either horned or hornless males

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irrespective of paternal phenotype (Hunt & Simmons, 1997; Moczek & Emlen, 2000). Furthermore, determination of fitness functions for both male types has shown that individual males maximize their fitness by adopting the appropriate life history (i.e. horned or hornless) given their condition (i.e. size; Hunt & Simmons, 2001). Conclusive evidence for alternative strategies is less common in the literature. In the ruff (*Philomachus pugnax*), independent and satellite reproductive strategies appear to be controlled by a single gene with two alleles – one allele giving rise to the independent strategy and the other allele giving rise to the satellite strategy (Lank *et al.*, 1995). It remains unclear, however, whether or not the two strategies have equal fitnesses (Widemo, 1998). A slightly more complex genetic model has been proposed for the marine isopod, *Paracerceis sculpta*, which is characterized by three male reproductive strategies (Shuster & Sassaman, 1997). The three strategies also appear to have equal fitnesses (Shuster & Wade, 1991; for other examples see Ryan *et al.*, 1992; Sinervo & Lively, 1996).

Foraging behaviour under the risk of predation can have important consequences on an individual's survivorship (Milinski & Heller, 1978; Lima & Dill, 1990; Lima, 1998). Thus, understanding the fitness consequences of foraging behaviour can help to differentiate between the mechanisms that govern alternative life histories. Foraging behaviour has been studied in several species that have alternative life histories. For example, in Atlantic salmon (*Salmo salar*), during the first winter of life, 'early migrant' individuals forage mostly during the day, whereas 'delayed migrant' individuals forage predominately at night; low light levels reduce the feeding efficiency at night, but presumably individuals foraging during this time are less prone to predation (Valdimarsson & Metcalfe, 1999). Early migrant individuals also appear to make fewer feeding attempts yet grow faster than delayed migrant individuals (Higgins & Talbot, 1985; Valdimarsson & Metcalfe, 1999). In bluegill (*Lepomis macrochirus*), we have previously found that offspring of 'cuckolder' males have higher conversion efficiency (efficiency of converting ingested food into soma) during the endogenous feeding stage and, shortly after the switch to exogenous feeding, take fewer risks during foraging than offspring of 'parental' males (Neff, 2004; Lister & Neff, 2006).

Here, we examine the consequences of foraging decision-making on the growth and survivorship of offspring sired by males of alternative life histories in bluegill. In Lake Opinicon (Ontario, Canada; 44° 16′ N, 76° 30′ W), parentals sexually mature at about 7 years of age and provide sole parental care for the developing eggs and larvae in their nest (Gross, 1982). Cuckolders mature precociously at 2 years of age and use a parasitic tactic to steal fertilizations from parentals during spawning. Cuckolders do not provide care to their young, but instead leave this to the parentals they parasitize. A common garden experiment has shown that the life histories have a degree of heritability as cuckolders produce about 50% more sons that become cuckolders themselves as compared to parentals (M. R. Gross unpublished data in Alcock, 1989, p. 412). In Lake Opinicon, about 16–21% of males mature precociously, whereas the other males follow the parental life history (Gross & Charnov, 1980; authors' unpublished data). Genetic paternity analysis of over a hundred broods collected from the lake has shown that parentals fertilize an average of 78% (range = 26–100%) of all eggs (Philipp & Gross, 1994; Fu *et al.*, 2001; Neff, 2001).

We used split *in vitro* fertilization to generate maternal half-sibs that differed in sire life history. This half-sib design is powerful because it controls for maternal effects when comparing paternal genetic effects of the two male life histories (Barber & Arnott, 2000). We released the offspring into a microcosm that contained *Hydra canadensis*. *Hydra canadensis* is a major predator of fish larvae and is estimated to kill over 20% of all bluegill larvae in Lake Opinicon (Elliott *et al.*, 1997). Approximately 2 months later, we collected all surviving juveniles and used microsatellite genetic markers to reconstruct the pedigrees. We then analysed the genetic and environmental components of growth rate and condition, and the potential trade-off between growth rate and survivorship. Finally, we used the pedigree data to calculate familial survivorship and used these data in conjunction with the paternity and frequency data to estimate the relative fitness of the two life histories.

