

## Tactic-specific benefits of polyandry in Chinook salmon *Oncorhynchus tshawytscha*

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(Received 20 June 2016, Accepted 18 October 2016)

This study examined whether polyandrous female Chinook salmon *Oncorhynchus tshawytscha* obtain benefits compared with monandrous females through an increase in hatching success. Both of the alternative reproductive tactics present in male *O. tshawytscha* (large hooknoses and small, precocious jacks) were used, such that eggs were either fertilized by a single male (from each tactic) or multiple males (using two males from the same or different tactics). The results show that fertilized eggs from the polyandrous treatments had a significantly higher hatching success than those from the monandrous treatments. It is also shown that sperm speed was positively related with offspring hatching success. Finally, there were tactic-specific effects on the benefits females received. The inclusion of jacks in any cross resulted in offspring with higher hatching success, with the cross that involved a male from each tactic providing offspring with the highest hatching success than any other cross. This study has important implications for the evolution of multiple mating and why it is so prevalent across taxa, while also providing knowledge on the evolution of mating systems, specifically those with alternative reproductive tactics.

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Key words: alternative reproductive tactics; genetic benefits; hatching success; multiple mating; sperm competition.

### INTRODUCTION

Polyandry, where females mate with multiple males within a single reproductive event, is a taxonomically widespread phenomenon and how it has evolved remains an important question for evolutionary biologists (Simmons, 2001; García-González & Simmons, 2005; Taylor *et al.*, 2014). Although mating is often very costly, through increased time and energy expenditure and increased risk of predator exposure, multiple mating is prevalent in many animal species. Thus, the benefits of multiple mating are presumed to exceed the associated costs (Jennions & Petrie, 2000). In species where females receive a material benefit during copulation with a male, or where the availability of viable sperm supply limits female reproductive success (Simmons, 2001), the benefits of polyandry are obvious. For example, it has been shown that in 58% of insect species, females ran out of sperm if only allowed to mate

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once (Ridley, 1988). Polyandry still occurs, however, in species where fertility does not increase by mating with multiple males, and nor do other direct benefits (*e.g.* nuptial gifts or parental care) to females exist. It is the frequency of polyandry in these non-resource-based mating systems that continues to puzzle evolutionary biologists, which has led to a number of theoretical hypotheses that attempt to explain these potential genetic (or indirect) benefits (Jennions & Petrie, 2000; Tregenza & Wedell, 2000; Simmons, 2001).

First, females can mate multiply as a bet-hedging strategy against poor male quality or uncertain future environments (Watson, 1991). By mating with multiple males, females reduce the risk of having all of their offspring sired by an unsuitable male, which could result in the complete loss of offspring and thus reproductive failure (Simons, 2011). Additionally, when future environmental conditions are unpredictable, polyandrous females benefit by increasing genetic diversity of offspring and thus lowering the risk of having no offspring suited to the future environments (Wedekind & Rüetschi, 2000; Simons, 2011; Seppälä *et al.*, 2012). Evidence for this hypothesis is limited and has only been shown to be a possible mechanism in small populations and in populations where there is a large proportion of unsuitable mates (Yasui, 2001; Yasui & Garcia-Gonzalez, 2016).

Second, polyandry and thus the promotion of sperm competition, provides opportunities for females to use post-copulatory mate choice mechanisms (*i.e.* cryptic female choice) to bias paternity towards more suitable males. This can be done in two ways: first, through a good-genes mechanism, referred to as the good-sperm hypothesis, whereby paternity is biased towards males of higher intrinsic quality, which is thought to increase offspring fitness (Yasui, 1997). The second possibility is through a compatible-genes mechanism, where a female would bias paternity towards males that have a more compatible genotype to the female. This is thought to be an aid in inbreeding avoidance or increasing heterozygosity, therefore resulting in offspring with higher fitness (Zeh & Zeh, 1996, 1997). Most research on the mechanisms driving polyandry for genetic benefits has focused on these two hypotheses, but the empirical evidence is limited and contradictory. For example, Simmons (2001) found no evidence for either the compatible-genes or good-sperm hypotheses in the field cricket *Teleogryllus oceanicus*. Female crickets did not skew paternity towards specific males as a means to increase offspring hatching success and a male cricket's success in sperm competition did not result in offspring with higher viability and performance (Simmons, 2001). A couple of studies, however, have shown support for the good-sperm hypothesis; Hosken *et al.* (2003) demonstrated that male yellow dung fly *Scatophaga stercoraria* that were able to displace the sperm of rival males in sperm competition produced offspring that had a faster development rate. Also, Fisher *et al.* (2006) found that in the brown antechinus *Antechinus stuartii*, males that had high paternity (*i.e.* were competitively superior in sperm competition) sired offspring that had greater survival.

