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Benefits of Multiple Mating in guppy (*Poecilia reticulata*)

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Acknowledgement

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Abstract

Polyandry is a behavior more common in nature than first assumed. During the last decade there have been increasing amounts of evidence that in absence of direct benefits, indirect benefits can offset the costs of polyandry. In the guppy, several studies showed that females adjust brood- and offspring size in response difference in mating system. These studies did however suffer from various problems, such as variation in density between the monandrous and polyandrous treatment which might be a confounding effect for the results. In this study, we tested whether female multiple mating affects offspring quality, and if this difference is resource dependent. The study had four treatments – monandrous high food, monandrous low food, polyandrous high food and polyandrous low food treatments. We found that reproductive success of females was affected by polyandry which suggests that females receive indirect benefits from the males. After birth there was no marked difference between females mated multiply and females mated singularly. Offspring size has a very low variation suggesting strong canalization off offspring size. Whatever variation left in offspring size is most likely due to maternal effects and not an effect of indirect benefits from the male.

Sammendrag

Polyandri er mer vanlig i naturen en først antatt. I løpet av det siste tiåret har bevismengden for at indirekte fordeler kan veie opp for kostnader ved polyandri, i fravær av direkte overførbare fordeler, vært økende. Hos guppyer viste flere studier at hunner er i stand til å justere størrelse på kullet og avkommet i respons av forskjeller i paringssystemet. Disse studiene har imidlertid flere problemer ved seg som for eksempel forskjell i tettheten mellom hunner som lever i polyandri og monogami. Dette kan være bakenforliggende faktorer for deres resultater. I dette eksperimentet testet vi om polyandri påvirket kvaliteten på avkommet, og om dette er påvirket av ressurstilgang. Vi hadde fire behandlinger i eksperimentet – monogame hunner med høy mattilgang, monogame hunner med lav mattilgang, polygame hunner med høy mattilgang og polygame hunner med lav mattilgang. Vi så at hunnens reproduktive suksess var påvirket av polyandri, noe som tyder på at hunner får indirekte fordeler fra hanner. Etter at avkommene var født fant vi ingen forskjell mellom hunner som lever i monogami eller polyandri. Avkommets størrelse hadde en svært lav varians, noe som tyder på at størrelse hos avkom er sterkt kanalisert. Variasjonen i avkommets størrelse skyldes mest sannsynlig påvirkninger fra mor og ikke indirekte effekter fra hannen.

Introduction

Sexual selection occurs because of differences among individuals to gain access to mates and their gametes (Andersson, 1994). Bateman (1948) studied the variation in reproductive success in male and female fruit flies (*Drosophila melanogaster*) and showed that male reproductive success varies more than females (figure 1). Males benefit from multiple mating because they can impregnate multiple partners, while females only have a certain amount of eggs, and do no benefit from re-mating once all their eggs have been fertilized (Bateman, 1948).

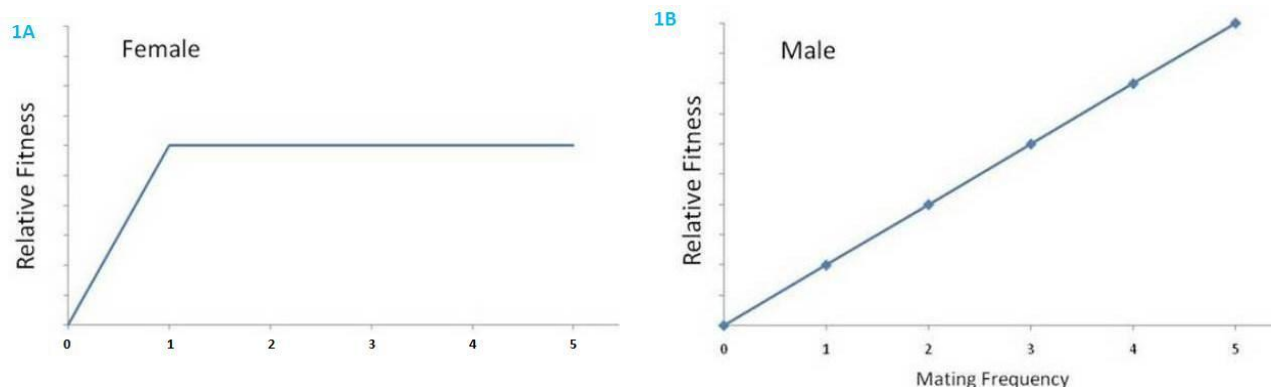


Figure 1. The Bateman principle. 1A) Relative female fitness are affected by mating frequency. After one mating, fitness remains constant as sperm from one male is enough to fertilize all her eggs. 1B) Males fitness increase as mating frequency increases (modified from Krasnec et al. 2012).

Females mating with more than one male during one mating sequence (polyandry) is a behavior more common in nature than first assumed (Andersson, 1994). This is an intriguing aspect of sexual conflict, as Bateman (1948) showed that most females can achieve full fertilization from one single male, and because mating often comes with some costs (Williams, 1966; Daly, 1978). For polyandry to evolve the costs of re-mating must be offset by the benefits (Petrie & Lipsitch, 1994). There are several ways in which females might benefit from polyandry. In some species, females mate multiply in order to secure fertilization of all their eggs (Tregenza & Wedell, 1998; Arnqvist & Nilsson, 2000). In other species, females receive direct benefits by gaining access to resources, from nuptial gifts or sperm transfer (Gwynne & Simmons, 1990; Arnqvist & Nilsson, 2000; Engqvist, 2007). In species with male biased mating systems, females might mate repeatedly to avoid costly harassment by males, a behavior that has been referred to as “convenience polyandry” (Thornhill & Alcock, 1983; Rowe, 1992; Rivera & Andres, 2002). However, not all polyandrous females receive direct benefits from mating multiply.

In absence of direct benefits, indirect benefits can offset the costs of polyandry. Over the last decade there have been increasing amounts of evidence to support the hypothesis that polyandry can evolve based on indirect /genetic benefits (Eberhard, 1996; Jennions & Petrie, 2000; Simmons, 2005). Higher genetic quality can result from either additive and/or non-additive genetic effects (Neff & Pitcher, 2005). Additive genetic effects often referred to as “good genes”, increase offspring attractiveness or viability by inheriting alleles from a male of high genetic quality (Jennions & Petrie, 2000). The “good gene” theory is related to the “sexy son” hypothesis introduced by Fisher (1930). The additive genetic effect assumes that all females will equally benefit by mating with a certain male. There are, however, reasons to believe that the ideal partner varies among females (Jennions & Petrie, 2000). The non-additive genetic effect, also referred to as “compatible genes”, increases fitness through favorable interactions between paternal and maternal genomes (Zeh & Zeh, 1996; Johnsen et al. 2000; Neff & Pitcher, 2005).