Materials and methods

Split *in vitro* fertilization

Breeding colonies were located in Lake Opinicon by daily snorkelling during the May to July breeding season of 2002. Over 2 days of spawning (11 and 25 June), 25 each of parentals, cuckolders and gravid females were collected using dip nets and transported to holding tanks in our aquarium facility at the Queen's University Biological Station. Each aquarium was supplied with fresh lake water via a flow-through system. Shortly after collection, maternal half-sibs were generated using split *in vitro* fertilization. Eggs were stripped from each female by applying gentle pressure to the abdominal region and milt was collected from males in 0.5 mL syringes in a similar manner. Each female's eggs were divided into two samples and one sample was fertilized with sperm from a cuckolder and the other sample was fertilized with sperm from a parental. Fertilization was accomplished by placing eggs and milt together in 50 mL of lake water in a 500 mL mason jar for 2 min. The jars were then filled with water and an air feed was placed in each jar to oxygenate the water. Each adult fish was weighed, length was measured and a fin clip was preserved in 95% ethanol for later use in microsatellite DNA analysis.

Water changes of about 150 mL were performed three to six times a day until just prior to 'swim-up' (when the offspring become freely swimming larvae), which occurred 8–9 days after fertilization. The numbers of larvae in each of the half-sib families were then counted using a grid-tray and released into our microcosm.

Microcosm

The microcosm consisted of a set of five interconnected pools filled with lake water (total volume ca. 65 000 L). Rocks and plants (*Myrophyllum spicatum*) were placed in patches in each of the four outer pools to mimic natural habitat. We introduced *H. canadensis* (approximately 60 per pool), which typically resides on *M. spicatum*, to create areas with plant cover and predators (risky habitat) and open areas with no predators (safe habitat) (Werner, 1967; Elliott *et al.*, 1997). The microcosm was equipped for water circulation within the pools and water changes of about 30% by volume were conducted bi-weekly with fresh water from the lake to ensure an adequate supply of zooplankton. In the first few months of life, bluegill larvae exhibit size selective feeding on zooplankton, where smaller individuals (< 6 mm) eat small nauplii and *Cyclops* spp. (0.1–0.3 mm) and larger individuals eat a greater variety of species (0.1–0.7 mm; Keast, 1980). We periodically sampled the zooplankton from the pools to confirm that the size range of zooplankton available was appropriate for the developing fish.

On 15 August, all surviving juveniles were collected from the microcosm and preserved in 95% ethanol. Thus, the offspring produced on 11 June were 65 days post-fertilization and the offspring produced on 25 June were 51 days post-fertilization.

Offspring phenotypic measurements and parentage

The wet weight was measured for each juvenile using a Mettler Toledo balance (0.0001 g) and the total body length was measured using Spi 2000 callipers (0.1 mm). For each juvenile, apparent growth rate (mm day^{-1}) was calculated from L/A, where L is total body length and A is age, and Fulton's condition factor was calculated from M/L^3, where M is wet mass (Sutton *et al.*, 2000; Neff & Cargnelli, 2004). We were unable to obtain the mass and hence condition factor for one juvenile.

The parentage of each juvenile was determined using microsatellite DNA analysis. First, DNA was isolated from the adults as well as the juveniles collected from the microcosm using a proteinase K digestion (Neff *et al.*, 2000). DNA concentration was quantified using a spectrophotometer. Second, we used a Whatman-Biometra T1 Thermocycler (Goettingen, Germany) to amplify the microsatellites with the following program: 60s at 92 °C; seven cycles of 30s at 92 °C, 30s at 54 °C and 30s at 72 °C; and 28 cycles of 15s at 92 °C, 30s at 54 °C and 20s at 72 °C. Each 10 μL polymerase chain reaction (PCR) contained approximately 75 ng of total DNA, 2 mM $MgCl_2$, 1× PCR buffer (Fisher Scientific, Ottawa, ON, Canada), 0.4 mM of each deoxynucleotide (Fisher), 0.25 units Taq DNA polymerase (Fisher) and 0.2 μM of each forward and reverse primer (Invitrogen Life Technologies, Burlington, ON, Canada). DNA was amplified using primers for the loci Lmar10, Lmar14 and RB7 (primers published in DeWoody *et al.*, 1998 and Schable *et al.*, 2002) and the forward primer was fluorescently labelled (Lmar14 by D3-PA green, Lmar10 and RB7 by D2-PA black; Beckman Coulter). The genotypes at these loci do not deviate from Hardy-Weinberg equilibrium in the Lake Opinicon population. PCR product was run following standard protocol for the CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA). Offspring were assigned to parents using PAPA version 1.1, a parentage assignment program based on a maximum likelihood method, with the typing error rate set at 0.01 (Duchesne *et al.*, 2002). Survivorship was then determined for each full-sib family by dividing the number of fish collected from the microcosm by the original number released into the microcosm.

