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Sperm plasticity to seawater temperatures in Atlantic cod *Gadus morhua* is affected more by population origin than individual environmental exposure

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ABSTRACT: Atlantic cod is a key species of the North Atlantic ecosystem whose distribution will likely be affected by climate change. Although general temperature effects on reproduction are known, there is a dearth of information on population and individual level life history and reproductive plasticity responses to temperature change. We tested the hypothesis that the sperm of Atlantic cod of different genetic backgrounds (southern versus more northerly Newfoundland and Labrador) and of different environmental histories (reared in indoor tanks and fed a forage diet versus reared in sea cages and fed pellets) have different average plastic responses to temperature. Male reproductive performance was examined at 4 temperatures by measuring sperm swimming characteristics at 10 and 30 s after motility activation. Genetic origin had a larger effect on sperm swimming characteristics than past environmental history. Moreover, groups derived from the southern population exhibited a more pronounced positive mean reaction norm than the group derived from the Newfoundland and Labrador population. This represents an example of cogradient variation as genotypes accentuate the thermal phenotypic plasticity. Even though there were differences between the groups in sperm swimming characteristics, this is unlikely sufficient to affect successful reproduction at the temperatures tested unless under sperm competition. Thus, sperm performance should not be a limiting factor for reproduction under predicted increases in sea surface temperatures.

KEY WORDS: Climate change · Sperm swimming · Seawater temperature · Thermal reaction norms · Local adaption · Environmental history · Phenotypic plasticity · Cogradient variation

INTRODUCTION

As a consequence of climate change, average sea surface temperatures (SSTs) have increased approximately 0.7°C over the past 100 yr, and, according to the Intergovernmental Panel on Climate Change (IPCC 2007), they are expected to increase another 1.5 to 3°C by the end of the 21st century. Obviously, this change will have impacts on marine species. Populations of many species will likely persist under suboptimal conditions, and to avoid extinction will have to cope with higher temperatures via plastic and/or evolutionary changes (reviewed by Hoffmann & Sgro 2011). Phenotypic plasticity occurs when the same genotype produces multiple phenotypes under different environmental conditions (Pigliucci 2005), and the function that describes the variation in the phenotypic response is termed a reaction norm (West-Eberhard 2003). An example is the thermal reaction norms that have likely evolved to maximize

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fitness under past climate conditions (Baumann & Conover 2011, Munday et al. 2012).

The extent of the impact of increased SSTs will vary among fish species through several factors, including physiological tolerance or capacity for acclimation and adaptation (Pankhurst & Munday 2011). As explained by these same authors, while temperature is determinant throughout the life cycle of an individual, it is determinant in the regulation of the entire reproductive process, affecting everything from early gametogenesis to larval hatching and juvenile growth and survival (e.g. Harrald et al. 2010, Wang et al. 2010). Thus, to be able to predict the effects of climate change on fish populations, it is important to examine reproductive performance under future temperature scenarios. Nonetheless, these issues are just beginning to be understood (e.g. Donelson et al. 2010, reviewed by Pankhurst & Munday 2011). One of the aspects affecting male reproduction is sperm motility and swimming characteristics. Following ejaculation, such characteristics are important in determining fertilization success (Cosson et al. 2008) and are affected by environmental parameters such as temperature (reviewed by Alavi & Cosson 2005). Usually, an increase in the temperature of the activation solution leads to an increase in sperm velocity, which is caused by increased flagellar beating and higher ATP consumption that subsequently results in a decrease in sperm longevity (Alavi & Cosson 2005, Cosson 2010).

