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Assessing the potential for post-ejaculatory female choice in a polyandrous beach-spawning fish

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Abstract

In species with limited opportunities for pre-ejaculatory sexual selection (behavioural components), post-ejaculatory mechanisms may provide opportunities for mate choice after gametes have been released. Recent evidence from a range of taxa has revealed that cryptic female choice (i.e., female-mediated differential fertilization bias), through chemical cues released with or from eggs, can differentially regulate the swimming characteristics of sperm from various males and ultimately determine male fertilization success under sperm competition. We assessed the potential role that such female-modulated chemical cues play in influencing sperm swimming characteristics in beach-spawning capelin (*Mallotus villosus*), an externally fertilizing fish that mates as couples (one male and one female) or threesomes (two males and one female) with presumably limited opportunities for pre-ejaculatory sexual selection. We assayed sperm swimming characteristics under varying doses and donor origins of egg cues and also examined the possibility of assortative mating based on body size. We found mating groups were not associated by size, larger males did not produce better quality ejaculates, and egg cues (regardless of dosage or donor identity) did not influence sperm swimming characteristics. Our findings suggest that intersexual pre-ejaculatory sexual selection and cryptic female choice mediated by female chemical cues are poorly developed in capelin, possibly due to unique natural selection constraints on reproduction.

KEYWORDS
cryptic female choice, fertilization, ovarian fluid, polyandry, sexual selection, sperm competition

1 | INTRODUCTION

Sexual selection occurs when certain individuals have advantages over others with respect to reproduction (Darwin, 1871). It is most obvious in sexually dimorphic species, where males either compete for access to mates (intrasexual competition) or females exert pre-ejaculatory mate choice (intersexual competition). In many species, females mate with multiple males within a single breeding episode (polyandry), and sexual selection can ensue after mating; sperm competition arises when ejaculates from different males compete to fertilize a female's eggs (Parker, 1970), and cryptic female choice occurs when females exert fertilization biases towards the

ejaculates of particular males (Eberhard, 1996; Firman, Gasparini, Manier, & Pizzari, 2017). Although both pre- and post-ejaculatory episodes of sexual selection are common in many taxa (Andersson & Simmons, 2006; Birkhead & Møller, 1998), the extent to which either dominates the mating system varies substantially among species (reviewed in Evans & Garcia-Gonzalez, 2016).

Across species, when pre-ejaculatory sexual selection is limited, post-ejaculatory sexual selection is generally pronounced (Parker, Ramm, Lehtonen, & Henshaw, 2018). In some cases, the opportunities for pre-ejaculatory interactions can be constrained, such as when female choice is undermined or because there are limited opportunities for mate assessment or intrasexual competition prior to mating. For example, high levels of sexual coercion experienced by female feral fowl (*Gallus gallus domesticus*) may limit their ability to exercise mate choice prior to ejaculation, but a female's ability to subsequently eject sperm from nonpreferred males potentially offers a mechanism to regain control over fertilization (Pizzari & Birkhead, 2000).

Unlike in most internally fertilizing species, females of many external fertilizers have limited control over which males release sperm in close proximity to their eggs. However, emerging evidence from several taxa confirms that even with limited or no control over mating partners, females are able to exert considerable influence over fertilization using post-ejaculatory mechanisms of gamete selection (reviewed in Kekäläinen & Evans, 2018). For example, mytilid mussels have egg-derived chemical signals that can differentially bias competitive fertilization success towards genetically compatible male gametes (Lymbery, Kennington, & Evans, 2017). Similarly, the ovarian fluid of female ocellated wrasse (*Symphodus ocellatus*) changes the outcome of sperm competition by favouring males with preferred phenotypes who produce fewer but higher-quality sperm in sperm competition (Alonzo, Stiver, & Marsh-Rollo, 2016). Thus, post-ejaculatory mechanisms of sexual selection afford females the opportunity of exerting cryptic female preferences for either intrinsically high-quality mates or those with whom they are genetically compatible (reviewed by Neff & Pitcher, 2005).

The capelin (*Mallotus villosus*) is an externally fertilizing marine fish that provides a highly tractable and experimentally versatile system for investigating potential gamete-level mechanisms of mate choice. The opportunities for pre-ejaculatory interactions between the sexes appear to be extremely limited. Although some capelin reproduce offshore, most populations spawn on beaches; they form 'couples' (a single female with a male) or 'threesomes' (a single female with one male on her left side and one male on her right side) and swim towards the beach in between breaking waves (Templeman, 1948), in a functionally two-dimensional aquatic environment. Male capelin do not appear to engage in physical combat and do not pursue females unless riding a wave onto the beach (Sleggs, 1933). Whereas females potentially assess thousands of males prior to spawning, the opportunity to evaluate individual mates seems to be limited to 2–3 s in between breaking waves. Nevertheless, it is possible that females assess certain male traits, such as body and fin size which are sexually dimorphic in capelin (Orbach, Donovan, & Purchase, 2019). These traits may be linked to male condition or genetic quality or confer advantages to females in