Statistical analysis

First, we performed a paired t-test to determine if the numbers of larvae released into the microcosm differed between half-sib families. Next, to determine if there were differences in growth rate and condition between offspring of cuckolders and parentals, we used mixed model analyses of variance with spawn date (11 or 25 June) and sire life history as fixed factors, and dam identity (maternal line) and the sire life history–dam identity interaction as random factors. Because the numbers of offspring within the paired half-sib families were unbalanced, we used a restricted maximum likelihood (REML) method, which is superior to a traditional estimator under such circumstances (Lynch & Walsh, 1998, p. 779).

Difference in survivorship between offspring of cuckolders and parentals was analysed using a Monte Carlo analysis (Manly, 1997). This analysis allowed us to incorporate differences in the numbers of larvae introduced into the microcosm from each family and as such, was more powerful than a binomial test. We set our null hypothesis as equal survivorship for each paired half-sib family and generated 50 000 data sets of the 25 paired families (50 families total). From each of these data sets, we calculated the ratio of pairs in which the cuckolder family had higher survivorship than the parental family. We then compared the observed ratio to these ratios to determine the P-value associated with the null hypothesis of equal survivorship.

To examine the potential trade-off between survivorship and growth rate, we used an ANCOVA with survivorship entered as the dependent variable, mean growth rate entered as the covariate and sire life history entered

as a fixed factor. We assessed maternal effects using linear regressions between dam body length or condition and mean offspring growth rate, condition, and survivorship. The survivorship data were arcsine square root transformed for these analyses, although the data are displayed in their untransformed state.

Finally, the survivorship data were then used in conjunction with the genetic paternity data from three previous studies (Philipp & Gross, 1994; Fu *et al.*, 2001; Neff, 2001) to estimate the relative fitness of the two life histories. Specifically, we used the life history model developed by Gross & Charnov (1980), which shows that the relative fitness of the two life histories can be calculated from:

$$\frac{\omega_c}{\omega_p} = \frac{q \times (1-h)}{(1-q) \times h}; \quad (1)$$

where ω denotes the fitness of cuckolders (c) or parentals (p), q denotes the proportion of all males that enter the parental life history and h denotes the proportion of eggs fertilized by all parentals. When the two life histories have equal fitnesses, eqn 1 simplifies to $q = h$ (i.e. the proportion of males entering the parental life history equals the proportion of all eggs fertilized by parentals). However, because previous estimates of q have been based on samples of 2 year old males, when the two life histories first diverge with respect to gonad development, the model assumes that there is no differential survivorship of cuckolder and parental offspring up to this age (see Gross & Charnov, 1980). When there is differential survivorship, h must be adjusted to represent the proportion of all 2 year olds fertilized by parentals:

$$\frac{\omega_c}{\omega_p} = \frac{q \times (1-h) \times s_c}{(1-q) \times h \times s_p}; \quad (2)$$

where s denotes the survivorship of cuckolder offspring or parental offspring to 2 years of age.

To obtain a confidence interval for the relative fitness calculation, we performed a second Monte Carlo analysis. This analysis re-sampled (with replacement) the original data for each variable in eqn 2. Specifically, q was estimated by re-sampling 139, 2-year-old males [58 males from Gross & Charnov (1980) and 81 males from the authors' unpublished data; there was no statistical difference in the proportion of cuckolders in the two samples: $\chi^2_1 = 0.51$, $P = 0.48$], h was estimated by re-sampling 106 broods for which paternity data exist (Philipp & Gross, 1994; Fu *et al.*, 2001; Neff, 2001) and s_c and s_p were estimated by re-sampling the 25 paired half-sib families in the present study. These values were then used to recalculate the relative fitness (ω_c/ω_p). The procedure was performed 50 000 times and the resulting values were used to extract the 95% confidence interval for the relative fitness calculation.