Offspring genetic quality can be enhanced through various mechanisms - before mating, before fertilization and/or after fertilization (Neff & Pitcher, 2005). Before mating (pre-copulatory) females can increase their offspring’s genetic quality by choosing to mate only with males that will contribute “good genes” (Jennions & Petrie, 2000) or compatible genes to their offspring (Thornhill, 1983; Andersson, 1994; Neff & Pitcher, 2005). In this case, polyandrous females may “trade-up” by mating with a second mate with good genes (Halliday, 1983; Gabor & Halliday, 1997; Jennions & Petrie, 2000; Pitcher et al. 2003) or for compatible genes (Johnsen et al. 2000) relative to their first mate. After sperm transfer females can influence the genetic quality of their offspring by “selecting” sperm carrying good or compatible genes to fertilize their eggs. This can be achieved by sperm competition and/or by female cryptic choice (Eberhard, 1996; Zeh & Zeh, 1997; Birkhead, 1998).

Conditions in parental environment, especially of the maternal environment play a major role in determining the offspring’s phenotype (Lynch & Ennis, 1983). By influencing condition of their offspring, parental investment enhances juvenile survival. Therefore, females can also influence the quality of their offspring through differential resource allocation. For example, by investing more resources in offspring of high genetic quality, parents can ensure that their surviving young are of the highest possible quality/condition (Zeh & Zeh, 1997; Neff & Pitcher, 2005). This is also known as the “differential allocation hypothesis” (Burley, 1988; Sheldon, 2000; Ratikainen & Kokko, 2010). Food availability could also have an effect as it influences the condition of the mother, which will also influence their capacity to further allocate resources to their offspring. For example, Reznick and Yang (1993) found that resource levels affect both female growth and reproduction in the guppy (*Poecilia reticulata*). Specifically, higher

food availability resulted in a significant increase in the number of offspring, independently of mother size, and less food resulted in an increase in the number of days between clutches. Females in better condition (e.g. high food availability) can afford to invest more resources to their offspring than females in poor condition. This suggests that females in good condition will have offspring of better quality.

In the guppy, several studies showed that females adjust brood- and offspring size in response to ecological factors, like food availability, competition and predation (Reznick & Yang, 1993; Bashey, 2008). Some studies also showed that females mated with several males produced larger offspring than females mated monandrously (Evans & Magurran, 2000; Ojanguren et al. 2005; Pélabon et al. 2011). This suggests that polyandry positively affects offspring size in this species. However, these studies suffer from various problems. For example, in the study by Ojanguren et al. (2005), females only had four days together with the male(s). This could result in sperm limitation, especially for females that were together with only one male. Sperm limitation could therefore affect clutch size (Evans & Magurran, 2000; Bayley et al. 2003), which again could affect offspring size (Skinner, 1985; Wilson & Lessells, 1994). Unfortunately, Ojanguren et al. (2005) did not correct for the effect of clutch size on offspring size and it remains difficult to estimate the exact effect of polyandry on offspring size. In addition these authors only observed offspring from the first clutch which can give a skewed result as the first clutch in guppy is generally smaller than later clutches. In the study from Larsen (2011) density during the mating period differed between monandrous and polyandrous females; females were either with only one male (monandrous treatment) or together with several males (polyandrous treatment). Density and operational sex ratio (OSR) are known to influence mating system and sexual selection (Emlen & Oring, 1977; Head & Brooks, 2006; Ojanguren & Magurran, 2007), as well as mating success or reproductive success (Jones et al. 2004; Jones et al. 2005; Klemme et al. 2007; Mills et al. 2007; Croshaw, 2010; Aronsen, 2012). Therefore, in the study by Larsen (2011), it is also difficult to distinguish the effect of multiple mating from the effects of density on the offspring size.

In this study, we tested whether female multiple mating affects offspring quality (size), and if this difference is resource dependent. If there is a difference in offspring quality due to multiple mating and if resource availability has an effect on offspring quality, this would suggest that the difference in offspring size results primarily from a differential investment by females. Alternatively, if size difference in offspring is unaffected by the resource availability, it would suggest that females receive genetic benefits from multiple mating and that the difference in offspring size between monandrous and polyandrous females is mainly genetic. In order to account for the numerous factors that can affect offspring size, we recorded the number of offspring in each clutch, as well as the number of clutches produced by females throughout six months.

Method



Figure 2. Guppies (*Poecilia reticulata*) are sexually dimorphic species with males being smaller and more colorful than females (Photo: Per-Harald Olsen).

Study species and population

The guppy (*Poecilia reticulata*) is a freshwater fish native to Trinidad and Tobago and the north-eastern parts of South America. Guppies are dimorphic, with males being smaller and more colorful than the larger and drab females (figure 2; Houde, 1997). Life history traits and ornamentation, as well as reproductive effort vary among populations and are partly dependant on environmental factors such as light, temperature, visibility, quality of the water and predation type (Endler, 1995; Houde, 1997; Magurran, 2005). Female guppies are ovoviparous, that is fertilization is internal and females give birth to fully developed offspring. Guppies mature by the age of 10-20 weeks (Magurran, 2005), and can give birth every third to fourth week after that (Reznick & Yang, 1993). Additionally, females are able to store sperm, which allows them to have several clutches from only one mating event. Mating system in the guppy is polygynandrous, with multi-male and multi-female polyandrous mating (Houde, 1997). Guppies have the highest reported frequency of multiple mating of all poeciliid species (Evans & Pilastro, 2011), and it is suggested to be the fish with the highest level of multiple fathers in a single clutch (Hain & Neff, 2007; Neff et al. 2008).

The guppies used in this experiment are laboratory-reared descendants from 500 wild caught individuals from the river Quare in Trinidad (10°39'N 61°12'W). The fish were collected in 1998 and transported to NTNU, Trondheim where they were placed in two large stock tanks with minimum interference. The populations in both tanks were always larger than 200 individuals. The fish were kept in stable laboratory conditions with a 12:12 hour light:dark cycle and water temperatures between 22°C and 26°C. They were fed *ad libitum*, once a day, alternating between commercially dried food flake (TetraMin) and newly hatched brine shrimp (*Artemia salina*).

Experimental fish

Juveniles were taken from stock aquariums and put into 5 liter aquariums. At sexual maturation, when gonopodium and color develop (Houde, 1997), males were returned to the stock aquariums and females were transferred individually to 1 liter aquariums to mature. We obtained a total of 160 females.