The highly polyandrous Chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1792) is an ideal system to test the potential for benefits of polyandry; *O. tshawytscha* are external fertilizers with large numbers of gametes per individual, therefore allowing for powerful maternal half-sib study designs that control for maternal effects. Furthermore, *O. tshawytscha* males exhibit alternative reproductive tactics (ART). The ARTs include large 4–5 year old guard-type males (known as hooknoses, due to the curved snout) and precocious 2 year old sneaking males (known as jacks) (Berejikian *et al.*,

2010; Butts *et al.*, 2012; Flannery *et al.*, 2013). Jacks have a smaller body size, which allows them to hide and elude aggressive hooknose males and employ a sneaking tactic to steal fertilizations from hooknoses. Although, like in most external fertilizers, females may not have much mate choice in the traditional sense (*i.e.* pre-copulatory), especially when jacks sneak into fertilizations, as it is unknown whether females are aware of their presence when they do sneak, females can still evolve selection mechanisms to bias fertilization towards certain males through mate choice at the gamete level (*i.e.* interaction between sperm–seminal plasma and egg–ovarian fluid) and induce post-copulatory selection (Rosengrave *et al.*, 2008; Yeates *et al.*, 2009). The presence of male ARTs provides an interesting system to study any possible tactic-specific effects of polyandry, which are rarely studied (Johnson & Brockmann, 2013). Also, because there is a dichotomy in the degree to which each male tactic relies upon sperm competition to gain fertilization success, study systems with male ARTs offer novel insight when examining post-copulatory mechanisms of sexual selection. Furthermore, a predictor of sperm competition success has already been established in *O. tshawytscha*; sperm speed is positively correlated with paternity success in sperm competition (S. J. Lehnert, I. A. E. Butts, E. Flannery, K. Peters, D. D. Heath & T. E. Pitcher, unpubl. data).

To test whether there are any indirect (genetic) benefits of polyandry in female *O. tshawytscha*, a split-clutch and split-ejaculate *in vitro* fertilization protocol was used producing eggs fertilized by either a single male (from each tactic) or two males (combinations of the same or different tactics). Resulting clutches were reared through to emergence and examined for offspring viability, as measured by hatching success, independent of fertilization success, in the different crosses. The relationship between male sperm competitiveness, measured as sperm speed, and offspring viability, measured as hatching success, was also examined to determine if superior sperm competitors (*i.e.* males with faster sperm) produce offspring with greater survival.

## MATERIALS AND METHODS

Spawning *O. tshawytscha* were collected between 29 September and 10 October 2014 from the Credit River, Mississauga, ON, Canada (43° 35' N; 79° 42' W), which flows into Lake Ontario. Hooknoses ( $n = 30$ , mean  $\pm$  s.e. mass,  $M = 8.3 \pm 0.3$  kg, range = 5.0–11.4 kg), jacks ( $n = 30$ , mean  $\pm$  s.e.  $M = 2.0 \pm 0.1$  kg, range = 0.4–3.2 kg) and females ( $n = 15$ , mean  $\pm$  s.e.  $M = 7.0 \pm 0.3$  kg, range = 5.0–8.9 kg) were collected by standard electrofishing techniques. Small body size and absence of secondary sexual characteristic (*e.g.* hooked snout and large teeth) were used to distinguish jacks from hooknoses. Gametes were collected by gently applying abdominal pressure on each individual, being careful there was no contamination with water, urine or faeces. Gametes were kept in a cooler at river water temperature (*c.* 11 °C) until analysis and fertilization occurred (up to 4 h later).

A total of 61 289 eggs were collected from  $n = 15$  females (mean  $\pm$  s.e. eggs per female =  $4093 \pm 300$ , range = 3532–4270) and were placed in a strainer to separate the eggs from the ovarian fluid. Eggs from each female were then divided into 20 clear-plastic (400 ml) containers (mean  $\pm$  s.e. eggs per container =  $204 \pm 4$ , range = 81–440 eggs), with each container of eggs representing a different replicated cross. For the monandrous crosses, 200  $\mu$ l of milt from two jacks ( $J_1$  and  $J_2$ ) and two hooknoses ( $H_1$  and  $H_2$ ) were used to individually fertilize subsets of eggs ( $n = 4$  crosses). For the polyandrous crosses, 100  $\mu$ l of milt from two of the same jacks and two of the same hooknoses (*i.e.* 200  $\mu$ l sperm total per polyandrous cross) were then added to the eggs simultaneously in all possible combinations, resulting in two within-tactic ( $J_1 + J_2$ ;  $n = 1$  and  $H_1 + H_2$ ;  $n = 1$ ) and four between-tactic ( $J_1 + H_1$ ,

$J_1 + H_2$ ,  $J_2 + H_1$  and  $J_2 + H_2$ ;  $n = 4$ ) crosses. Only sperm volume and not sperm number were controlled for as it has been shown that there is no difference in sperm density between jacks and hooknoses (Flannery *et al.*, 2013). All crosses were replicated twice, resulting in 20 (10 crosses  $\times$  2 replicates) crosses per female (20 crosses  $\times$  15 females = 300 crosses total). A different set of four males (two jacks and two hooknoses) was used for each of the 15 females (therefore a total of  $n = 60$  males were used), with no male being used again in crosses involving a different female. A 25% ovarian fluid solution (50 ml of ovarian fluid: 150 ml river water; hereafter referred to as activation medium) was used to activate the sperm, which is a dilution in the range of dilutions (10–50%) used for previous studies (Rosengrave *et al.*, 2008; Butts *et al.*, 2012; Evans *et al.*, 2013). It is unknown, however, what the concentration of ovarian fluid is under natural spawning conditions. Ovarian fluid was used in this study in an attempt to mimic natural spawning conditions and because of its role in cryptic female choice (Rosengrave *et al.*, 2008; Evans *et al.*, 2013). Sperm was pipetted into the stream of activation medium as it was poured onto eggs, thus initializing sperm activation and equal opportunity for multiple males (for polyandrous crosses only) to fertilize the eggs. Eggs were then placed into a recirculating incubation system held at 11°C. The incubation system contained two stacks of incubation trays, with each stack containing up to eight trays that were each divided into 16 cells per tray so crosses could remain separate. Eggs were left undisturbed for a week after they were fertilized, after which daily checks were performed and the number of non-viable eggs (white coloured) were counted, removed and frozen from each cell of the divided incubation trays. Eggs hatched after 40 days (440 degree days) and eyed eggs were counted a few days before hatch to get a proxy of hatching success. Non-viable eggs (frozen) were placed in 5% acetic acid solution (Hoysak & Liley, 2001) to determine if eggs were fertilized or not. If the eggs turned completely clear, they were deemed to be unfertilized, while fertilized eggs had a visible, small white mass inside the egg. Unfertilized eggs ( $n = 177$ ) were removed (resulting in an overall 99.7% fertilization success) from data analyses to avoid any confounding effects related to difference in fertility among males.