All means are reported ± 1 SE. Statistical tests were performed using JMP (v. 4.0.4; SAS Institute, Cary, NC, USA).

Results

There was no significant difference in the numbers of larvae sired by cuckolders or parentals that were released into the microcosm (cuckolder families: 381 ± 49; parental families: 357 ± 56; paired $t_{24} = 0.53$, $P = 0.60$). Based on the juveniles collected from the microcosm, the PAPA software successfully assigned 97% ($n = 254$ of 262) to a parent pair (at the 95% CI); the remaining 3% could not be assigned to a single parent pair and were removed from further analysis.

Cuckolder offspring grew an average of 0.333 ± 0.011 mm day^{-1} to a total length of 18.7 ± 0.8 mm and had a condition of $0.672 \times 10^{-5} \pm 0.019 \times 10^{-5}$ g mm^{-3} ($n = 20$ families; Table 1). Parental offspring similarly grew an average of 0.328 ± 0.019 mm day^{-1} to a total length of 18.7 ± 1.3 mm and had a condition of $0.666 \times 10^{-5} \pm 0.036 \times 10^{-5}$ g mm^{-3} ($n = 19$ families). The mixed model ANOVA revealed no difference in the growth rates of offspring from cuckolder and parental sires ($F_{1,16} = 0.82$, $P = 0.38$; Fig. 1a). However, there was a significant effect of dam identity ($F_{20,16} = 3.27$, $P = 0.010$), indicating that some half-sib families contained faster growing individuals than other half-sib families. Dam identity explained 25% of the total variance in growth rate. There was no interaction between sire life history and dam identity on growth rate ($F_{16,215} = 0.16$, $P = 1.0$). The spawn date also was not significant ($F_{1,215} = 2.10$, $P = 0.15$).

There was no significant difference in the condition of offspring from cuckolder and parental sires ($F_{1,16} = 0.001$, $P = 0.97$; Fig. 1b), and there was no significant effect of dam identity ($F_{20,16} = 1.20$, $P = 0.36$) or the interaction term ($F_{16,214} = 0.98$, $P = 0.48$). The spawn date was significant ($F_{1,214} = 22.0$, $P < 0.001$), with individuals from the earlier spawn date being in better condition than those from the later spawn date.

Of the 50 families released into microcosm, there were surviving offspring from 20 of the cuckolder families and 19 of the parental families. Of the 25 dams, three (12%) had no surviving offspring, three (12%) had surviving offspring with only the cuckolder sire, two (8%) had surviving offspring with only the parental sire and the remaining 17 (68%) had surviving offspring with both sires. Mean family survivorship for cuckolder offspring was 1.6% ± 0.4% and for parental offspring was 1.1% ± 0.3% ($n = 25$ families for each). Among the 22 dams that had surviving offspring, we found that cuckolder offspring had higher survivorship than parental offspring in 15 half-sib families and lower survivorship in seven half-sib families (Fig. 1c). Thus, cuckolder offspring had higher survivorship than parental offspring in 2.14 (= 15/7) times as many of the paired half-sib families, which was significantly > 1 (Monte Carlo analysis: $P = 0.032$).

Although not statistically significant, there was a positive relationship between survivorship and mean growth rate (ANCOVA: $r^2 = 0.13$, $F_{1,36} = 3.63$,

Table 1 Summary of the offspring measurements for larval bluegill (*Lepomis macrochirus*). Half-sibling families were sired by either a parental (P) or cuckolder (C) male and the data include the number of offspring introduced to the microcosm (n1), surviving offspring (n2), survivorship (Surv.), mean total body length, apparent growth rate and condition.