In the present study, we used sperm swimming characteristics to analyze male reproductive plasticity in response to expected increases in ocean temperatures in different populations of Atlantic cod *Gadus morhua*. As suggested by Purchase and colleagues (Purchase et al. 2010, Purchase & Moreau 2012), sperm swimming may be used to examine reproductive thermal plasticity in male fish with external fertilization because (1) a single ejaculate contains billions of cells with similar genetic background that can be divided and exposed to different conditions, (2) sperm swimming characteristics are correlated with fertilization success in several species (e.g. Atlantic cod; Skjæraasen et al. 2009, Butts et al. 2011) and (3) there are tools available, such as computer assisted sperm analysis (CASA) systems that allow for the precise measurement of sperm swimming characteristics, making detection of plasticity possible. Atlantic cod was chosen as our study species for several reasons. First, it is a eurythermal species, occupying a wide range of temperatures from −1.5 to 19°C, but this narrows to 1–8°C during the spawning season (Righton et al. 2010), making it a prime candidate to express thermal plasticity. Second, it is a key species of the North Atlantic ecosystem, both ecologically and economically (Mieszkowska et al. 2009), whose distribution is expected to be affected by increasing SST driven by climate change (Drinkwater 2005). Third, distinct populations show multi-gene patterns of temperature-related variation indicative of adaptation to local thermal regimes (Bradbury et al. 2010, Behrens et al. 2012). Fourth, several studies suggest potential temperature effects on cod reproductive performance (Kjesbu et al. 2010, Morgan et al. 2010, Purchase et al. 2010). Fifth, Atlantic cod sperm biology has been well researched, providing a good basis by which to design experiments to test for thermal responses (Trippel & Morgan 1994a, Skjæraasen et al. 2009, Butts et al. 2010, Purchase et al. 2010). Finally, sperm performance plasticity to environmental conditions may be highly variable among individual males (Purchase & Moreau 2012), including Atlantic cod, as Purchase et al. (2010) found great inter-individual variability in cod sperm phenotypic plasticity to temperature, both in terms of velocity and longevity. However, the fish in their study all originated from the same area (Bay of Fundy, New Brunswick, Canada) and, thus, population differences remain unknown.

We focused our attention on 2 evolutionary distinct northwest Atlantic cod designatable units (DU) as identified by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2010); the southern population and the Newfoundland and Labrador population. For these populations, Drinkwater (2005) predicted that an increase in SST above 3°C would cause the southern cod to decrease or collapse and the Newfoundland and Labrador cod to increase in abundance. A large thermal gradient exists between these 2 areas (Brander 1994) and Bradbury et al. (2010) showed that cod from the southern population have mostly 'warmer' temperature-associated SNPs (single nucleotide polymorphisms), while cod from the Newfoundland and Labrador population have mostly 'cold' alleles. These polymorphisms may be partly responsible for the differences in several phenotypic traits, such as growth (Purchase & Brown 2000, 2001, Salvanes et al. 2004, Harrald et al. 2010), juvenile food conversion efficiency (Purchase & Brown 2000), morphology and allometry (Marcil et al. 2006) and metabolic plasticity to temperature (Grabowski et al. 2009), among Atlantic cod populations. Some of these phenotypic changes are considered an example of countergradient variation (CnGV), which occurs when genotypes oppose envi-

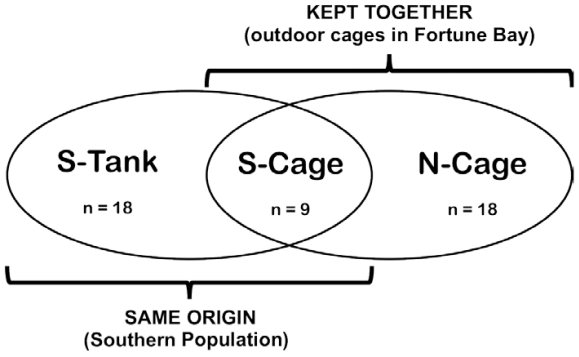

Fig. 1. Schematic representation of the Atlantic cod groups' genetic background and environmental history. Below each group name is the number of males that contributed sperm samples (n)