For each male, sperm speed was assessed using 0.1  $\mu$ l sperm sample that was pipetted into a chamber of a 2X-CEL glass slide (Hamilton Thorne; [www.hamiltonthorne.com](http://www.hamiltonthorne.com)), covered with a glass coverslip (22 mm  $\times$  22 mm) and activated with 15  $\mu$ l of the activation medium, using the ovarian fluid of the female that the male was paired with during fertilization. Sperm and activation medium were kept at 11°C during analyses using a chilling block. Activated sperm were video recorded using a CCD B/W video camera module (XC-ST50, Sony, Japan; <https://pro.sony.com/bbsc/ssr/product-XCST50/>) at 50 Hz vertical frequency, mounted on a Olympus CX41 microscope ([www.olympus-lifescience.com](http://www.olympus-lifescience.com)) that was equipped with a  $\times 10$  negative-phase objective. Video-recordings were analysed using the HTM-CEROS 12 sperm-tracking software package (Hamilton Thorne). Curvilinear sperm speed (VCL; average speed on the actual point-to-point track followed by the sperm cell; Gage *et al.*, 2004) at 5 s post-activation was the metric used to represent sperm speed and these estimates correspond to the mean of all motile cells analysed.

All data were analysed using R software 2.15.1 ([www.r-project.org](http://www.r-project.org)). To investigate whether offspring hatching success differed between monandrous and polyandrous crosses and whether there were any tactic-specific effects, two different generalized linear mixed models (GLMM) were used for binomial data (eggs that hatched were scored as 1, eggs that did not hatch were scored as 0) with a logit-linked function using the *glmer* function in the *lme4* package in R (Bates *et al.*, 2009). Data from both GLMM models showed no overdispersion ( $\phi = 1$ ). The first GLMM had cross type (two levels: monandry or polyandry) as the fixed factor in the model while female identity, cross replicate and incubation tray position were random factors. Models were compared (by likelihood-ratio tests) with and without each random factor to determine which factors significantly contributed to the variance observed for offspring hatching success and the per cent variance explained was calculated for each factor. Next, models were compared with and without the fixed factor to determine the effect of cross type on hatching success. To investigate whether male alternative reproductive tactic had an effect on hatching success (a tactic-specific effect) in both monandrous and polyandrous matings a second GLMM was used with a similar approach as above, except the model included tactic-specific crosses as the fixed factor (five levels: J, H, J + J, H + H and H + J). Models were compared as described above and Tukey *post hoc* analyses were performed to compare differences between cross types. In both of the above GLMMs, male identity was omitted as a random factor as it is not possible to

assign a specific cross to each male owing to the nature of the design (*i.e.* polyandrous crosses have two different males). To examine whether there was a positive relationship between sperm competitive ability and viability of the offspring, a GLMM was used with hatching success of monandrous crosses as the dependent variable, sperm speed and sperm density (to control for possible differences in sperm number between males) as fixed factors and female identity as a random factor. Models were compared with and without male sperm speed to determine significance. Due to high sperm density in some video recordings that prevented accurate analyses, one jack and four hooknose males were not included in the analysis (resulting in  $n = 55$  males:  $n = 29$  jacks,  $n = 26$  hooknoses). All data are presented as means  $\pm$  S.E.

## RESULTS

Offspring from polyandrous crosses had significantly higher hatching success than offspring from monandrous crosses [ $\chi^2 = 72.42$ , d.f. = 1,  $P < 0.001$ ; Fig. 1(a)]. All three random factors (female identification, tray and replicate) in the model were significant (Table I). The significance of these random factors is not surprising given the large sample size of the present study, as well as it is common in such studies for there to be strong maternal and tray effects (Houde *et al.*, 2015). Furthermore, by including these factors in the model as random effects, they are controlled for when testing for the fixed effect, thus they do not bias the results. When examining tactic-specific differences within the polyandrous and monandrous crosses, significant tactic effect was found [ $\chi^2 = 315.53$ , d.f. = 1,  $P < 0.001$ ; Fig. 1(b)] and again the three random factors were significant (Table I). *Post hoc* analysis showed that there was a significant difference in hatching success (all  $P < 0.05$ ) between all cross types, with the exception of one comparison between the crosses involving a single jack and two jacks [J *v.* J + J;  $P > 0.5$ ; Fig. 1(b)]. To determine if the H + J cross was driving the differences in hatching success between monandry and polyandry crosses, the GLMM was rerun excluding the H + J cross, therefore only comparing four cross types [two cross types within each monandry (H and J) and polyandry treatment (H + H and J + J)] and equal number of crosses of jack and hooknoses per comparison. The results did not change as the effect of polyandry was still significant ( $\chi^2 = 25.24$ , d.f. = 1,  $P < 0.001$ ). Both jack and hooknose sperm speed (based on measurements from a mean = 94 sperm cells from each male) was significantly related to hatching success while controlling for any sperm density and female identity effects [hooknose:  $\chi^2 = 7.7$ , d.f. = 1,  $P < 0.01$ ; Fig. 2(a); jack:  $\chi^2 = 4.3$ , d.f. = 1,  $P < 0.05$ ; Fig. 2(b)].