Impact Statement

This study provides novel insights into modes of sexual selection in capelin—a marine fish that exhibits the rare reproductive strategy of beach-spawning. We hypothesized that opportunities for pre-ejaculatory sexual selection are limited and based on body size, whereas post-ejaculatory section is present in the form of cryptic female choice. We found mating groups were not associated by size, larger males did not produce better quality ejaculates, and egg cues (regardless of dosage or donor identity) did not appear to influence sperm swimming characteristics. Whereas sperm competition is probable, unique natural selection constraints on gametes of this species may constrain sexual selection.

terms of his ability to help bury eggs deeply in the substrate (Orbach et al., 2019). It is also possible that post-ejaculatory sexual selection occurs. Unlike any other externally fertilizing vertebrate described to date, capelin sperm are pre-activated inside the male prior to ejaculation (Beirão, Lewis, Wringe, & Purchase, 2018). Capelin eggs are also unusual because of their adhesiveness (Templeman, 1948). These distinctive adaptations suggest strong selection pressures act on gametes. We anticipate the potential for some pre-ejaculatory sexual selection to occur in capelin, but that due to the described constraints, post-ejaculatory mechanisms to be more prevalent.

Using a natural population of beach-spawning capelin, we tested the hypothesis that pre-ejaculatory sexual selection is based on body size, as there may be advantages conferred to females by mating with relatively large or similar sized males. To determine whether larger males have superior sperm, we assessed the relationship between male body size and sperm swimming characteristics. We then tested whether chemicals released with the female's externally spawned eggs provide opportunities for post-ejaculatory mate choice. Specifically, we assessed whether potential female cues cause any dose-dependent changes in sperm swimming characteristics (Zadmajid, Myers, Sørensen, & Butts, 2019). Finally, we conducted a cross-classified (factorial) experiment to determine whether egg-derived chemicals provide the opportunity for females to differentially favour certain males' sperm over others. In this way, we asked whether female cues provide a putative mechanism of compatibility selection in capelin, as increasingly reported for a number of externally and internally fertilizing vertebrate and invertebrate species (Kekäläinen & Evans, 2018).

2 | MATERIALS AND METHODS

2.1 | Sampling of spawning capelin

Capelin were collected from Bellevue Beach, Newfoundland, Canada (47.6296°N, 53.7334°W). Fish used to determine whether females select

mates based on male size were sampled using dip nets as spawning couples or threesomes during July 2008, 2010, 2015 and 2018. Spawning fish used to assess female cues and sperm swimming characteristics were collected as groups by cast net between 19 July 2018 and 23 July 2018. These latter samples were separated by sex and kept in aerated coolers containing water from the spawning location. Gametes were stripped within 24 hr of capture. The genital pore was dried, and the ejaculate was collected in a micropipette by applying pressure to the abdomen. The ejaculate was transferred to an Eppendorf tube and maintained at 5°C. All ejaculate samples were analysed within 1 hr of stripping. Eggs were deposited directly into plastic dishes. All fish were anesthetized in diluted clove oil and euthanized with a knife through the brain prior to sampling. Lengths were measured from the tip of the rostrum to the fork in the caudal fin. All procedures followed the Canadian Council on Animal Care guidelines for the use of research animals (Memorial University protocols 08-01-CP, 11-05-CP, 14-07-CP, 15-04-CP).

2.2 | Sperm swimming assessment

2.2.1 | Preparation of egg water

In many externally fertilizing fishes, ovarian fluid (i.e., coelomic or peritoneal fluid) is released with eggs (Zadmajid et al., 2019) and can mediate sperm swimming characteristics and potentially facilitate cryptic female choice (e.g., Alonzo et al., 2016; Elofsson, Van Look, Sundell, Sundh, & Borg, 2006; Lehnert, Butts, et al., 2017; Lehnert, Devlin, Heath, & Pitcher, 2017; Poli, Immler, & Gasparini, 2019; Rosengrave, Gemmell, Metcalf, McBride, & Montgomerie, 2008; Urbach, Folstad, & Rudolfsen, 2005). Capelin have low quantities of ovarian fluid (~1% of total egg volume; Beirão, Lewis, et al., 2018) compared to closely related salmonids (10%–30% total egg volume; Lahnsteiner, Weismann, & Patzner, 1999), which might limit opportunities for post-ejaculatory sexual selection. However, capelin eggs instantly stick to substrate (Templeman, 1948) and it is possible the adhesive mechanism affects sperm, as it does in anurans (Burnett, Sugiyama, Bieber, & Chandler, 2011; Tholl et al., 2011). To create a solution with female cues, equal volumes of water (0.5 ml) and eggs (0.5 ml) were added to an Eppendorf tube, shaken thoroughly for 10 s to mix, and the resulting solution was extracted. Thus, our 'egg water' (EW) solution contained the small proportion of ovarian fluid present, plus any cues released by the eggs. Shaking of eggs does not break them up as they are adapted to incubate on the beach with high surf. We observed sperm swimming in different test solutions made using 15 psu degassed tap water and 0.1% BSA (Beirão, Lewis, et al., 2018; Beirão, Litt, & Purchase, 2018). We chose to set treatment water at 15 psu because sperm swimming ability is greatly reduced at higher salinities in this species (Beirão, Lewis, et al., 2018).