ronmental influences, leading to reduced phenotypic variation along a gradient (as reviewed by Conover et al. 2009). Purchase & Brown (2000), for example, showed that for the same temperature Atlantic cod larvae from a more northerly population had higher growth rates than those from a more southerly population. Less common are patterns of cogradient variation (CoGV) that occur when genotype influences accentuate environmental plasticity (Conover et al. 2009). Using captive wild fish, Harrald et al. (2010) found such a pattern in growth rates of 2 groups of cod from Scotland. Nonetheless, these studies disregard the possible effect that an individual's environmental history can have on its phenotypic traits, including those involved with reproductive performance. Instead, most studies try to control for previous environmental history by either using the F_1 generation of cultured fish (e.g. Grabowski et al. 2009) or allowing individuals to acclimate to a common garden environment (e.g. Harrald et al. 2010). In the second case, there may be lingering effects of different environmental histories prior to introduction to the common garden and the degree to which this will affect the plastic response and hide genotypic differences is unknown.

To address some of these outstanding uncertainties, we tested the hypothesis that Atlantic cod of differing genetic backgrounds (F_1 individuals bred from wild fish of different populations) and of differing environmental histories have different average plastic responses to temperature in terms of sperm swimming characteristics. As both factors were expected to have some effect, we were most interested in resolving their relative importance. Broadly, we were also interested in contributing to knowledge of the effects of increasing ocean water temperatures on the reproductive performance of high latitude fish and how this may vary among populations.

MATERIALS AND METHODS

Fish origin and stocking conditions

Three groups of male Atlantic cod differing in either environmental history (i.e. rearing conditions) or genotype were used in this experiment (Fig. 1). The genetic background of the populations is described in the COSEWIC report on the Atlantic Cod (COSEWIC 2010). The Newfoundland and Labrador group were spawned in 2008 from fish captured in Smith Sound, in Trinity Bay, Newfoundland (Fig. 2) (Northwest Atlantic Fishery Organization [NAFO] division 3L). In 2009, these were placed in sea cages

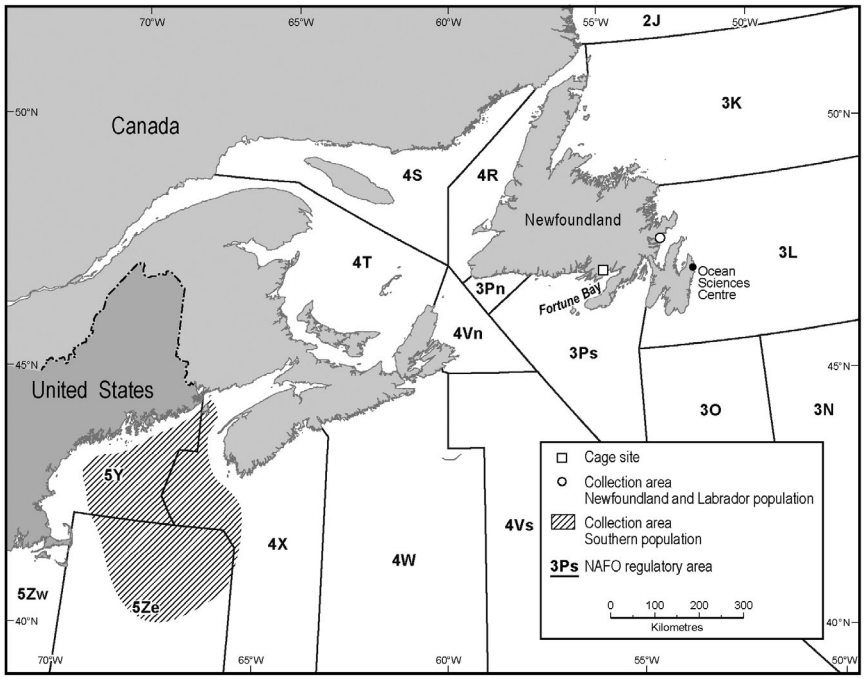

Fig. 2. Sampling location of the wild parents and grandparents of the cultured Atlantic cod used in the 3 groups. NAFO (Northwest Atlantic Fisheries Organization) divisions and location of the sea cages and the Ocean Science Center (indoor tanks)