## DISCUSSION

In the present study, the split-clutch fertilization trials show that in *O. tshawytscha*, polyandrous females may obtain small, but significant, benefits by producing offspring with a higher hatching success compared with monandrous females (74.4 and 69.5% respectively), while controlling for differences in fertilization success. These results are consistent with the meta-analysis completed by Simmons (2005), in which there was a small, but significant positive effect of polyandry on embryo viability, as measured by hatching success across insect species. Other studies on the benefits of polyandry in Salmonidae, however, produced contradictory results. Kekäläinen *et al.* (2010) found that in Arctic charr *Salvelinus alpinus* (L. 1758) polyandrous females produced offspring with lower mortality, whereas in Atlantic salmon *Salmo salar* L. 1758 it was

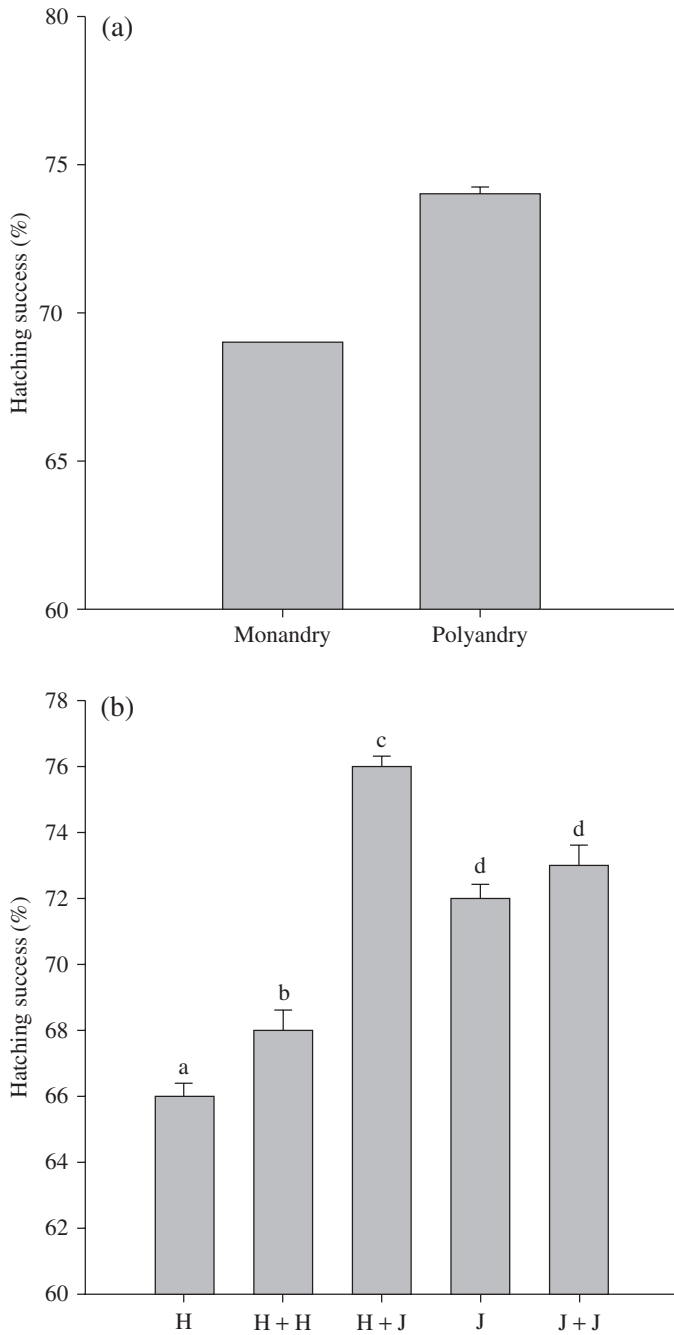


FIG. 1. Mean  $\pm$  S.E. hatching success from (a) monandrous or polyandrous crosses and (b) tactic-specific crosses of female *Oncorhynchus tshawytscha* with hooknose (H) and jack (J) males. Different lower-case letters indicate significant difference:  $P < 0.05$ .



TABLE I. Binomial models for hatching success from monandrous and polyandrous crosses and cross types involving both reproductive tactics of *Oncorhynchus tshawytscha*. Female identification (ID), tray and replicate were treated as random effects in the model. Variance components (and s.d.) were estimated using restricted maximum likelihood, and the significance of variance components were tested using likelihood-ratio tests. Note that  $\pi^2/3 \approx 3.3$  is the underlying residual variance of a binomial model with logit link function (Nakagawa & Schielzeth, 2010)