Aquariums were separated with thin wooden plank walls that prevented females to see each other and therefore avoiding dominance interaction (Borg et al. 2006; Borg et al. 2012). From the time females were taken out from stock population, until two weeks before the mating period was initiated, they were fed *ad libitum*, once a day, altering between commercially dried food flake(TetraMin) and newly hatched brine shrimp (*Artemia salina*).

Approximately three months after females were isolated they were photographed using a standardized assembly that contained a Canon EOS 300D digital camera with remote control, two mounted lights on each side of the camera and a white background. Fish were put in cold water (8-10°C) for a short amount of time right before photographing in order to slow them down. After the photo was taken, fish were placed back in their aquarium at normal water temperature (22-26°C) where they rapidly recovered. The fish did not show any signs of injury or sickness after this treatment. For each photo session a scale was photographed for size calibration. The size of all fish was measured from the photos using ImageJ, version 1.45s (Rasband, 1997-2012). We measured total and standard length, the standard length being defined from the tip of the jaw to the base of the tail (appendix 1).

An equal amount of adult male guppies (n=160) was sampled from the stock aquariums, photographed and placed individually in 1 liter aquariums where they had an acclimation period of 7 days. During this period males were fed *ad libitum*, once a day, altering between commercially dried food flakes (TetraMin) and newly hatched brine shrimp (*Artemia salina*).

Experimental treatments

In this study, we manipulated breeding regime and resource level, with two levels each, giving a total of four different treatments, polyandrous high food level, monandrous high food level, polyandrous low food level, and monandrous low food level. Monandrous females were mated with only one male and polyandrous females were mated with multiple males. For the high food level treatment we wanted the fish to be in good condition while for the low food treatment we wanted the fish to have poor condition but still get enough food to reproduce. Fish were sorted by size and subsequently assigned to a treatment in order to minimize size difference among treatments (Table 1).

Table1. Mean size of females with their standard errors (SE). We found no significant difference in models with or without the effect of treatments on length of females ($F_{2}=0.05$, $p=0.95$).

Treatment	Monandrous	Polyandrous
Low resource availability	20.91mm \pm 0.54mm	20.78mm \pm 0.54mm
High resource availability	21.11mm \pm 0.50mm	20.92mm \pm 0.52mm

Each treatment had an equal amount of males and females ($n=40$). The aquariums were randomly placed into numbered shelves to avoid confounding effect of placement. Females in the experiment were together with either one or four different males, on at a time. Fish in the polyandrous treatment were divided into ten blocks, each containing four males and four females. Fish were allocated to each block in order to minimize differences in size among blocks. Each morning the males in the polyandrous treatment were swapped between the four females in their block. In the monandrous treatment the males were removed and placed back with the same female every day in order to produce the same level of disturbance in both breeding-regime treatments.

In order to establish the amount of food provided to females for the food treatments, a pilot study was conducted. From this study we concluded that the *ad libitum* amount of food for an adult, female guppy was 0.008 grams per day; this was set as the daily food amount for the high resource treatment. In the limited-resource treatment, females received half of this amount, the amount being doubled when males and females were together. The food for one week for each female was weighed beforehand and stored in small eppendorf tubes.

Ethical permit

The study was conducted with consent from the Norwegian Animal Research Authority (FOTS ID#937).

The experiment

After females had been photographed, they were placed on their assigned food treatments. The original plan was to feed them once a day. However, this was adjusted to every second day due to difficulties of dividing the food in similar portions throughout the week. Two weeks after the food treatment was started, we began the mating period. The mating period lasted for 20 days after which the males were removed. All of the males were photographed and killed in ice and stored individually in ethanol for later DNA analysis. After the males had been removed, females were left in their aquariums to give birth. Java moss (*Vesicularia dubyana*) was added to provide shelter for the juveniles. Offspring were photographed together with their mother the day after birth. Offspring were killed and stored for later DNA analysis;

the mother was placed back in her aquarium. We ended the experiment six months after the males had been removed in accordance with earlier studies (Egset, 2011; Larsen, 2011). During this period we record the number of clutch produced, the size of each clutch, and the size of each offspring for each female. The standard length of the offspring was measured from the photos using the morphometric software tpsDig version 2.05 (Rohlf, 2005) and tpsUtil version 1.26 (Rohlf, 2004).

Statistical analyses

All analyses were done in R version 2.14.1 (R Development Core Team, 2011), for support of R we used RStudio version 0.97.314 (RStudio Team, 2012). For linear mixed-effects models (LMM) we used the *lmer* function from the *lme4* package (Bates et al. 2011). For analyses of not-normally distributed data with negative binomial errors we used the *glm.nb* function in the *MASS* package (Vanables & Ripley, 2002). For type III analysis of variance we used the function *Anova* from the *car* package (Fox & Sanford, 2011).

We used *generalized linear models* (GLM) to test the effect of the treatments and female length on the probability to reproduce (fertility) and number of offspring and clutches produced (fecundity). For the analysis of fertility, we analyzed the probability to reproduce with a GLM with binomial error. The number of clutches and number of offspring produced were analyzed using GLM with a poisson or a negative binomial error structure when data were over dispersed. We used the *Akaike's Information Criterion* (AIC) to find the best model. Age of the mother was not considered in the models as it is correlated with size in guppies. Mother size was entered in the model as covariates.

We performed a variance component analysis to find the sources of variation in offspring size. We used a LMM fitted by *maximum likelihood* (ML). Clutch number was nested within mother within treatments. For the analyses of offspring size we used a LMM fitted by ML for model selection. We tested the effect of treatment, mother length and clutch size on offspring size. Clutch number nested within mother was set as a random factor. The best model was selected using the AIC. Computations of slopes and the intercepts were done on models fitted by *restricted maximum likelihood* (REML). The best model according to model selection was mean centered and standardized. We mean centered female size and clutch size to get the mean offspring size in each treatment. Mean centering variables changes the values, but not the scale. The variation in mother length and clutch size was standardized. When we standardize we change the values and scale in order to compare coefficients for predictors that were measured on different scales.

To test if female growth were affected by offspring production (maternal investment) we did a type III anova. We used a type III anova because we have unbalanced data with significant correlations. Growth is estimated as the difference in length between the start and end of the study. For maternal investment we multiplied the mothers' mean offspring size with the total offspring number.

Results

Female fertility

The probability to produce at least one clutch tended to increase with female length (table 2).

Reproducing females were on average 5.5% larger than non-reproducing females (figure 3a). This effect was similar in all treatments (no interaction effects). Additionally, the probability of producing at least one offspring was on average 47.8% higher in polyandrous females than in monandrous ones, but this effect was affected by the difference in resource level (figure 3b). While the probability to reproduce increase by 18% with high food availability for the polyandrous females, it decreases by 33% in the monandrous females. Fertility of monandrous females on the other hand was 18% lower in high food availability than in low food availability.