in Fortune Bay, Newfoundland (Fig. 2), and fed commercial feed pellets (hereafter referred to as N-Cage). The second group were southern population cod, spawned in 2009 from sets of parents and grandparents captured in the Gulf of Maine (Fig. 2) (NAFO divisions 4X, 5Ze and 5Y); and in 2010 placed in cages directly adjacent to those of the N-Cage fish and fed the same food (hereafter referred to as S-Cage). The third group originated from the same southern population broodstock and spawning, but were raised in indoor aquaculture flow-through tanks at Memorial University of Newfoundland's Ocean Science Centre (OSC; 47° 63′ N, 52° 66′ W) and fed a forage diet (herring *Clupea harengus*, mackerel *Scomber scombrus* and squid *Illex* spp.) (hereafter referred to as S-Tank). For additional information on the groups see Table 1.

In order to avoid any influence of environmental conditions during the final stages of gonadal development, and only test for the separate effects of population and previous environmental histories, the following conditions were created: 4 mo before the start of the sperm experiment, the 2 groups raised in outdoor cages were brought to the OSC and placed together with the S-Tank group in a 25.5 m^3 round tank under the same conditions. In total, the tank contained 171 fish (see Table 1 for the number of fish of each group), in a sex ratio of 1.3:1 (male: female). The fish were fed a forage diet (herring, mackerel and squid). The tank was provided with constant aeration and water exchange of 27% h^{-1}, and the temperature was maintained at a constant (±SEM) 5.3 ± 0.1°C. Rearing and experimentation at the OSC were conducted in accordance to protocol 12-09-IF approved by the Memorial University Animal Care Committee following the regulations of the Canadian Council on Animal Care for the treatment and welfare of animals.

Semen sampling

Semen samples were collected from each group (n = 18 for N-Cage, n = 9 for S-Cage and n = 18 for S-Tank) during their spawning seasons (S group samples were obtained from mid-March to early April and N-Cage group samples from mid-April to early May 2012). We only collected samples during the groups' peak reproductive period to minimize effects related to seasonal variability during the spawning season (see e.g. Butts et al. 2010). Whenever possible, 2 semen samples were collected at least 1 wk apart for each male to further control for seasonal variability in sperm quality. The urogenital papilla was carefully cleaned and dried and semen collected with the help of a syringe after applying slight pressure on the abdomen. The first 1 ml of semen was discarded to help avoid seawater, urine and feces contamination. Samples were kept in 2 ml syringes at 3 to 6°C and covered with parafilm until arrival at the lab (<2 h).

Table 1. Description of the 3 groups in terms of group size (the number of individuals from the group present in the common tank at the beginning of the experiment), individuals' mean length, Fulton's condition factor and mean semen sample osmolality (only for individuals who contributed sperm samples to this study). Values are ± standard error of the mean (SEM)

	Group size	Mean length (cm)	Fulton's *K* index	Osmolality (mOsm kg^{-1})
S-Tank	25	54.56 ± 0.83	1.58 ± 0.15	388.2 ± 2.8
S-Cage	72	47.06 ± 2.41	1.46 ± 0.06	364.2 ± 6.2
N-Cage	74	61.68 ± 0.86	1.19 ± 0.03	385.2 ± 2.6

Quantification of sperm swimming characteristics

Sperm swimming characteristics were analyzed within 10 h of collection. Before each experimental trial, osmolality was verified to identify samples contaminated with urine, and samples with osmolalities outside of the 340 to 415 mOsm kg^{-1} range reported for this species (Butts et al. 2010) were discarded. Motility was also visually evaluated under the microscope and a few semen samples with less than 70% motile cells were rejected. Rejected samples were not biased by group, but rather suggest contamination during sampling. Semen samples and activation solution (UV filtered seawater, pH 7.6 to 7.7, salinity 31 to 32 psu and supplemented with 1% w/v bovine serum albumin to prevent the sperm from adhering to the slide/coverslip; see e.g. Purchase et al. 2010) were maintained at the test temperature (3, 6, 9 or 12°C) using an electronic chilling/heating dry bath (Echotherm IC20XT). The microscope plate was chilled to the desired temperature with a customized Physitemp TS-4 system. The experimental temperatures were chosen to represent those common for Atlantic cod spawning in nature (Righton et al. 2010), as well as stressful environmental conditions (12°C). Sperm were pre-diluted (1:20) in a non-activating saline solution (2/3 freshwater and 1/3 seawater) (Rouxel et al. 2008), and activated in a 10-well slide by adding 1 µl of pre-diluted sperm to 15 µl of activation solu-