2.2.2 | EW dose–response experimental design

We tested whether egg water (EW) affects sperm swimming characteristics (and thus potentially functions as a female 'cue') and whether there is a dose-dependent response. Nineteen replicates (groups) of males and females were used (Table S1). The ejaculate from each male was tested in the EW of a randomly selected unique female. Each male was tested with a 'high-dose' (50% EW as described above), a 'low-dose' (12.5% EW) and a 'control' (water only).

2.2.3 | Gamete donor identity experimental design

We determined whether sperm swimming characteristics depend on the identities of specific combinations of EW-donor ID and sperm-donor ID. We set up a series of 2 × 2 factorial crosses (n = 11), each involving two randomly selected females and males that were crossed in all four combinations (Table S2). This enabled us to evaluate the importance of interacting male-by-female effects, which would be consistent with cryptic female choice favouring specific males, mediated by substances in the female's cues (Evans, Garcia-Gonzalez, Almbro, Robinson, & Fitzpatrick, 2012; Lymbery et al., 2017; Poli et al., 2019; Rosengrave et al., 2008). Males and females were housed in separate holding tanks, and no attention was paid to body size during retrieval from the holding tanks. The individuals used in this experiment were different than those used to explore the EW dose–response. We used the 50% EW as described above.

2.2.4 | Sperm swimming test

Sperm swimming characteristics were recorded at 100 frames per second using a Prosilica GE680 monochrome camera (Allied Vision Technologies), connected to an inverted Leica DM IL LED microscope, with a 20× phase contrast objective. The microscope plate and glass slides (MicroTool 2 chambers, Cytonix) were prechilled to ~8°C (temperature at spawning beach) with a customized Physitemp TS-4 system. Ejaculate (0.1 µl) was added to the slide chamber followed by 4 µl of the appropriate treatment solution.

Waves wash gametes away within seconds of ejaculation, and capelin sperm swimming ability decreases rapidly over time (Beirão, Lewis, et al., 2018). We therefore analysed sperm swimming characteristics between 6 s (earliest possible given experimental design) and 20 s after the ejaculate came into contact with the treatment solution. Videos were analysed with a computer-assisted sperm analysis (CASA) plugin for ImageJ (Wilson-Leedy & Ingermann, 2007, modified by Purchase & Earle, 2012) and sampled in half-second intervals. CASA plugin input parameters were modified from Beirão, Lewis, et al. (2018) so the software could differentiate motile and drifting sperm (Table S3). Measurements of sperm characteristics were based on an average of 141 + 79 SD sperm tracks per unique video. We analysed the percentage of sperm cells that were motile, and of these motile cells, we analysed the curvilinear swimming velocity (VCL) and path linearity (LIN, Figures S1 and S2). These parameters have been associated with fertilization success in other species (e.g., Boschetto, Gasparini, & Pilastro, 2011; Fitzpatrick, Simmons, & Evans, 2012; Gage et

	Year	T	df	p
Body size of males in spawning groups vs. not in spawning groups	2008	0.66	105	.51
	2010	0.94	36	.35
	2015	0.11	92	.91
	2018	0.18	69	.85
Body size of males in couples vs. in threesomes	2008	1.17	26	.25
	2010	0.96	25	.35
	2015	0.45	47	.66
	2018	–	–	–
Female body size vs. male body size	2008	0.58	16	.57
	2010	1.26	17	.22
	2015	1.13	26	.27
	2018	0.25	16	.81

Note: Independent *t* tests were used to assess differences in body size among males collected in spawning groups, and those collected randomly at the beach at the same time, as well as comparison of body size among males collected spawning as couples or as threesomes. Correlation analysis was used to compare body size of females and males within active spawning groups (see Martials and Methods for details). Each year of collection was analysed separately (only couples were collected in 2018).

al., 2004; Malo et al., 2005). Two technical replicates of mixing sperm and test solution were completed for each male/treatment in the EW dose–response experiment, and three technical replicates for each male–female pairing were completed in the gamete donor identity experiment. These technical replicates consisted of unique mixing/videos of independent aliquots of ejaculate and EW from the same individuals.

2.3 | Statistical analyses

To determine whether females select spawning partners based on body size, we assessed each of the four years of collections independently. We used independent *t* tests to determine whether there is a difference in body size between males collected as active beach-spawning groups (couples and threesomes) compared with males collected at the same time but not part of a spawning group. Independent *t* tests were also used to compare body size of males found spawning as couples or as threesomes. To determine whether larger females select larger spawning partners, we assessed the correlation between female and male body size of capelin collected in active spawning groups. Assuming that females would select for larger bodied males, the smaller male within each threesome was removed from the correlations to avoid issues of non-independence between the two males present in the same spawning group. Body size was log_e-transformed prior to analysis to improve assumptions of normality and homogeneity of variance. All raw data used for statistical analysis is provided in the Supporting Information.