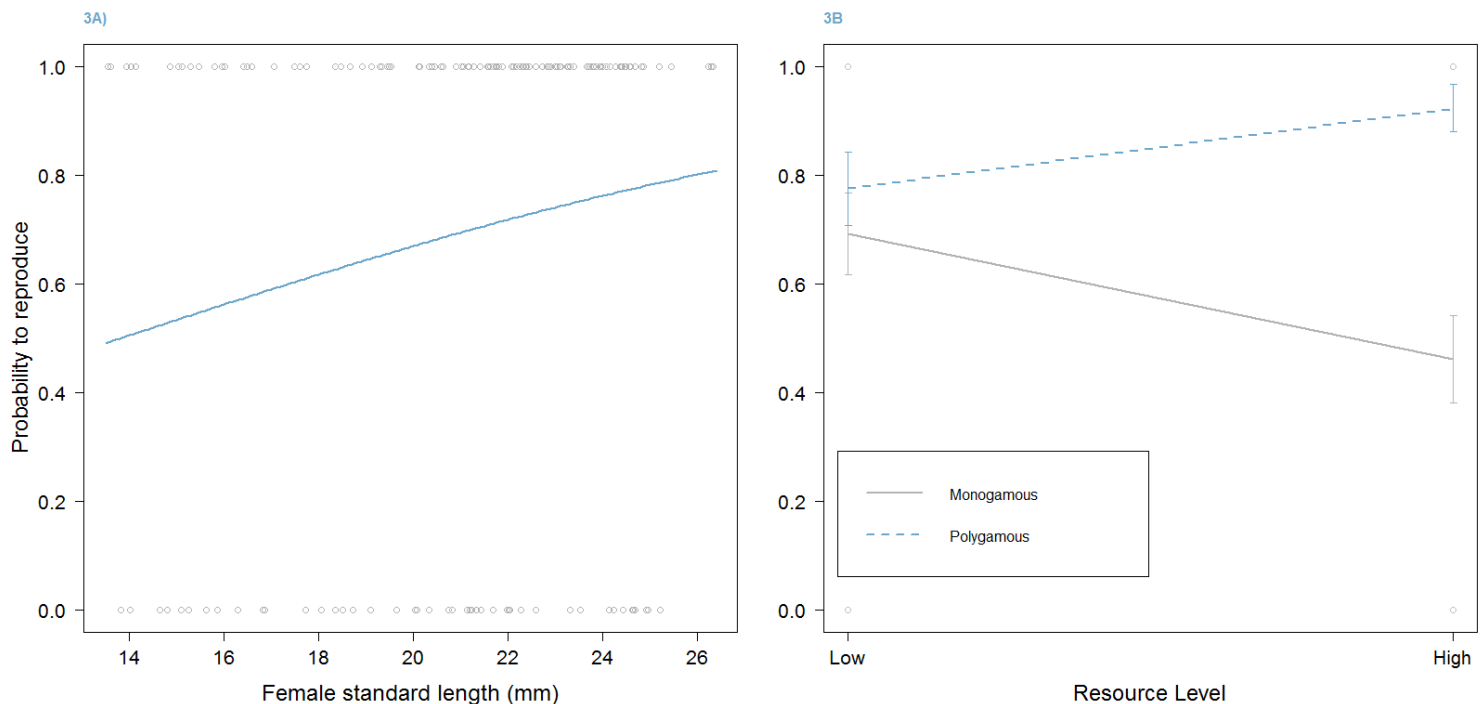


Figure3. Effect of breeding regime, food level and female standard length on the probability to reproduce, tested with a GLM (table 2). A) Relationship between the probability to reproduce and female standard length (p-value=0.05; mean and SE non-reproducing females: 20.12 ± 0.52 mm, mean size reproducing females: 21.23 ± 0.30 mm). B) Probability to reproduce is positively affected by differences in resource level (p-value=0.04) in interaction with mating system (p-value=0.01; mean and SE monandrous females (solid line): low food: 0.69 ± 0.08 ; high food: 0.46 ± 0.08 . mean and SE polyandrous females (dashed line): low food: 0.78 ± 0.07 ; high food: 0.92 ± 0.04). Overall high food availability had a negative effect on reproductive success

Table 2. Parameter estimate ($\beta \pm SE$) for the effect of breeding regime, food level and female standard length on the probability to reproduce. The test was conducted using a GLM with binomial error structure. Foodtreatment2 is equal to high food treatment and breedingregime2 is equal to the polygamous treatment.

Effect	$\beta \pm SE$	\bar{x} transformed (e^β)	z-value	p-value
Intercept	-1.58 \pm 1.27	0.21	-1.27	0.21
Factor(Breedingregime)2	0.49 \pm 0.52	1.63	0.52	0.35
Factor(Foodtreatment)2	-0.97 \pm 0.48	0.38	0.48	0.04
Female standard length	0.11 \pm 0.06	1.12	0.06	0.05
Fctr(Breedingregime2:Foodtreatment2)	2.22 \pm 0.86	9.207330866	0.86	0.01

Fecundity

Females produced between 1 and 6 clutches (mean \pm SE = 2.15 \pm 0.10). In total monandrous females produced respectively 57 and 63 clutches in the low food and high food treatment, and polyandrous females produced 42 and 79 clutches in low and high food treatment, respectively. We did not find any statistically significant effects of treatments or mother length on number of clutches produced (table 3).

Table 3. Parameter estimate ($\beta \pm SE$) for the effect of female standard length, mating system and food treatment on number of clutches produced per female (clutch size). This was tested using GLM with poisson family. Interactions effects were tested but are not presented in the table. None of the interactions were statistical significant (not shown).

Effect	$\beta \pm SE$	\bar{x} transformed (e^β)	z-value	p-value
Intercept	0.26 \pm 0.48	1.30	0.54	0.59
Female length	0.02 \pm 0.02	1.02	1.09	0.30
Factor(food treatment)2	0.08 \pm 0.13	1.08	0.58	0.57
Factor(breeding regime)2	-0.03 \pm 0.13	0.97	-0.24	0.81

Females had between 1 and 21 offspring per clutch (mean \pm SE = 9.33 \pm 0.62). The total number of offspring produced by a female was not statistically significantly different in the two mating treatments, but increased with female standard length similarly in both food treatments (table 4; figure 4).

Table4. Parameter estimate ($\beta \pm SE$) for the effect of female standard length, mating system and food treatment on the total number of offspring produced. This was tested using GLM with poisson family.