tion and immediately covering the slide with a coverslip. Video was acquired with a Prosilica GE680 monochrome camera (Allied Vision Technologies) attached to an inverted Leica microscope DM IL LED (Leica microsystems) using a 20× phase-contrast lens and recorded from 10 to 31 s after motility activation using Prostream software (http://prostream.southernvisionsystems.com/) at 100 frames per second (fps).

Videos were analyzed for sperm swimming characteristics at 10 and 30 s post-activation with the open CASA software developed by Wilson-Leedy & Ingermann (2007) and modified by Purchase & Earle (2012). Input parameters (see Table A1 in the Appendix) were chosen so that the software could differentiate drifting from motile sperm. Because we were interested in the temperature effect on overall sperm motility, we were more liberal than some other studies when choosing the parameters by which to consider a sperm motile. For example, we used minimum VSL (defined below) of 1 µm s^{-1} rather than 4 µm s^{-1} as used by Tuset et al. (2008), although our approach generally follows that of Purchase et al. (2010), using different software. The entire process was repeated 3 times for every semen sample at each temperature. Based on their biological meaning and accuracy (Wilson-Leedy & Ingermann 2007), the following parameters obtained with the CASA plugin were compared among groups of fish: VCL (curvilinear velocity, velocity according to the actual path; µm s^{-1}), VSL (straight line velocity, velocity according to the straight path, i.e. displacement; µm s^{-1}), VAP (velocity according to the smoothed path; µm s^{-1}), LIN (linearity; % calculated from VSL/VAP), and WOB (wobble; % calculated from VAP/VCL). Of note, high WOB values from this software should be interpreted as having relatively low side to side motion.

Data analysis

All statistical analyses were conducted using R 2.15.1 (R Development Core Team 2012) and the R package agricolae (de Mendiburu 2012). A total of 936 video records were analyzed (45 males × 1–2 sperm samples per male × 4 temperatures × 3 replicates), giving data on a total of 95 584 motile spermatozoa. Results of the 3 replicates per semen sample were averaged and semen samples from the same male taken on different days were averaged and this value used in the statistical model. Using the individual reaction norms from each fish, we calculated the coefficient of variation (CV) of the CASA parameters for the 3 groups. In addition to the parameters obtained with CASA, we also performed a cluster analysis to group sperm cells across all treatments and males with similar swimming features, using the K-means algorithm. We decided to use 4 clusters after analyzing a plot of within groups sum of squares by the number of clusters. Of the 4 clusters, the slowest sperm represent the first cluster, with mean VCL of 67.69 µm s^{-1} and WOB 37%, and the fastest sperm with 'straighter' movement were gathered in the fourth cluster, with a mean VCL of 151.28 µm s^{-1} and WOB 80% (Table 2). VCL (Skjæraasen et al. 2009) and the percentage of progressive sperm (Rudolfsen et al. 2008), closely related to WOB, are the parameters that better correlate with fertilization in Atlantic cod, and thus we focused on this fourth cluster for analysis of the different treatments (hereafter referred to as the high quality cluster).

Table 2. Description of the 4 clusters obtained in this study. Each cluster is described by the mean values for the 5 CASA software parameters used in the analysis: VCL (curvilinear velocity), VSL (straight line velocity), VAP (velocity according to the smoothed path), WOB (wobble) and LIN (linearity). The 4th cluster (in **bold**) was considered the high quality cluster

Cluster	VCL (µm s^{-1})	VAP (µm s^{-1})	VSL (µm s^{-1})	LIN %	WOB %
1	67.69	23.85	21.36	87	37
2	137.04	89.58	81.45	91	67
3	111.07	58.15	53.70	92	54
4	**151.28**	**119.66**	**112.09**	**94**	**80**

We also performed a principal component analysis to help identify patterns of variation using all of the CASA parameters. The principal components were calculated from the correlation matrix. Based on the explained variance, we focused on the first 2 components. The first (PC1) explained 69% of the variation and described the velocity parameters (VAP, VCL and VSL), while PC2 explained 20% of the variation and was related to LIN (see Table A2 in the Appendix).