	Effect ( <i>n</i> )	Variance components		Likelihood ratio test		Phenotypic variance (%)
		Variance	s.d.	$\chi^2$	<i>P</i>	
Effect of monandry or polyandry on hatching success	Female ID (15)	0.64	0.80	2027	<0.001	15.8
	Tray (20)	0.10	0.32	230.8	<0.001	2.5
	Replicate (2)	0.001	0.036	5.094	<0.05	0.02
	Error	$\pi^2/3$				81.7
Effect of cross type on hatching success	Female ID (15)	0.64	0.80	1933	<0.001	15.9
	Tray (20)	0.084	0.29	229.1	<0.001	2.1
	Replicate (2)	0.0014	0.038	5.642	<0.05	0.03
	Error	$\pi^2/3$				82

found that polyandrous females received no offspring fitness benefits compared with monandrous females (Lumley *et al.*, 2016). A possible explanation for the increase in offspring hatching success detected in the present study could be that females take advantage of sperm competition among males in order to ensure that a male of higher quality will fertilize the majority of her eggs by being superior in sperm competition against other males (Yasui, 1997). A positive relationship was found between sperm speed, a metric correlated with sperm competition success in *O. tshawytscha* (S. J. Lehnert, I. A. E. Butts, E. Flannery, K. Peters, D. D. Heath & T. E. Pitcher, unpubl. data) and *S. salar* (Gage *et al.*, 2004) and hatching success for both hooknoses and jacks, suggesting that females could potentially use post-spawning processes to bias fertilization towards males with better sperm quality, which may indicate higher genetic quality (Rudolfson *et al.*, 2006). Although this finding does provide some evidence supporting the good-sperm hypothesis, it cannot be ruled out that the compatible-genes hypothesis is the mechanism under operation in addition to or instead of the good-sperm hypothesis. In order to tease apart the two potential mechanisms, which may not be mutually-exclusive, a design that incorporates full factorial crosses into the current maternal half-sib design would be needed (Pitcher & Neff, 2007; Houde & Pitcher, 2016). Despite this, the present study does show that female *O. tshawytscha* benefit from polyandrous mating and that this benefit is most likely an indirect genetic benefit. The only possible direct benefit that the females could be obtaining is that if by mating multiply, they are overcoming a limited sperm supply from individual males and therefore benefiting from an increase in fertilization success. This was not explicitly tested in the present study, but this seems unlikely as a single male *O. tshawytscha* should provide enough sperm to fertilize all eggs from a given female (Macfarlane *et al.*, 2009). Additionally, fertilization success of the eggs was scored and any non-fertilized eggs were removed from the data, thus the hatching success data are independent of fertilization success, so it can be ruled out that females are obtaining any direct fertility benefit

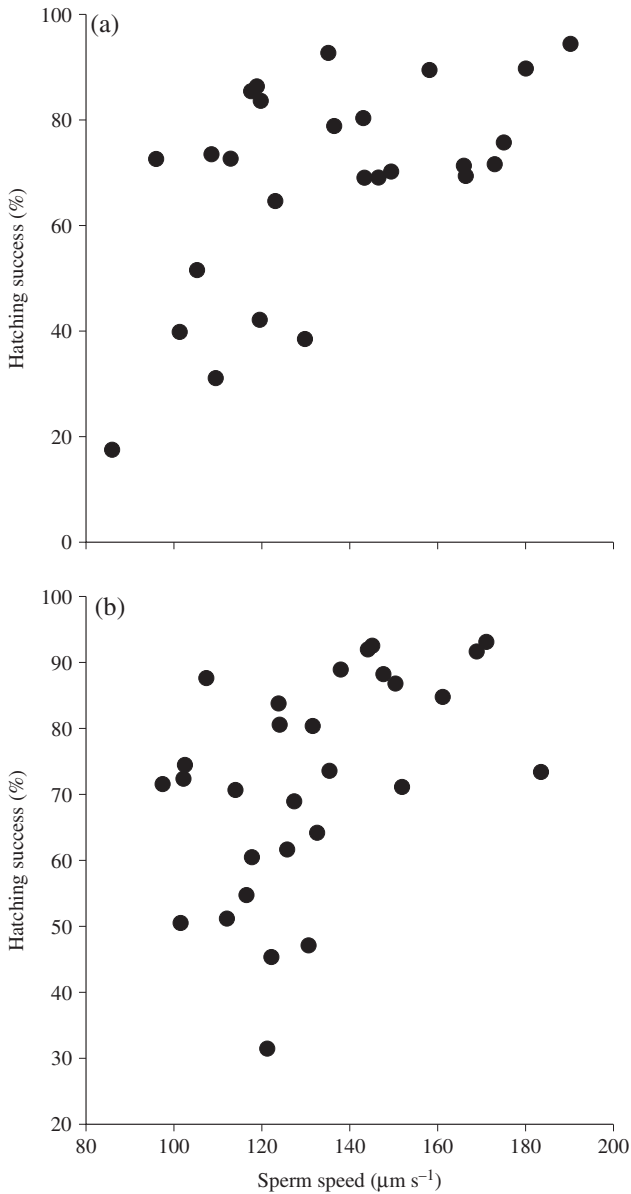


FIG. 2. Relationship between hatching success of monandrous crosses for (a) hooknose and (b) jack male with female *Oncorhynchus tshawytscha* and corresponding sperm speed.

and thus the increase in hatching success of offspring from polyandrous females is most probably a genetic benefit.