The relationship between male body size (Tables S1 and S2) and sperm swimming characteristics was assessed by correlation, using
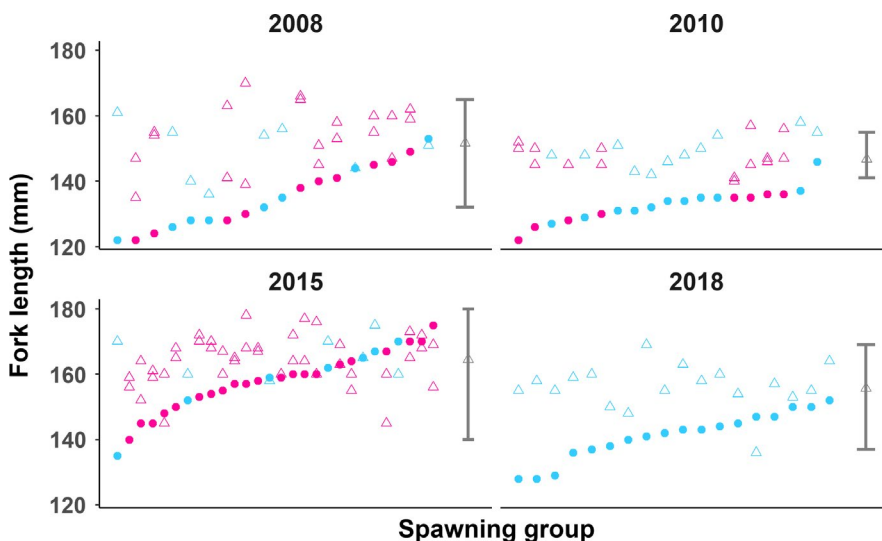

FIGURE 1 Fork length (mm) of female (filled circles) and male (open triangles) capelin in natural beach-spawning groups. Each panel shows a single collection completed in different years, with spawning groups arranged horizontally by female size. The relative abundance of couples (blue) and threesomes (pink) is representative of what was present on the beach, with the exception of 2018, when only couples were targeted for collection. Mean and total range of randomly collected males not from natural spawning groups are shown in grey

TABLE 2 Summary statistics of mixed model analyses testing the effect of treatment (e.g., 15 psu water, low-dose egg water, high-dose egg water) on capelin sperm swimming characteristics (percent motility, curvilinear velocity [VCL] and path linearity [LIN])

	Fixed effects					Random effects				
	Variable	Mean Sq.	df	F	p	Variable	Variance	LLR	df	p
% motility	log(Time)	9.16	1, 18.1	83.2	<.00001	Male–female group	3.75	104	3	<.00001
	Treatment	0.022	2, 96.9	0.195	.82	Technical replicate	2.08	5,546	3	<.00001
	log(Time) × Treatment	0.0.13	2, 96.8	0.119	.89					
VCL	log(Time)	0.9030	1, 18.0	105	.00001	Male–female group	0.32	115	3	<.00001
	Treatment	0.0010	2, 93.4	0.060	.94	Technical replicate	0.12	1,528	3	<.00001
	log(Time) × Treatment	0.0046	2, 94.8	0.266	.77					
LIN	log(Time)	8.89	1, 18.2	100	<.00001	Male–female group	0.19	79	3	<.00001
	Treatment	0.025	2, 96.6	0.282	.76	Technical replicate	0.16	1,616	3	<.00001
	log(Time) × Treatment	0.014	2, 95.4	0.158	.85					

Note: Swimming characteristics were assessed every 0.5 s between 6 and 20 s after contact with test solution. A total of 19 randomly selected male–female groupings were tested under each treatment, with two technical replicates for each treatment-group combination. The significance of fixed effect terms was assessed using the Satterthwaite approximation. The significance of random effect terms was assessed using likelihood ratio tests, where the likelihood ratio test statistic (LLR) is −2 × the difference in log-likelihoods of models with and without each random effect (random slopes and intercepts tested simultaneously—see main text). The total variance associated with each random effect is shown.
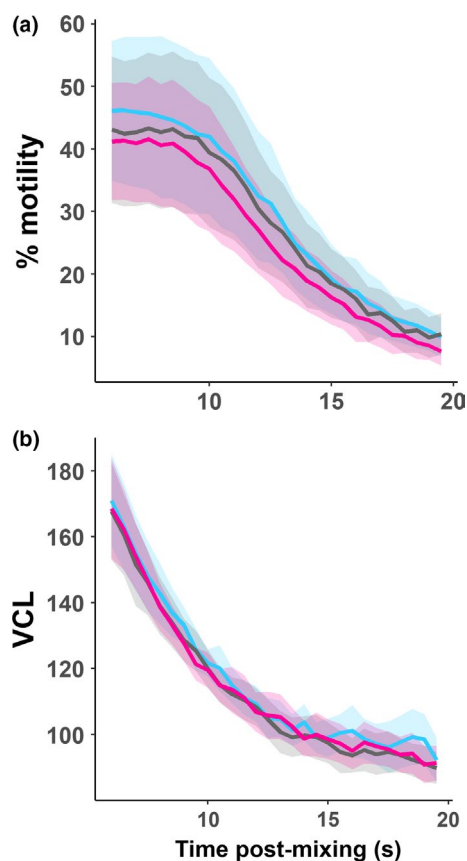
FIGURE 2 Effect of female egg water (EW) treatments on capelin sperm (a) percent motility, (b) curvilinear velocity (VCL), from 6 to 20 s post-mixing with solution. Pink = high-dose EW, grey = low-dose EW, blue = water only control. Solid lines indicate the average of 19 unique male–female combinations, and bands indicate +2 *SE*

41 unique males from the EW dose–response (50% EW used) and gamete donor identity experiments. The 50% EW was used as it was the only solution in which every male was tested and thus enabled the highest sample size for this analysis.