Effect	$\beta \pm SE$	\bar{x} transformed (e^β)	z-value	p-value
Intercept	-1.10 \pm 0.62	0.33	-1.80	0.07
Female length	0.15 \pm 0.0	1.16	5.31	<<0.001
Factor(Foodtreatment)2	1.90 \pm 0.91	6.69	2.10	0.04
Female length:factor(Foodtreatment)2	-0.08 \pm 0.04	0.92	-1.90	0.06

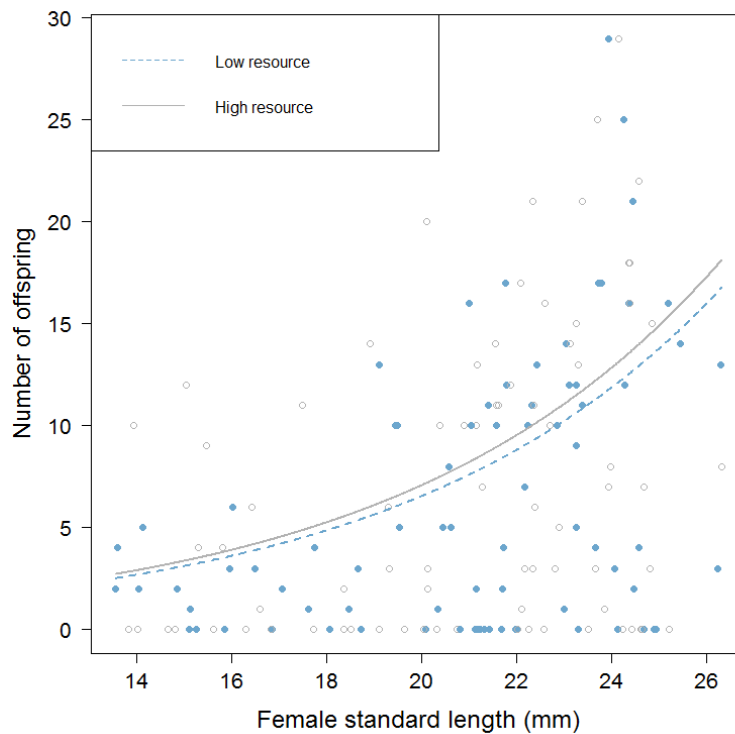


Figure4. The effect of female length and food level on total number of offspring produced, tested with a GLM (table 4). Total number of offspring produced were positively affected by female standard length ($p\text{-value} < 0.001$). Females in high resource level (solid line) had slightly more offspring than females in low resource level ($p\text{-value} = 0.04$; dashed line). The positive effect of female standard length on the total number of offspring produced tended to be negatively affected by high resource level (interaction effect, $p\text{-value} = 0.06$).

Offspring size

Females produced offspring of similar size in all treatments (appendix 2; $\text{mean} \pm \text{SE} = 6.97 \pm 0.01$).

Coefficients of variation (CV), defined as the ratio of the *standard deviation* (σ) to the *mean* (μ), showed that there is almost no variation in offspring size ($\text{CV} = 4.6\%$). A variance component analysis on offspring size showed that the major source of variation came from female length and the clutch size, while almost no variance was explained by the treatments (table 5). Offspring size increased with the length of the mother and decreased with clutch size. The positive effect of mother length was less pronounced under high food availability (figure 5a). The negative effect of clutch size was less pronounced in large and polyandrous females (figure 5b). There was also a positive, statistically significant interaction between clutch size and female length (table 6) indicating that the negative effect of clutch size on offspring size decreases for large females.

Table5. Variance component analysis of offspring standard length. Effect of mating regime and food level are treated together as the effect of treatments (Low food monandrous, high food monandrous, low food polyandrous, high food polyandrous).

Source of variation	Variance in offspring size	% Variance
Treatments	1.1×10^{-11}	9.0×10^{-9}
Mother (treatment)	0.05	42.98
Clutch (mother)	0.02	17.00
Residual	0.05	40.02