The present study also revealed tactic-specific effects of monandry and polyandry on offspring hatching success of *O. tshawytscha*. Overall, it was found that there seems to be a benefit to females when jacks are present in spawning events, as crosses involving a jack produced offspring with higher hatching success. Within the



monandrous crosses of the present study, there was approximately an 8% difference in offspring hatching success between a single jack and a single hooknose (72.3 and 66.6%, respectively). Within the polyandrous crosses, the cross involving two hooknoses (H + H) had the lowest offspring hatching success (68.3%) compared with the two other polyandrous crosses that included either two jacks (J + J; 73.2%) or a hooknose and a jack (H + J; 76.3%). This is consistent with the only other study that examined tactic-specific benefits of polyandry, where Johnson & Brockmann (2013) found that in horseshoe crabs *Limulus polyphemus*, sneaker males provide benefits through increased offspring developmental success to polyandrous females but not to monandrous females. Paternity analysis of the offspring from these polyandrous crosses would have provided useful insight into whether a particular male sired more offspring during sperm competition and thus whether there is the potential for the compatible genes hypothesis to be in operation. In addition, having the paternity data would allow further investigation into the relationship between sperm competitiveness and hatching success by relating these two metrics for the polyandrous crosses in addition to the monandrous crosses, thus providing more conclusive evidence for the good-sperm hypothesis.

It should be noted that some of the crosses used in the experiment are more ecologically relevant than the others as, in the wild, some crosses occur very frequently and some occur rarely, if at all (Berejikian *et al.*, 2010). In the wild, a female often mates with a single hooknose, multiple hooknoses, or one or more hooknoses with one or more sneaking jacks. In the present experiment, these spawning events represent H, H + H and H + J respectively, whereas spawning events with one (J) or two jacks and no hooknoses (J + J) are unlikely to occur in nature. Of these ecologically relevant crosses, the present data show there is an increase in hatching success (6–8%) when comparing only hooknose crosses (both monandrous and polyandrous; H and H + H) to the cross involving both a jack and a hooknose (H + J). It is unknown if females are aware of the presence of jacks and whether a jack will sneak during a given spawning event, however, it has been shown that when no hooknoses are present and only jacks, a female will delay spawning until a hooknose presents itself (Berejikian *et al.*, 2000). A possible explanation of this increase in hatching success is that jacks, hooknoses and females mature at different times. Jacks mature precociously at 2 years old, while hooknoses and females mature at ages 4+ years, so during a spawning season, the jacks present are from an entirely different year class than the hooknoses and females (Flannery *et al.*, 2013). Therefore, the H + J cross presents a female with the broadest genetic continuum compared with other crosses and reduces the risk of a female only mating with closely related individuals, as no jack present would be closely related to the female as they are from a completely different year class. The older hooknoses may have increased DNA damage due to increased mutations during the additional 2 years before maturation compared with jacks. By mating with multiple males, including males differing in age, it provides a female with the ability to limit the amount of eggs fertilized by older males, which could have decreased sperm quality compared with younger males (Radwan, 2003). In the present study population, however, there do not appear to be any age-related effects on sperm competitiveness, as jack and hooknose males share equal paternity in natural mimicked *in vitro* conditions (Flannery, 2011; S. J. Lehnert, I. A. E. Butts, E. Flannery, K. Peters, D. D. Heath & T. E. Pitcher, unpubl. data). Nevertheless, DNA mutation and age related effects have not been examined in fishes and whether older males have any negative effects on offspring quality is unknown.

It is important to note that in the wild, it is unknown if females have any control on which males are participating in spawning events and thus through the nature of the mating system, polyandry might have evolved in this system not through the female obtaining indirect benefits, but because of males attempting to maximize their reproductive success. Nevertheless, females could still evolve to use this type of mating system to exhibit post-copulatory selection to try and bias paternity towards certain males. For example, evidence suggests that females of many species may bias paternity in favour of males with specific genotypes (Palumbi, 1999; Yeates *et al.*, 2009; Butts *et al.*, 2012; Løvlie *et al.*, 2013). In fishes, polymorphic genes involved in the immune response are often a target for female mate choice (Yeates *et al.*, 2009) and in the present study population, there are interesting patterns between the females, jacks and hooknoses in terms of their major histocompatibility (MH) genes (S. J. Lehnert, I. A. E. Butts, E. Flannery, K. Peters, D. D. Heath & T. E. Pitcher, unpubl. data). These genes are involved in the immune response, where females may select mates with more divergent genotypes relative to their own, therefore providing offspring with more diverse MH genes, which may increase fitness through better defence against a wider range of pathogens (Aeschlimann *et al.*, 2003; Consuegra & de Leaniz, 2008; Neff *et al.*, 2008). In the same population of *O. tshawytscha*, females and hooknoses do not differ significantly at the MH genes (MH II $\beta$ ); however, females and jacks do differ significantly (S. J. Lehnert, I. A. E. Butts, E. Flannery, K. Peters, D. D. Heath & T. E. Pitcher, unpubl. data). Therefore, females could be employing post-spawning mechanisms (cryptic female choice), *via* ovarian fluid (Rosengrave *et al.*, 2008) or egg-sperm recognition (Yeates *et al.*, 2009), to bias paternity in favour of a genetically superior, or compatible male to increase embryo viability (Neff & Pitcher, 2005). For example, Pitcher & Neff (2006) found (in the same wild population of *O. tshawytscha*) that MH class IIB alleles contribute to both additive (good genes) and non-additive (compatible genes) genetic effects on viability, including hatching success and early juvenile survivorship (Wedekind *et al.*, 2004; Pitcher & Neff, 2007).

In conclusion, female *O. tshawytscha* benefit when mating with multiple males by producing more hatched offspring. There is preliminary evidence that may support a good-sperm process occurring in that males with faster sperm produced offspring with higher hatching success, but further study is needed to determine the mechanism under operation. The benefits observed in this study are also tactic-specific, with the precocious jack males providing a greater benefit generally, but the presence of both jack and hooknose males provide the female with the greatest benefit. This study not only has obvious implications to the evolution of polyandry and mating systems, but can have important implications to fish conservation breeding programmes as protocols that adopt multiple males and jacks could improve such programmes.