The statistical analyses for the EW dose–response and gamete donor identity experiments were conducted using a mixed modelling approach. Models were fit with restricted maximum likelihood (REML) using the 'lme4' package (Bates, Maechler, Bolker, & Walker, 2014) in R 3.4.3 (R Core Team, 2017). Time elapsed since the ejaculate was mixed with the test solution was included as a covariate because capelin sperm swimming declines rapidly with time (Beirão, Lewis, et al., 2018). The significance of fixed effects was assessed using the 'lmerT-est' package (Kuznetsova, Brockhoff, & Christensen, 2017) and the Satterthwaite approximation for degrees of freedom. The significance of random effects was assessed using likelihood ratio tests. We report the total variance associated with each random effect parameter and the likelihood ratio test statistic (−2x the differences in log-likelihood of the full model and reduced model). Unless otherwise specified, the comparison of full and reduced models tests both slope and intercept effects simultaneously. Time and VCL were $\log_e$-transformed, and percent motility and LIN were logit transformed prior to analysis to improve model assumptions.

2.3.1 | EW dose–response

Treatment (50% EW, 12.5% EW and control water), time elapsed after ejaculate was mixed with the test solution (6–20 s), and the interaction of treatment and time was modelled as fixed effects. To account for differences among unique male–female replicate groups of fish (19) and among technical replicate videos within a

	Variable	Variance	LLR	df	p
% motility	Male ID	1.79	30.5	2	<.00001
	Female ID	~0	~0	1	~.99
	Male ID × Female ID	0.13	1.26	1	.26
	Technical replicate	1.34	1,722	2	<.00001
VCL	Male ID	0.126	37.3	2	<.00001
	Female ID	~0	~0	1	~.99
	Male ID × Female ID	~0	~0	1	~.99
	Technical replicate	0.146	608	2	<.00001
LIN	Male ID	0.064	11.7	2	.0029
	Female ID	0.007	2.60	1	.11
	Male ID × Female ID	~0	~0	1	~.99
	Technical replicate	0.168	216	2	<.00001

TABLE 3 Summary statistics of mixed model analysis testing the effect of the identity of the female cues capelin sperm are exposed to on sperm swimming characteristics (percent motility, curvilinear velocity (VCL) and path linearity (LIN))

Note: Variation in sperm swimming characteristics is assessed using 11 unique crossings of two randomly selected males and two randomly selected females (see main text). The variance attributed to random intercept effects of male ID, female ID, the interaction between male and female ID, and technical replicates (three per combination) are reported. Random slope terms associated with male ID and technical replicates are included in the model; however, since the variance attributed with each slope term was small relative to the random intercept terms, here we only report variance associated with intercepts. The significance of random intercept terms was assessed using likelihood ratio tests, where the likelihood ratio test statistic (LLR) is −2 × the difference in log-likelihoods of models with and without the associated random intercept term.

treatment-group combination (2), group identity and technical replicates nested within group identity were included as random slopes and intercepts. The effect of treatment on each sperm swimming characteristic was plotted across time as the average of all male–female replicate groupings.

2.3.2 | Gamete donor identity

The interacting effects of EW-donor identity and male sperm-donor identity on sperm swimming characteristics were analysed using 11 unique sets (blocks) of two randomly selected males and two randomly selected females which were fully crossed. Time elapsed since ejaculates were mixed with solution was modelled as a fixed effect. To account for differences in the rate of decline in sperm swimming characteristics over time, male identity and technical video replicates (three per cross) were both included as random slope effects. Since we were interested in the relative impact of male identity and female identity on sperm swimming characteristics, male identity, female identity, the interaction between male and female identities, and technical video replicates were tested as random intercepts. We report the total variance associated with each random intercept effect.

3 | RESULTS

3.1 | Female selection for male body size

Samples taken over four years indicate that males captured in spawning groups were of similar size to males collected randomly

at the beach ($p > .05$; Table 1; Figure 1). There was no difference in a male's size whether he spawned in a couple (alone with a female) or in a threesome with a female and another male ($p > .05$; Table 1). Relatively large females were not exclusively associated with relatively large males during spawning events ($p > .05$; Table 1; Figure 1).

3.2 | Sperm swimming characteristics and male body length

Sperm swimming characteristics were not correlated with male body length using all fish from the 2018 experiments (percent motility: $r_{39} = 0.27$, $p = .08$; VCL: $r_{39} = 0.05$, $p = .75$; LIN: $r_{39} = 0.001$, $p = .99$).

3.3 | EW dose–response

Egg water had no effect on any metric of sperm swimming characteristics; sperm swam similarly in 50% EW, 12.5% EW and control water ($p > .05$; Table 2, Figure 2, Figure S1). Most of the variation in sperm swimming characteristics was driven by differences in individual male ejaculate quality (Table 2).