Differences between the groups for the different variables were analyzed with a mixed-model nested ANOVA considering the different temperatures as repeated measures on a given male. Both temperature and group were considered fixed factors, while male was considered random and nested within group. Except for PC2 at 30 s post-activation, all the other dependent variables had a significant interaction between group and temperature. Thus, to further examine this, the ANOVA models were simpli-

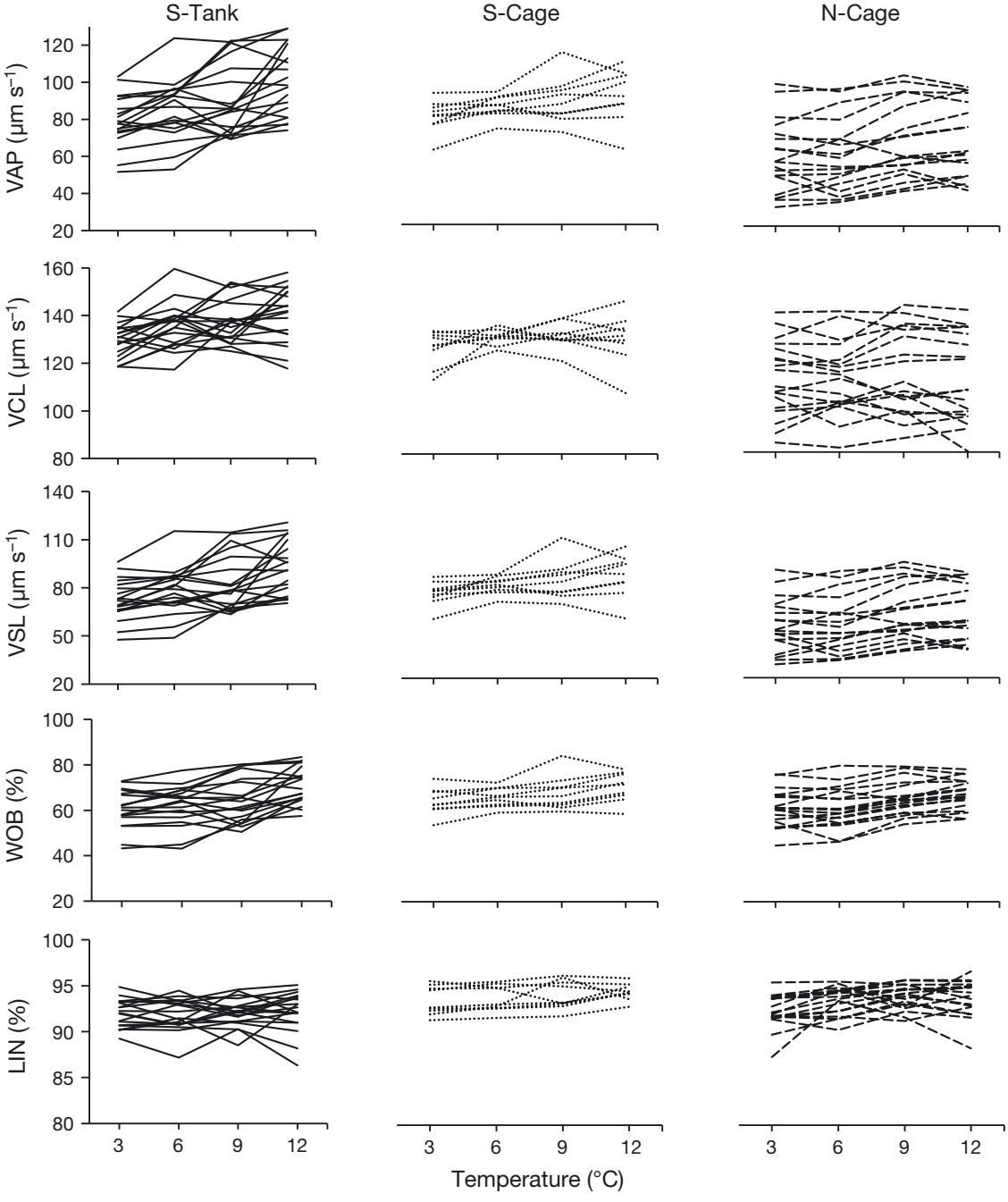

Fig. 3. Individual thermal reaction norms in terms of VAP (velocity according to the smooth path), VCL (curvilinear velocity), VSL (straight line velocity), WOB (wobble) and LIN (linearity) at 10 s after motility activation for the 3 different groups (S-Tank, S-Cage and N-Cage; see Fig. 1)

fied by creating one for each tested temperature and Tukey's Honestly Significant Difference (HSD) was applied for multiple comparisons. Differences were considered significant at $p < 0.05$. Percentile data was normalized through arcsine square root transformation. Numeric results are expressed as arithmetic means ± standard error of the mean (SEM).

RESULTS

Average phenotypic plasticity of sperm swimming characteristics to the tested temperatures differed among the 3 groups and among males within the same group. In general, the N-Cage males had the lowest reaction norms for the tested temperatures for