We thank the Ontario Ministry for Natural Resources and Forestry (OMNRF) for assistance in the field with the collection of fish. We thank the University of Toronto Koffler Scientific Reserve (KSR) for providing lodging and laboratory space where our work was carried out. We thank J. Smith, A. Forest and C. Audet from the University of Windsor for help in the field and laboratory. We thank K. Wellband for help with statistical analyses and S. Lehnert and for help with statistical analyses and insightful comments on the manuscript. This study was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), Canada Foundation for Innovation, Ontario Research Fund and an Early Research Award to T.E.P.

## References

- Aeschlimann, P. B., Häberli, M. H., Reusch, T. B. H., Boehm, T. & Milinski, M. (2003). Female sticklebacks *Gasterosteus aculeatus* use self-reference to optimize MHC allele number during mate selection. *Behavioral Ecology and Sociobiology* **54**, 119–126.
- Berejikian, B. A., Tezak, E. P. & LaRae, A. L. (2000). Female mate choice and spawning behaviour of Chinook salmon under experimental conditions. *Journal of Fish Biology* **57**, 647–661.
- Berejikian, B. A., Van Doornik, D. M., Endicott, R. C., Hoffnagle, T. L., Tezak, E. P., Moore, M. E. & Atkins, J. (2010). Mating success of alternative male phenotypes and evidence for frequency-dependent selection in Chinook salmon *Oncorhynchus tshawytscha*. *Canadian Journal of Fisheries and Aquatic Sciences* **67**, 1933–1941.
- Butts, I. A. E., Love, O. P., Farwell, M. & Pitcher, T. E. (2012). Primary and secondary sexual characters in alternative reproductive tactics of Chinook salmon: associations with androgens and the maturation-inducing steroid. *General and Comparative Endocrinology* **175**, 449–456.
- Consuegra, S. & de Leaniz, C. G. (2008). MHC-mediated mate choice increases parasite resistance in salmon. *Proceedings of the Royal Society B* **275**, 1397–1403.
- Evans, J. P., Rosengrave, P., Gasparini, C. & Gemmel, N. J. (2013). Delineating the roles of males and females in sperm competition. *Proceedings of the Royal Society B* **280**, 20132047.
- Fisher, D. O., Double, M. C., Blomberg, S. P., Jennions, M. D. & Cockburn, A. (2006). Post-mating sexual selection increases lifetime fitness of polyandrous females in the wild. *Nature* **444**, 89–92.
- Flannery, E. (2011). Sperm competition and the alternative reproductive tactics of Chinook salmon (*Oncorhynchus tshawytscha*). Master's Thesis, Department of Biological Sciences, University of Windsor, Windsor, ON, Canada. Available at <http://scholar.uwindsor.ca/cgi/viewcontent.cgi?article=1065&context=etd/>
- Flannery, E., Butts, I. A. E., Słowińska, M., Ciereszko, A. & Pitcher, T. E. (2013). Reproductive investment patterns, sperm characteristics and seminal plasma physiology in alternative reproductive tactics of Chinook salmon (*Oncorhynchus tshawytscha*). *Biological Journal of the Linnean Society* **108**, 99–108.
- Gage, M. J. G., Macfarlane, C. P., Yeates, S., Ward, R. G., Searle, J. B. & Parker, G. A. (2004). Spermatozoal traits and sperm competition in Atlantic salmon: relative sperm velocity is the primary determinant of fertilization success. *Current Biology* **14**, 44–47.
- García-González, F. & Simmons, L. W. (2005). The evolution of polyandry: intrinsic sire effects contribute to embryo viability. *Journal of Evolutionary Biology* **18**, 1097–1103.
- Hosken, D., Garner, T., Tregenza, T., Wedell, N. & Ward, P. (2003). Superior sperm competitors sire higher-quality young. *Proceedings of the Royal Society B* **270**, 1933–1938.
- Houde, A. L. S. & Pitcher, T. E. (2016). fullfact: an R package for the analysis of genetic and maternal variance components from full factorial mating designs. *Ecology and Evolution* **6**, 1656–1665.
- Houde, A. L. S., Black, C. A., Wilson, C. C., Pitcher, T. E. & Neff, B. D. (2015). Genetic and maternal effects on juvenile survival and fitness-related traits in three populations of Atlantic salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **72**, 751–758.
- Hoysak, D. J. & Liley, N. R. (2001). Fertilization dynamics in sockeye salmon and a comparison of sperm from alternative male phenotypes. *Journal of Fish Biology* **58**, 1286–1300.
- Jennions, M. D. & Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. *Biological Reviews* **75**, 21–64.
- Johnson, S. L. & Brockmann, H. J. (2013). Parental effects on early development: testing for indirect benefits of polyandry. *Behavioral Ecology* **24**, 1218–1228.
- Kekäläinen, J., Rudolfson, G., Janhunen, M., Figenschou, M., Peuhkuri, N., Tamper, N. & Kortet, R. (2010). Genetic and potential non-genetic benefits increase offspring fitness of polyandrous females in non-resource based mating system. *BMC Evolutionary Biology* **10**, 20.
- Løvlie, H., Gillingham, M. A. F., Worley, K., Pizzari, T. & Richardson, D. S. (2013). Cryptic female choice favours sperm from major histocompatibility complex- dissimilar males. *Proceedings of the Royal Society B* **280**, 20131296.