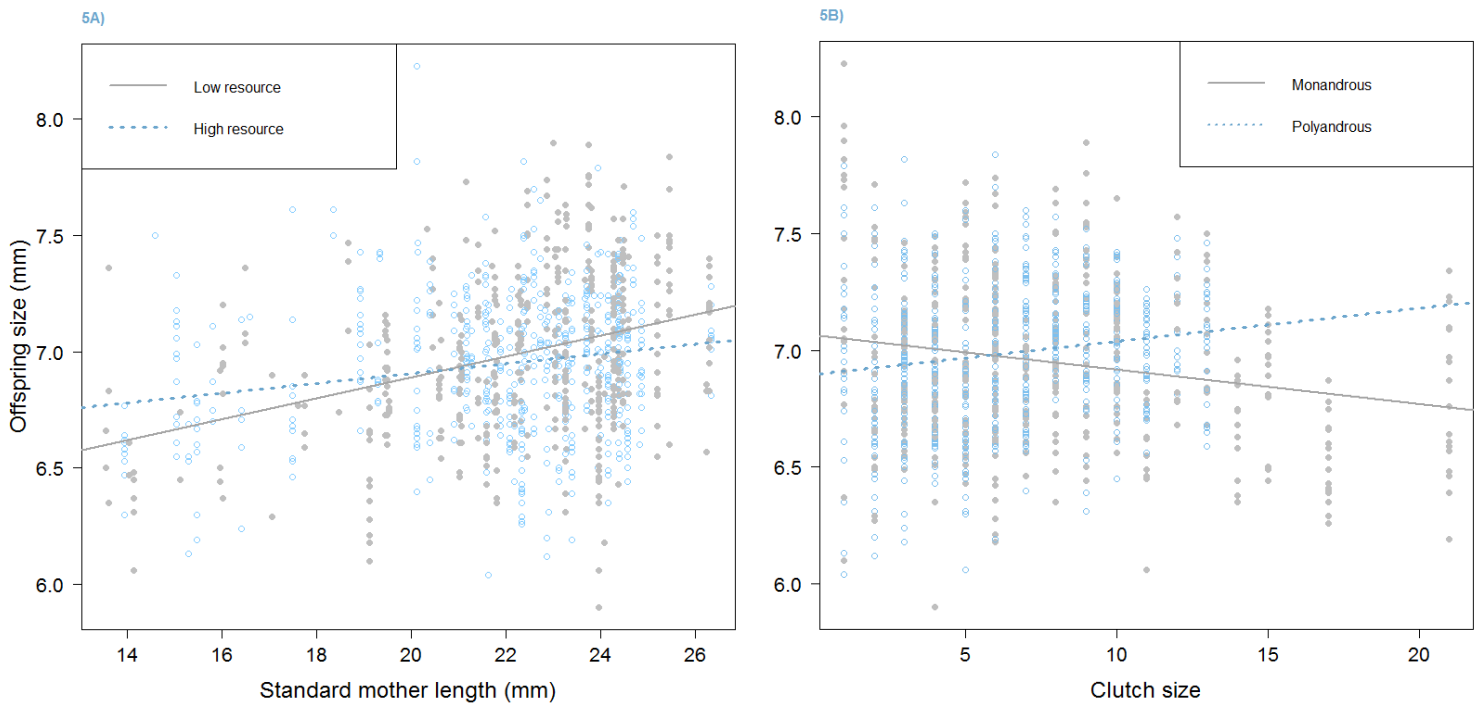


Figure5. Plots produced from the results of LMM on offspring size (table 5). A) Offspring size is affected by mother standard length and food level - low (solid line) and high (dashed line) resource level. 4b) Offspring size is affected by clutch size and mating system - monandrous (solid line) and polyandrous (dashed line) females.

Table6. Results for fixed effects of the best LMM on offspring size, model selection based on AIC (appendix 3). The variables were mean centered and standardized. Parameter estimate ($\beta \pm SE$) for the effect of monandrous treatment (BR1), polyandrous treatment (BR2), high food treatment (FT2), standardized female standard length (F.StL), standardized clutch size (StC), and the significant interactions.

Fixed effect	$\beta \pm SE$	t-value
BR1	7.00 ± 0.05	157.02
BR2	7.00 ± 0.04	164.03
FT2	-0.02 ± 0.05	-0.34
F.StL	0.17 ± 0.03	4.9
StC	-0.06 ± 0.02	-2.27
FT2:F.StL	-0.07 ± 0.04	-1.77
BR2:StC	0.08 ± 0.04	2.07
F.StL:StC	0.07 ± 0.03	2.78

Maternal investment

Female growth was affected by food treatment (figure 6). Females in the high food treatment grew 5% more than the females in low food treatment. Maternal investment had no statistically significant effect on female growth (not shown; table 7).

Table7. Mean growth of females in the treatments. Growth was entered as difference in size from the end of the mating period until the end of the experiment (6 months). Only reproducing females were used for these calculations.

Treatment	Monandrous	Polyandrous
Low resource availability	0.77 ± 0.33	1.19 ± 0.33
High resource availability	2.10 ± 0.37	2.02 ± 0.29

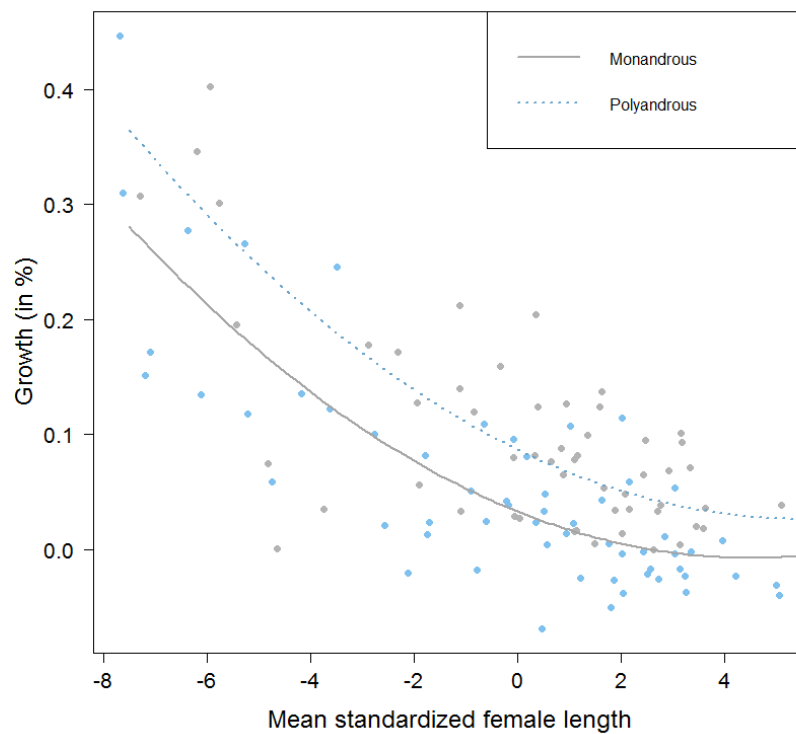


Figure6. Relationship between the female length and the growth expressed in percentage of length increase compared to the initial length. The estimates of the relationship for the females in the low food treatment are $\text{Growth} = 0.033 (+/- 0.001) - 0.018 (+/- 0.002) * \text{female length} + 0.002 (+/0.0005) * \text{female length}^2$; and for the high food treatment $\text{Growth} = 0.087 (+/- 0.001) - 0.018 (+/- 0.002) * \text{female length} + 0.002 (+/0.0005) * \text{female length}^2$ (r^2 of the model = 0.62).

Discussion

Previous studies investigating the effects of polyandry on offspring quality (size) indicated that polyandry might have benefits for females. These studies did not consider the possible confounding factors such as density and maternal effects. In this study we tested whether food availability and polyandry affected the female reproductive output by placing females in different treatments using a full-factorial design where we accounted for differences in density. We found that polyandry had a positive effect on female fertility. It also affected offspring size but only in interaction with female size. Polyandry did not have an effect on female fecundity, however. Resource availability had a negative effect on reproductive success in females but positively affected the number of offspring produced as well as decreasing the effect of mother length.

Female fertility and fecundity

We saw that polyandrous females had a higher reproductive success than monandrous females in interaction with food level (figure 2b). High food level significantly decreased the reproductive success of monandrous females. We suggest that female guppies mated singularly wait with reproduction when in good environment for a chance to mate with a male with “good” or more “compatible” genes. In poor environment (low food treatment) however, females accept higher risks during mate choice as their *residual reproductive value* (RRV) diminish and therefore prefer to reproduce rapidly before their condition deteriorate too much. This is known as the terminal investment hypothesis (Clutton-Brock, 1984). This effect of food deprivation was also found in another study by Javois and Tamaru (2004), supporting our theory. If this is true it would presume that females are controlling sperm use and are able to delay reproduction, suggesting that the difference in fertility does not result from the amount of sperm transferred but from a female decision. This is supported by our results because once a female reproduced there was no effect of the mating treatment on the number of offspring produced. We found an increase in total number of offspring produced in large females, this effect was not a result of more clutches (table 3), meaning that the observed increase in total offspring number comes from a larger clutch size.