Parents		Offspring					
Dam	Sire	n1	n2	Surv. (%)	Length (mm)	Growth rate (mm day^{-1})	Condition ($\times 10^{-5}$ g mm^{-3})
June 11							
1	P	195	10	5.1	24 ± 1	0.37 ± 0.02	0.81 ± 0.02
	C	380	5	1.3	21 ± 2	0.32 ± 0.03	0.76 ± 0.06
2	P	187	4	2.1	24 ± 4	0.37 ± 0.05	0.78 ± 0.09
	C	387	10	2.6	24 ± 1	0.37 ± 0.01	0.80 ± 0.02
3	P	357	2	0.6	29 ± 6	0.44 ± 0.09	1.01 ± 0.21
	C	211	0	0.0	–	–	–
4	P	108	0	0.0	–	–	–
	C	117	2	1.7	21 ± 2	0.32 ± 0.03	0.70 ± 0.10
5	P	172	1	0.6	20 ± 0	0.30 ± 0.00	0.67 ± 0.00
	C	382	6	1.6	23 ± 1	0.35 ± 0.01	0.75 ± 0.02
6	P	389	2	0.5	22 ± 4	0.34 ± 0.06	0.84 ± 0.10
	C	97	8	8.2	25 ± 1	0.39 ± 0.02	0.78 ± 0.02
7	P	77	0	0.0	–	–	–
	C	75	0	0.0	–	–	–
8	P	77	0	0.0	–	–	–
	C	44	0	0.0	–	–	–
9	P	465	3	0.6	22 ± 2	0.34 ± 0.02	0.78 ± 0.06
	C	136	2	1.5	20 ± 1	0.31 ± 0.02	0.75 ± 0.01
10	P	256	2	0.8	22 ± 0	0.34 ± 0.00	0.74 ± 0.01
	C	226	3	1.3	22 ± 1	0.35 ± 0.02	0.75 ± 0.02
11	P	10	0	0.0	–	–	–
	C	147	0	0.0	–	–	–
June 25							
12	P	354	5	1.4	15 ± 2	0.30 ± 0.04	0.59 ± 0.04
	C	556	15	2.7	15 ± 1	0.30 ± 0.02	0.59 ± 0.03
13	P	862	7	0.8	22 ± 1	0.44 ± 0.02	0.76 ± 0.04
	C	819	9	1.1	17 ± 1	0.33 ± 0.03	0.63 ± 0.04
14	P	770	3	0.4	11 ± 2	0.22 ± 0.03	0.48 ± 0.17
	C	645	5	0.8	12 ± 0	0.23 ± 0.01	0.53 ± 0.02
15	P	572	5	0.9	13 ± 1	0.25 ± 0.02	0.46 ± 0.06
	C	595	5	0.8	11 ± 1	0.22 ± 0.00	0.46 ± 0.03
16	P	1083	3	0.3	12 ± 0	0.23 ± 0.01	0.38 ± 0.04
	C	666	1	0.2	22 ± 0	0.44 ± 0.00	0.69 ± 0.00
17	P	551	4	0.7	21 ± 2	0.41 ± 0.03	0.71 ± 0.05
	C	510	11	2.2	19 ± 1	0.37 ± 0.02	0.69 ± 0.02
18	P	334	3	0.9	18 ± 1	0.36 ± 0.01	0.66 ± 0.01
	C	383	8	2.1	18 ± 2	0.35 ± 0.03	0.64 ± 0.04
19	P	450	1	0.2	10 ± 0	0.20 ± 0.00	0.50 ± 0.00
	C	460	2	0.4	17 ± 4	0.33 ± 0.07	0.64 ± 0.03
20	P	58	2	3.4	12 ± 0	0.25 ± 0.00	0.50 ± 0.05
	C	859	1	0.1	16 ± 0	0.31 ± 0.00	0.61 ± 0.00
21	P	771	51	6.6	21 ± 0	0.40 ± 0.01	0.73 ± 0.01
	C	732	30	4.1	20 ± 1	0.39 ± 0.02	0.70 ± 0.02
22	P	93	1	1.1	22 ± 0	0.44 ± 0.00	0.69 ± 0.00
	C	115	0	0.0	–	–	–
23	P	277	0	0.0	–	–	–
	C	447	5	1.1	15 ± 2	0.30 ± 0.04	0.65 ± 0.06
24	P	231	1	0.4	11 ± 0	0.22 ± 0.00	0.55 ± 0.00
	C	278	8	2.9	18 ± 2	0.35 ± 0.04	0.66 ± 0.05
25	P	225	0	0.0	–	–	–
	C	264	8	3.0	18 ± 2	0.36 ± 0.03	0.64 ± 0.02