- Lumley, A. J., Diamon, S. E., Einum, S., Yeates, S. E., Peruffo, D., Emerson, B. C. & Gage, M. J. G. (2016). Post-copulatory opportunities for sperm competition and cryptic female choice provide no offspring fitness benefits in externally fertilizing salmon. *Royal Society Open Science* **3**, 150709.
- Macfarlane, C. P., Hoysak, D. J., Liley, N. R. & Gage, M. J. G. (2009). *In vitro* fertilization experiments using sockeye salmon reveal that bigger eggs are more fertilizable under sperm limitation. *Proceedings of the Royal Society B* **276**, 2503–2507.
- Nakagawa, S. & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews* **85**, 935–956.
- Neff, B. D. & Pitcher, T. E. (2005). Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology* **14**, 19–38.
- Neff, B. D., Garner, S. R., Heath, J. W. & Heath, D. D. (2008). The MHC and non-random mating in a captive population of Chinook salmon. *Heredity* **101**, 175–185.
- Palumbi, S. R. (1999). All males are not created equal: fertility differences depend on gamete recognition polymorphisms in sea urchins. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 12632–12637.
- Pitcher, T. E. & Neff, B. D. (2006). MHC class IIB alleles contribute to both additive and non-additive genetic effects on survival in Chinook salmon. *Molecular Ecology* **15**, 2357–2365.
- Pitcher, T. E. & Neff, B. D. (2007). Genetic quality and offspring performance in Chinook salmon: implications for supportive breeding. *Conservation Genetics* **8**, 607–616.
- Radwan, J. (2003). Male age, germline mutations and the benefits of polyandry. *Ecology Letters* **6**, 581–586.
- Ridley, M. (1988). Mating frequency and fecundity in insects. *Biological Reviews* **63**, 509–549.
- Rosengrave, P., Gemmell, N. J., Metcalf, V. J., McBride, K. & Montgomerie, R. (2008). A mechanism for cryptic female choice in Chinook salmon. *Behavioral Ecology* **19**, 1179–1185.
- Rudolfson, G., Figenschou, L., Folstad, I., Tveiten, H. & Figenschou, M. (2006). Rapid adjustments of sperm characteristics in relation to social status. *Proceedings of the Royal Society B* **273**, 325–332.
- Seppälä, O., Karvonen, A., Rellstab, C., Louhi, K.-R. & Jokela, J. (2012). Reciprocal interaction matrix reveals complex genetic and dose-dependent specificity among coinfecting parasites. *The American Naturalist* **180**, 306–315.
- Simmons, L. W. (2001). The evolution of polyandry: an examination of the genetic incompatibility and good-sperm hypotheses. *Journal of Evolutionary Biology* **14**, 585–594.
- Simmons, L. W. (2005). The evolution of sperm competition: sperm competition, sperm selection and offspring viability. *Annual Review of Ecology, Evolution, and Systematics* **36**, 125–146.
- Simons, A. M. (2011). Modes of response to environmental change and the elusive empirical evidence for bet hedging. *Proceedings of the Royal Society B* **278**, 1601–1609.
- Taylor, M. L., Price, T. A. R. & Wedell, N. (2014). Polyandry in nature: a global analysis. *Trends in Ecology & Evolution* **29**, 376–383.
- Tregenza, T. & Wedell, N. (2000). Genetic compatibility, mate choice and patterns of parentage. *Molecular Ecology* **9**, 1013–1027.
- Watson, P. J. (1991). Multiple paternity as genetic bet-hedging in female sierra dome spiders, *Linyphia litigiosa* (Linyphiidae). *Animal Behavior* **41**, 343–360.
- Wedekind, C. & Rüetschi, A. (2000). Parasite heterogeneity affects infection success and the occurrence of within-host competition: an experimental study with a cestode. *Evolutionary Ecology Research* **2**, 1031–1043.
- Wedekind, C., Walker, M., Portmann, J., Cenni, B., Müller, R. & Binz, T. (2004). MHC-linked susceptibility to a bacterial infection, but no MHC-linked cryptic female choice in whitefish. *Journal of Evolutionary Biology* **17**, 11–18.
- Yasui, Y. (1997). A “good-sperm” model can explain the evolution of costly multiple mating by females. *American Naturalist* **149**, 573–584.
- Yasui, Y. (2001). Female multiple mating as a genetic bet-hedging strategy when mate choice criteria are unreliable. *Ecological Research* **16**, 605–616.
- Yasui, Y. & Garcia-Gonzalez, G. (2016). Bet-hedging as a mechanism for the evolution of polyandry, revisited. *Evolution* **70**, 385–397.

- Yeates, S. E., Einum, S., Fleming, I. A., Megens, H., Stet, R. J. M., Hindar, K., Holt, W. V., Van Look, K. J. W. & Gage, M. J. G. (2009). Atlantic salmon eggs favour sperm in competition that have similar major histocompatibility alleles. *Proceedings of the Royal Society B* **276**, 559–566.
- Zeh, J. A. & Zeh, D. W. (1996). The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proceedings of the Royal Society B* **263**, 1711–1717.
- Zeh, J. A. & Zeh, D. W. (1997). The evolution of polyandry II: post-copulatory defences against genetic incompatibility. *Proceedings of the Royal Society B* **264**, 69–75.

### Electronic Reference

- Bates, D., Maechler, M. & Bolker, B. (2009). lme4: linear mixed-effects models using S4 classes R package version 0999999-2. Available at <http://cran.r-project.org/web/package/lme4/>