Our results differed markedly from the study by Pélabon et al. (2011) who did not find any marked difference on fertility in polyandrous and monandrous females. Evans and Magurran (2000) however, found an increase in fertility for polyandrous females. But since their study differed from ours in several aspects it is quite difficult to directly draw any conclusions from the results. In their study females had a shorter mating period of only 4 days and additionally they only considered the effect of the first clutch, which could have had an effect on their results (page 5, introduction).

Offspring quality (size)

We found no direct effects of the treatments on offspring size. The results from CV showed that there was a very low variance in offspring size (<5%). This suggests a very strong canalization of offspring size at birth. Canalization of offspring size might have evolved as a response to high predation pressure both from predators and cannibalism.

Variation in offspring size was mostly from the variation within clutch and among females due to the size difference among females. This suggested that most of the variation in offspring size were due to maternal effects, and therefore non-genetic. Offspring size increased with the length of the mother but was less pronounced under high resource availability, suggesting that small females allocate more resources when in better condition, as expected from theory on differential allocation (Zeh & Zeh, 1996; Neff & Pitcher, 2005).

Clutch size negatively affected offspring size in the monandrous females but not in the polyandrous females. Looking at the data (figure 4b) it seems like that this observation could be a result of unbalanced data. Females from polyandrous treatments do not produce very large clutches (>15 offspring), which could affect the results. Removing these large clutches from the model and running it again gives us very similar results (not shown). We suggest that this effect could be from sibling rivalry. If multiply mated females have multiple paternity as was found by Evans and Magurran (2000) and Elgee et al. (2012), offspring in polyandrous females would be less related to each other than offspring sired by a single father. This could lead to a stronger rivalry between the offspring that would have to compete more and therefore take more of the mother's resources (Hepper, 1986; Mock & Parker, 1997).

These results are very different from the once observed by Ojanguren et al. (2005) and Pélabon et al. (2012) where they found that polyandrous females had larger offspring than monandrous females. These studies differ from our study in several ways. In the study by Ojanguren et al. (2005) they only mated the females for four hours each day for four consecutive days, which possible could have resulted in sperm depletion in the monandrous females. In addition to this they only looked at the first clutch, and only used data from 10 females in each of the treatments, where they choose to use only females that had a clutch size larger than four. In our study we have considered the entire data from a long term effect of polyandry making it difficult to compare these results. The study by Pélabon et al. (2012) also looked on the long term effect of polyandry, however in this study they did not account for the density, and their results could therefore have been from a confounding effect of differences in density in the polyandrous and monandrous females.

Maternal investment

The amount of growth during the course of the experiment was negatively affected by the size of the female. Larger females grew less than smaller ones, as expected if smaller fish were younger and were therefore still in their growing phase. This effect was non-linear, however, as indicated by the statistically significant quadratic term (Figure 5). This is also expected due to the asymptotic nature of the growth in fish. The food treatment strongly affected the growth of the fish, the growth being reduced by nearly 5% when food availability was reduced. This reduction in growth was similar for females of all sizes (no interaction effect). Maternal investment did not affect female growth in either treatment, suggesting that females do not compensate between growth and reproduction.

Conclusion

Reproductive success of females was affected by polyandry which suggests that females receive indirect benefits from the males. After birth there was no marked difference between females mated multiply and females mated singularly. Offspring size has a very low variation suggesting strong canalization of offspring size. Whatever variation left in offspring size is most likely maternal effects and not a effect of indirect benefits from the male.

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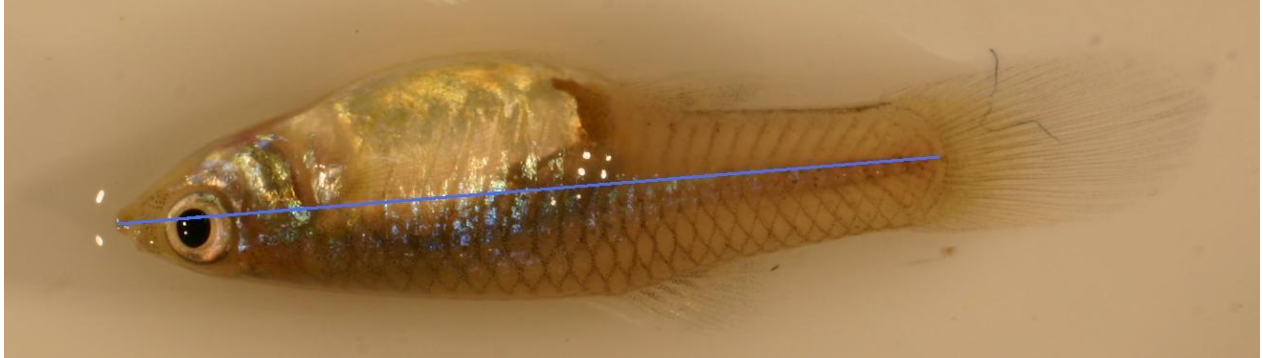
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Appendices

Appendix 1



Picture illustrating how we measured the standard length of the fish. Standard length of fish is defined as from the tip of the jaw to the base of the tail.

Appendix 2

Appendix2. Average offspring size (mm) with standard errors (SE) for all four treatments.

Treatment	Monandrous	Polyandrous
High resource availability	6.91 ±0.02 mm	6.98 ±0.07 mm
Low resource availability	6.99 ±0.02 mm	7.00 ±0.02 mm

Appendix 3

Model selection analysis of the relationship between treatments (breeding regime (BR) 1 and 2 are respectively monandrous and polyandrous treatment), female standard length (SD.L), clutch size (CS) and the interactions. The best models are bold typeface, with § being the selected model. Degree of freedom (d.f.), AIC, relative AIC (ΔAIC), Akaike weights (w_i).