Table 3. Coefficient of variation for VCL, VAP, VSL, LIN and WOB of the 3 groups, 10 s after motility activation

	Temp. (°C)	S-Tank	S-Cage	N-Cage
VCL	3	6.88	6.06	15.28
	6	7.29	3.45	15.13
	9	7.82	6.10	17.53
	12	9.11	9.09	17.13
VAP	3	19.94	13.07	35.21
	6	19.31	10.58	36.28
	9	22.84	16.37	32.73
	12	19.95	17.68	31.01
VSL	3	19.83	12.62	34.27
	6	18.83	10.42	35.06
	9	22.91	16.62	31.65
	12	19.44	1760	29.82
LIN	3	1.79	1.71	2.04
	6	2.09	1.55	1.82
	9	2.24	2.05	1.78
	12	2.42	1.40	2.11
WOB	3	15.39	10.48	21.73
	6	14.71	9.29	22.95
	9	16.90	11.66	17.23
	12	12.14	10.25	14.89

sperm velocities, VAP, VCL and VSL (Fig. 3). Furthermore, this group had the highest CV among individuals (except for LIN) (Table 3).

The ANOVA procedure for the high quality cluster detected a significant interaction between temperature and group at 10 s and 30 s after sperm motility activation (Table 4). Thus, the main effects were not interpreted and separate ANOVAs at each temperature were conducted. At 10 s, significant group effects were detected at each temperature (for 3, 6, 9 and 12°C, $F_{2,44} \geq 8.019$, $p \leq 0.001$) and the same happened at 30 s (for 3, 6, 9 and 12°C, $F_{2,44} \geq 7.719$, $p \leq 0.001$). At both times, N-Cage males had a lower percentage of spermatozoa in the high quality cluster