3.4 | Gamete donor identity

Similar to the EW dose–response results, differences in sperm swimming characteristics were primarily driven by variation
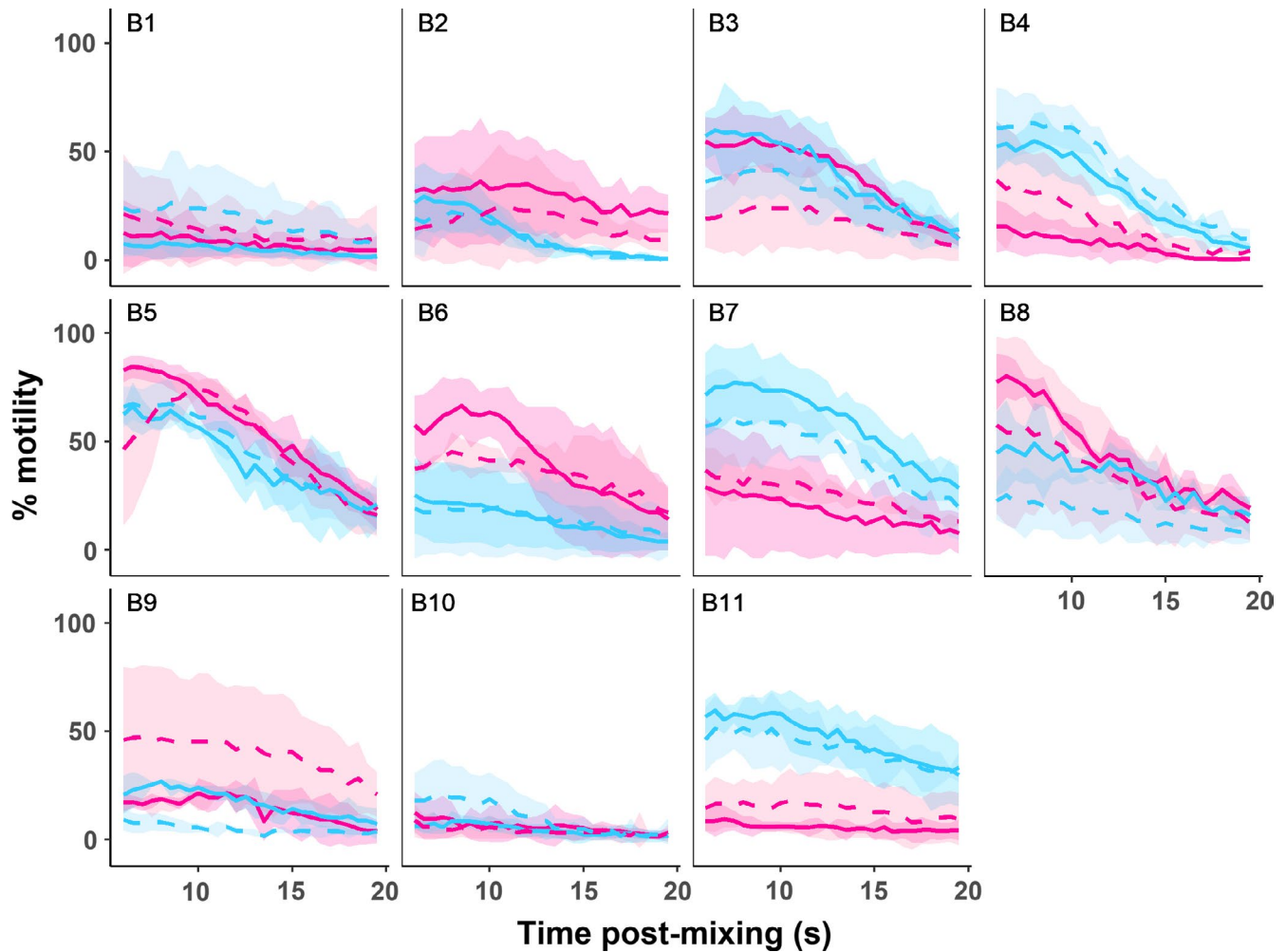
FIGURE 3 Percent motility of sperm from 6 to 20 s post-mixing with egg water (EW). Each panel shows values from two males and two females that were crossed (males and females unique to each panel). Colours (blue, pink) distinguish between males, and line types (solid, dashed) distinguish between females. Error bands are +2 *SE* based on three technical replicates. If most variation is driven by male quality, lines of similar colour should appear closer together than lines of similar type, within a panel

among males, not female EW (Table 3, Figures 3 and 4, Figure S2). The interaction between males and females was not significant (*p* > .05; Table 3).

4 | DISCUSSION

Our results indicate that beach-spawning female capelin do not selectively spawn with males based on absolute or relative body size. Combined with the limited time available between breaking waves to assess other potential mate attributes, there appear to be limited opportunities for pre-ejaculatory mate choice. We therefore hypothesized that post-ejaculatory mate choice occurs. However, our experiments examining sperm swimming characteristics and egg water interactions did not support the hypothesis that females have the ability to exert post-ejaculatory mate choice. Whereas it is probable that sperm competition plays an important role in this system, our study was not designed to evaluate this component of post-ejaculatory sexual selection, and its importance in capelin remains unknown.