Model	d.f.	AIC	ΔAIC	w_i
§ BR + FT + SD.L + CS + FT:SD.L + BR:CS +SD.L:CS	7	157.48	0.00	0.500
BR + FT + SD.L + CS + BR:SD.L + FT:SD.L + BR:CS +SD.L:CS	8	158.38	0.90	0.242
BR + FT + SD.L + CS + BR:CS + FT:SD.L + FT:CS + SD.L:CS	8	159.01	1.53	0.150
BR + FT + SD.L + CS + BR:FT + FT:SD.L + BR:CS + SD.L:CS	8	159.13	1.65	0.124
BR + FT + SD.L + CS + BR:SD.L + BR:CS + FT:SD.L + FT:CS + SD.L:CS	10	159.90	2.42	0.078
BR + FT + SD.L + CS + BR:CS + SD.L:CS	6	160.02	2.55	0.068
BR + FT + SD.L + CS + BR:CS + FT:CS +SD.L:CS	7	160.56	3.08	0.049
BR + FT + SD.L + CS + BR:FT + BR:CS + FT:SD.L + FT:CS + SD.L:CS	10	160.68	3.20	0.044
BR + FT + SD.L + CS + BR:FT + BR:CS +SD.L:CS	7	161.26	3.78	0.032
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + BR:CS + FT:CS +SD.L:CS	10	161.60	4.13	0.033
BR + FT + SD.L + CS + BR:SD.L + BR:CS + FT:CS +SD.L:CS	8	161.79	4.31	0.035
BR + FT + SD.L + CS + BR:FT + BR:CS + FT:CS +SD.L:CS	8	161.93	4.45	0.032
BR + FT + SD.L + CS + FT:SD.L + BR:CS	6	162.20	4.73	0.031
BR + FT + SD.L + CS + FT:SD.L + BR:CS + FT:CS	7	162.52	5.04	0.026
BR + FT + SD.L + CS + BR:FT + BR:SD.L + BR:CS + SD.L:CS	8	162.56	5.08	0.036
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:FT:SD.L	11	162.56	5.08	0.031
BR + FT + SD.L + CS + BR:CS + FT:CS	6	162.85	5.37	0.036
BR + FT + SD.L + CS + FT:SD.L +SD.L:CS	6	162.93	5.45	0.036
BR + FT + SD.L + CS + BR:FT + BR:SD.L + BR:CS + FT:CS + SD.L:CS	10	163.18	5.70	0.031
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:FT:CS	11	163.38	5.90	0.031
BR + FT + SD.L + CS + BR:SD.L + FT:SD.L + BR:CS + FT:CS	8	163.46	5.98	0.027
BR + FT + SD.L + CS + BR:SD.L + FT:SD.L + BR:CS + FT:CS	8	163.46	5.98	0.032
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:SD.L:CS	11	163.53	6.05	0.030
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + BR:CS + FT:CS + SD.L:CS + FT:SD.L:CS	11	163.60	6.12	0.028
BR + FT + SD.L + CS + BR:CS	5	163.66	6.18	0.029
BR + FT + SD.L + CS + BR:SD.L + FT:SD.L + FT:CS +SD.L:CS	8	163.68	6.20	0.029
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + SD.L:CS	8	163.84	6.36	0.026
BR + FT + SD.L + CS + FT:SD.L + FT:CS +SD.L:CS	7	164.06	6.58	0.026
BR + FT + SD.L + CS + BR:FT + FT:SD.L + BR:CS + FT:CS	8	164.13	6.65	0.025
BR + FT + SD.L + CS + BR:FT + BR:CS + FT:CS	7	164.21	6.73	0.023
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:FT:SD.L + BR:FT:CS	12	164.49	7.02	0.024
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:FT:SD.L + FT:SD.L:CS	12	164.55	7.07	0.022
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + BR:CS +	8	164.74	7.27	0.019
BR + FT + SD.L + CS + BR:SD.L + BR:CS	6	164.95	7.47	0.018
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + FT:CS + SD.L:CS	10	165.09	7.61	0.017
BR + FT + SD.L + CS + BR:FT + BR:SD.L + BR:CS + FT:SD.L + FT:CS	10	165.10	7.63	0.016
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:FT:CS + BR:SD.L:CS	12	165.24	7.76	0.016
BR + FT + SD.L + CS + BR:FT + FT:SD.L + FT:CS +SD.L:CS	8	165.37	7.89	0.015
BR + FT + SD.L + CS + BR:FT + BR:SD.L + BR:CS + FT:CS	8	165.44	7.96	0.015
BR + FT + SD.L + CS + FT:CS +SD.L:CS	6	165.51	8.03	0.015
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:SD.L:CS + FT:SD.L:CS	12	165.53	8.05	0.015
BR + FT + SD.L + CS + SD.L:CS	5	165.60	8.12	0.015
BR + FT + SD.L + CS + FT:SD.L	5	165.70	8.22	0.014

BR + FT + SD.L + CS + FT:SD.L + FT:CS	6	165.72	8.24	0.013
BR + FT + SD.L + CS + BR:SD.L + SD.L:CS	6	165.75	8.27	0.014
BR + FT + SD.L + CS + FT:CS	5	166.19	8.71	0.011
BR + FT + SD.L + CS + BR:FT +SD.L:CS	6	166.26	8.78	0.011
BR + FT + SD.L + CS + BR:FT + FT:CS +SD.L:CS	7	166.41	8.93	0.010
BR + FT + SD.L + CS + BR:SD.L + FT:CS	6	166.44	8.96	0.010
BR + FT + SD.L + CS + BR:FT + BR:SD.L +FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:FT:SD.L + BR:FT:CS + BR:SD.L:CS	13	166.45	8.97	0.011
BR + FT + SD.L + CS + BR:FT + BR:SD.L +FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:FT:SD.L + BR:FT:CS + FT:SD.L:CS	13	166.47	9.00	0.011
BR + FT + SD.L + CS + BR:FT + BR:SD.L +FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:FT:SD.L + BR:SD.L:CS + FT:SD.L:CS	13	166.52	9.05	0.010
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:CS + SD.L:CS	8	166.62	9.15	0.009
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:CS +SD.L:CS	8	166.62	9.15	0.009
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L	7	166.83	9.35	0.008
BR + FT + SD.L + CS + BR:FT + BR:SD.L + FT:SD.L + FT:CS	8	166.99	9.51	0.008
BR + FT + SD.L + CS + BR:FT + FT:CS	6	167.14	9.67	0.007
BR + FT + SD.L + CS + BR:FT + BR:SD.L +FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:FT:CS + BR:SD.L:CS + FT:SD.L:CS	13	167.22	9.74	0.007
BR + FT + SD.L + CS	4	167.42	9.94	0.006
BR + FT + SD.L + CS + BR:SD.L	5	167.72	10.25	0.005
BR + FT + SD.L + CS + BR:FT	5	168.10	10.62	0.005
BR + FT + SD.L + CS + BR:FT + BR:SD.L +FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:FT:SD.L + BR:FTCS + BR:SD.L:CS + FT:SD.L:CS	14	168.43	10.95	0.004
BR + FT + SD.L + CS + BR:FT + BR:SD.L	6	168.49	11.01	0.004
BR + FT + SD.L + CS + BR:FT + BR:SD.L +FT:SD.L + BR:CS + FT:CS + SD.L:CS + BR:FT:SD.L + BR:FTCS + BR:SD.L:CS + FT:SD.L:CS	15		12.88	0.002
+ BR:FT:SD.L:CS		170.36		