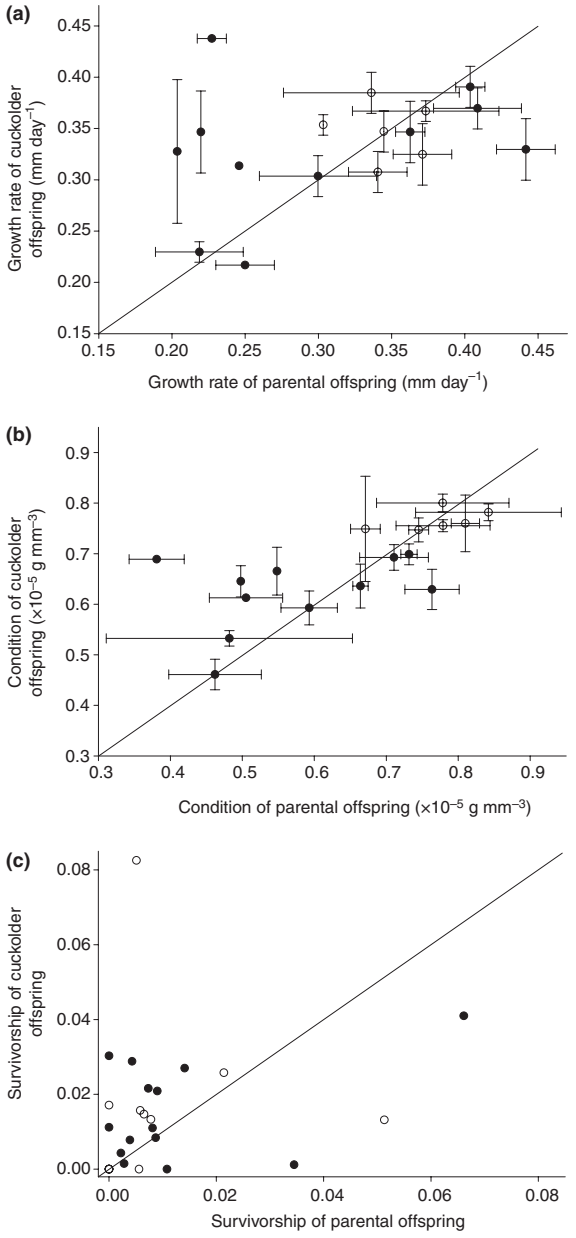

Fig. 1 Paired half-sib family means for (a) apparent growth rate, (b) Fulton's condition factor and (c) survivorship in bluegill (*Lepomis macrochirus*). The *x*-axes represent values from families sired by parental males and the *y*-axes represent values from families sired by cuckolder males. The diagonal lines represent equal values for each half-sib family. Overall, individuals had higher mean growth rate in none of the cuckolder families as compared to higher mean growth rate in eight of the parental families, higher mean condition in eight of the cuckolder families as compared to nine of the parental families, and higher survivorship in 15 of the cuckolder families as compared to seven of the parental families. Open circles represent families spawned on 11 June and filled circles represent families spawned on 25 June. The error bars represent ± 1 SE. The data point in the lower left corner of (c) represents three dams that had no surviving offspring with either sire type.

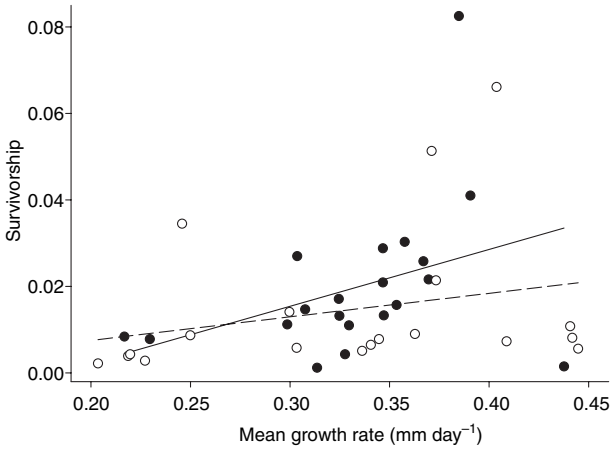

Fig. 2 The relationship between mean apparent growth rate and mean survivorship for full-sib families in bluegill (*Lepomis macrochirus*). Filled circles and solid lines represent cuckolder families and open circles and dashed lines represent parental families. The equations of the lines are: cuckolder, survivorship = 0.132 × mean growth rate − 0.024; parental, survivorship = 0.054 × mean growth rate + 0.003. The lines are from linear regressions, although the statistical analyses used ANCOVA (see text).