Pre-ejaculatory sexual selection based on body size and other size-related morphological traits is common across taxa (Blanckenhorn, Mühlhäuser, Morf, Reusch, & Reuter, 2000; Howard, Martens, Innis, Drnevich, & Hale, 1998). We report that across 4 years of data collection, beach-spawning male capelin were on average larger than females, confirming the well-established sexual size dimorphism for this species (Orbach et al., 2019; Vandeperre & Methven, 2007). Unlike in another osmerid (*Plecoglossus altivelis*), where females mate most frequently with males of a similar body size to their own (Iguchi & Maekawa, 1993), female capelin did not selectively spawn with individual (couples) or pairs of males (threesomes) based on absolute or relatively large body size. For both of these species, high-density spawning aggregations and the absence of male territories may explain the lack of female pre-ejaculatory mate choice for relatively larger-sized males. However, females could potentially select mates based on other traits in the brief few seconds between breaking waves, such as fin size (Petersson, Järvi, Olsén, Mayer, & Hedenskog, 1999), behaviour (Chen et al., 2018), or scent (Milinski, 2003).
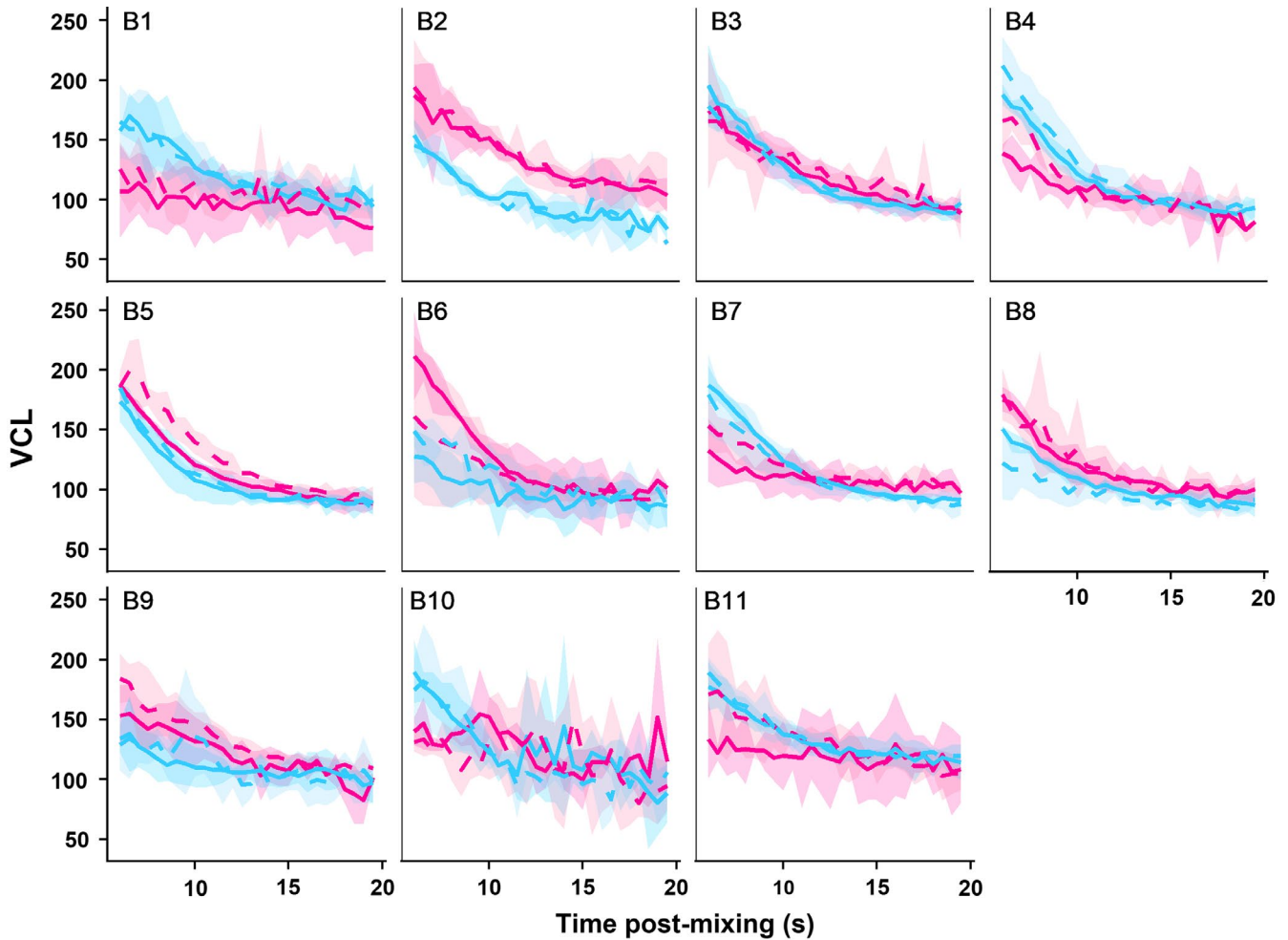
FIGURE 4 Curvilinear velocity of sperm (VCL) from 6 to 20 s post-mixing with egg water (EW). Each panel shows values from two males and two females that were crossed (males and females unique to each panel). Colours (blue, pink) distinguish between males, and line types (solid, dashed) distinguish between females. Error bands are +2 *SE* based on three technical replicates. If most variation is driven by male quality, lines of similar colour should appear closer together than lines of similar type, within a panel

Across species with limited pre-ejaculatory sexual selection, there is generally pronounced post-ejaculation selection (Parker et al., 2018). We therefore expected to find post-ejaculation selection in the form of cryptic female choice mediated by egg cues in capelin. However, we found no evidence that egg water influences sperm swimming characteristics in relation to its concentration (dose) or as a consequence of female–male identity interaction, despite contrary findings in other fishes (Butts, Prokopchuk, Kašpar, Cosson, & Pitcher, 2017; Elofsson et al., 2006; Lahnsteiner, 2002; Lehnert, Butts, et al., 2017; Lehnert, Devlin, et al., 2017; Litvak & Trippel, 1998; Poli et al., 2019; Rosengrave et al., 2008; Turner & Montgomerie, 2002; Urbach et al., 2005; Yeates et al., 2013). As capelin often spawn on the beach in threesomes, sperm competition risk (probability of competition with one other male; Parker, Ball, Stockley, & Gage, 1997) is likely to be high, but intensity (the number of competing males; Parker, Ball, Stockley, & Gage, 1996) will be low. Males produce small amounts of ejaculate, as their gonadosomatic index (weight of testes relative to body size) is comparatively low (~1%; Lewis, 2013) for fishes (Stockley, Gage, Parker, & Møller, 1997).

In many species, larger individuals produce more sperm, and if sperm are released proportionally, larger males often garner a higher share of paternity under a fair-raffle model of sperm competition (Parker, 1990). We found larger male capelin do not produce higher-quality ejaculates, suggesting that if a loaded-raffle model exists (Parker, 1990), it is not based on male size.

The lack of obvious egg water-mediated effect on sperm traits in this species is unexpected, although we acknowledge that cryptic female choice mediated by egg water may be revealed if fertilization is assessed or other sperm traits were measured, such as longevity. Both pre- and post-ejaculatory sexual selections are well established in the closely related Salmonidae, but there are key differences in gametes of the smelt family (Osmeridae) which might limit the possibility of cryptic female choice. For example, capelin have structurally different sperm than salmonids (Beirão, Lewis, & Purchase, 2015), and unlike salmonids (10%–30% total egg volume; Lahnsteiner et al., 1999), there is very little ovarian fluid (~1% of total egg volume) released with capelin eggs (Beirão, Lewis, et al., 2018). To be thorough, our experiment was designed to evaluate the potential influences of

both ovarian fluid and any chemicals released from eggs (Morisawa, Tanimoto, & Ohtake, 1992), including the adhesive egg coat (Burnett et al., 2011; Tholl et al., 2011). The ovarian fluid concentration that capelin sperm experienced in our work was lower than other studies that used high and likely unnatural concentrations, even for a salmonid (e.g., 25%–50% ovarian fluid, Turner & Montgomerie, 2002; Lehnert, Butts, et al., 2017). However, the amount we used was likely unnaturally high, not low for this species. The ovarian fluid was diluted and is higher than probably exists at the site of sperm contact in nature, as the fish would substantially disperse gametes as they vigorously move gravel while spawning in 2–3 cm of water. Although waves wash sperm away from eggs quickly, our analyses tracked potential affects for 20 s. This time constraint does not seem to be a problem in salmonids that spawn in fast flowing rivers. Capelin sperm are unique in that they are the only known externally fertilizing vertebrate to release sperm that are pre-activated in the male and are subsequently deactivated on contact with sea water (Beirão, Lewis, et al., 2018). This may limit the ability for sperm to respond to chemical cues released by the female. Furthermore, the salinity of sea water at spawning sites is also problematic for capelin embryo development (Purchase, 2018). It is possible that these natural selection limitations on capelin gametes are so severe that opportunities for sexual selection are constrained. Future detailed experimentation is needed to explore this possibility as we note that the relationship between sperm swimming characteristics and fertilization was not measured in our study.

In conclusion, we report measurements of pre-ejaculatory sexual selection on body size and test for a potential mechanism of female based post-ejaculatory sexual selection. Our results suggest that females do not exhibit preferences for male body size, and we found no evidence that they exploit post-ejaculatory mechanisms via egg cues to mediate sperm swimming characteristics. It remains unclear whether pre-ejaculatory sexual selection that is not size-related maintains the striking sexual dimorphism observed in capelin (Orbach et al., 2019) or whether more subtle mechanisms of post-ejaculatory selection via cryptic female choice would be detected when sperm compete for fertilizations. We hope that the present work will stimulate future studies that investigate potential male–female interactions under the more natural conditions of sperm competition (e.g., EW doses) as well as those that further test for putative mechanisms of cryptic female choice in this system (e.g., genes linked to major histocompatibility complex: MHC) which may facilitate the selection of genetically compatible mates (e.g., Milinski, 2003).

CONFLICT OF INTEREST
The authors declare they have no conflicts of interest.

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