$P = 0.065$), and this relationship was similar for both cuckolder and parental life histories ($P > 0.23$ for main and interaction effects; Fig. 2). There was no relationship between dam body length or condition and mean offspring growth rate, condition or survivorship ($P > 0.09$ for each).

Using eqn 2, and assuming that q equals 82% (=114/139; Gross & Charnov, 1980, authors' unpublished data), h equals 78% (Philipp & Gross, 1994; Fu *et al.*, 2001; Neff, 2001), and s_c and s_p equal 0.016 and 0.011, respectively, the fitness of cuckolders relative to parentals was estimated to be 1.87 {= [0.82 × (1–0.78) × 0.016]/[(1–0.82) × 0.78 × 0.011]}. The Monte Carlo analysis provided a 95% confidence interval of 0.86–4.5 (Fig. 3).

Discussion

We used a quantitative breeding design and a semi-natural microcosm to examine the performance of juvenile bluegill. We found that dam identity explained 25% of the variation in offspring apparent growth rate, but this variation was not attributed to dam body length or condition. The dam effect may instead relate to differences in egg size or quality (e.g. Heath *et al.*, 1999). We also found that offspring from sires that differ in life history ('parental' and 'cuckolder') have similar apparent growth rates and body condition, but their families differ in survivorship. Our split *in vitro* fertilization design, which controls for dam effects, revealed that families sired by cuckolders had 1.4 times (=0.016/0.011) higher survivorship than families sired by parentals.

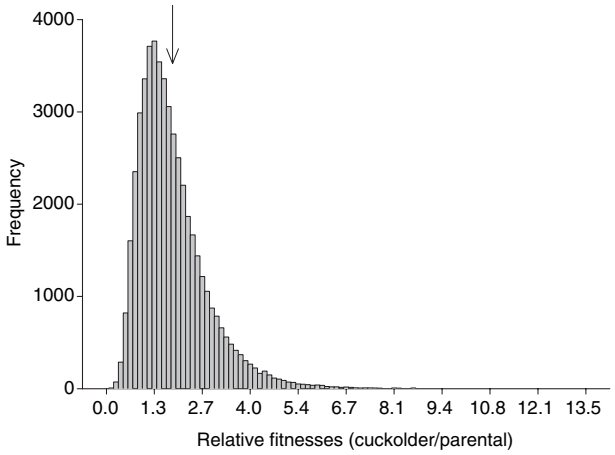

Fig. 3 Histogram of the Monte Carlo analysis of relative fitnesses for cuckolder and parental life histories in bluegill (*Lepomis macrochirus*). The bars represent the frequency of values of relative fitnesses among the 50 000 iterations. The mean relative fitnesses value was 1.87 (arrow) with a 95% CI of 0.86–4.5.

The difference in survivorship between parental and cuckolder families may relate to differences in their willingness to take risks during foraging. Using a dichotomous choice experiment with risky and safe feeding habitats, we have previously found that cuckolder offspring were less willing than parental offspring to forage in the risky habitat even when the latter habitat was more profitable (Lister & Neff, 2006). Thus, the higher survivorship in cuckolder families observed in the present study may relate to a similar unwillingness of these individuals to forage in risky habitats. The difference in survivorship might also relate to an increased ability of cuckolder offspring to escape predation. For example, Neff (2004) found that cuckolder offspring raised in either the field or laboratory were longer than parental offspring when they switched to exogenous feeding, and the increased body size of cuckolder offspring was estimated to provide up to 3.5 times higher survivorship from predation by *H. canadensis*. Furthermore, the difference in conversion efficiency between offspring of cuckolders vs. parentals (Neff, 2004), assuming that it continues during exogenous feeding, may explain why cuckolder offspring were not smaller or in lower condition than parental offspring.

There was no apparent trade-off between survivorship and growth rate among our juvenile bluegill. Several empirical studies indicate that in order for an individual to attain a higher growth rate, food intake must increase and consequently survivorship typically decreases (e.g. Fraser & Gilliam, 1992; Biro *et al.*, 2004). However, we found a positive relationship ($P = 0.065$) between apparent growth rate and survivorship for both parental offspring and cuckolder offspring. Studies on fishes indicate that vulnerability to predation decreases as size increases (Ware, 1975; Reznick *et al.*, 1990; Persson *et al.*, 1996). Indeed, our predator *H. canadensis* does select for smaller prey (< 10 mm; Neff, 2004; also see Elliott *et al.*, 1997). Thus, it is possible that in our study, offspring with higher growth rates surpassed some threshold of susceptibility to predation sooner than those with lower growth rates. Consequently, over the course of our study, families with higher mean growth rates may not have experienced reduced survivorship.

The differential survivorship between cuckolder and parental offspring has important implications for understanding the mechanism governing the life histories. Specifically, if the two life histories represent a pair of alternative strategies, then equal fitnesses are required to ensure evolutionary stability (Maynard Smith, 1982; Gross, 1996). If the two life histories instead represent a conditional strategy, then unequal fitnesses are expected (Gross, 1996). Gross & Charnov (1980) developed a life history model to calculate the relative fitnesses of cuckolder and parentals (also see Gross, 1982). Their original application of the model to bluegill showed that the proportion of males entering the parental life history was similar in value to the proportion of eggs fertilized by all parentals. Thus, their calculation suggested that the two life histories had close to equal fitness (i.e. $q = h$, see eqn 1). However, their calculation assumed that there was no differential survivorship of offspring fertilized by parentals vs. cuckolders up to 2 years of age.

Incorporating the differential survivorship estimated in our study, we calculated that cuckolders actually have 1.87 (95% CI = 0.86–4.5) times higher fitness than parentals. Some caution is warranted when interpreting this value because it was not significantly > 1 (Monte Carlo analysis: $P = 0.059$). Furthermore, our survivorship values are based on a microcosm and not the more complex natural environment of Lake Opinicon. For example, although our microcosm included a major predator of bluegill larvae, *H. canadensis* (Elliott *et al.*, 1997), it did not include other predators such as other fishes. Nevertheless, if the fitness of the cuckolder life history is in fact nearly two-fold higher than the fitness of the parental life history, then the life histories could not have evolved as alternative strategies (i.e. a genetic polymorphism) because there would be strong selection against the parental life history. The life histories may instead represent a conditional strategy whereby there is a 'decision' gene that is monomorphic in the population and serves to shunt individuals into one or the other life history based on their condition or status (Gross, 1996). In this case, parental males would be 'making the best of a bad situation.' The differential fitness calculated for bluegill would also be in sharp contrast to the equal fitnesses calculated for alternative life histories in the marine isopod, swordtail and side-blotched lizard (Shuster & Wade, 1991; Ryan *et al.*, 1992; Sinervo & Lively, 1996).

If the male bluegill life histories represent a conditional strategy, then it is interesting that the life histories also display a moderate level of heritability. In several other

species, an individuals body size or growth rate is a key component of the 'condition' that governs which life history is adopted (e.g. Hunt & Simmons, 1997; Hutchings & Jones, 1998). We have previously argued that conversion efficiency – the ability of an individual to convert ingested food into soma – also may play an important role in governing which life history is adopted (Lister & Neff, 2006). Growth rate, body size and conversion efficiency all are likely to have an additive genetic component and thereby show some degree of heritability. The heritability of life history observed in bluegill may thus be an indirect effect of the inheritance of 'condition' (i.e. conversion efficiency; see Lister & Neff, 2006 for further discussion). Modelling has shown that such inheritance does not preclude evolutionary stability of a conditional strategy (Repka & Gross, 1995; Gross & Repka, 1998).

In conclusion, there was no difference in the apparent growth rates of offspring sired by males of cuckolder and parental life histories in bluegill. However, families of cuckolders had higher survivorship than those of parentals. In conjunction with paternity data, the survivorship data suggest that cuckolders have higher fitness than parentals, and as such, the life histories may represent a conditional strategy.

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