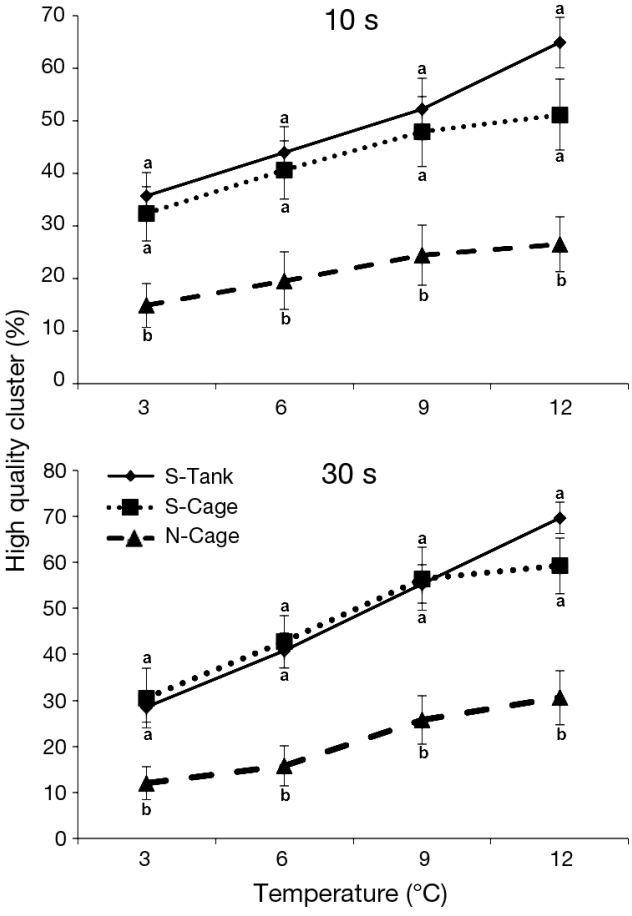

Fig. 4. Mean percentage of spermatozoa in the high quality cluster for the different groups, (top panel) 10 and (lower panel) 30 s after motility activation at different temperatures. Different letters represent significant differences between the groups for the same temperature. Significant differences detected with Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$). Symbols and whiskers represent mean values ± standard error of the mean (SEM)

Table 4. Nested repeated-measures ANOVAs for the high quality cluster analyses at 10 and 30 s after motility activation. 'Source' is a code for the different terms, 'df' is degrees of freedom, 'Error' refers to which 'Source' is used in the denominator of the *F*-test

Source	Term	df	Error	10 s		30 s	
				F	p	*F*	p
1	Group	2	2	10.770	<0.001	15.210	<0.001
2	Male (Group)	44					
3	Temperature	3	5	57.552	<0.001	86.769	<0.001
4	Group × Temperature	6	5	2.412	0.030	2.753	0.015
5	Temperature × Male (Group)	132					

than both S groups, which did not differ (Fig. 4). Phenotypic plasticity in the percentage of spermatozoa in the high quality cluster is also evident as percentages increase with increasing temperature for all groups (Fig. 4). Nonetheless, both S groups appeared to show greater plasticity to temperature than the N-Cage males.

For the principal component analysis, we also found a significant interaction between temperature and group for both PC1 and PC2 at 10 s, but only for PC1 at 30 s (Table 5). PC1 describes sperm velocity and sepa-

Table 5. Nested repeated-measures ANOVAs for both PC1 and PC2 at 10 and 30 s after motility activation. 'Source' is a code for the different terms, 'df' is degrees of freedom, 'Error' refers to which 'Source' is used in the denominator of the *F*-test

Source	Term	df	Error	10 s				30 s			
				PC1		PC2		PC1		PC2	
				F	p	*F*	p	*F*	p	*F*	p
1	Group	2	2	12.070	<0.001	7.328	0.002	10.090	<0.001	8.010	0.001
2	Male (Group)	44									
3	Temperature	3	5	29.208	<0.001	1.572	0.199	21.590	<0.001	0.879	0.454
4	Group × temperature	6	5	2.771	0.014	3.357	0.004	2.252	0.042	1.141	0.342
5	Temperature × Male (Group)	132									

rate ANOVAs conducted at each temperature showed consistent significant differences in scores between groups at both 10 s (for 3, 6, 9 and 12°C, $F_{2,44} \geq 7.612$, $p \leq 0.001$) and 30 s (for 3, 6, 9 and 12°C, $F_{2,44} \geq 5.125$, $p \leq 0.009$), with the N-Cage group differing from the 2 S groups, which did not differ from each other (Figs. 5 & 6). This indicates that this parameter was affected by group genotype but not by environmental history. On the other hand, for PC2, which mainly describes LIN and accounts for only 20% of the variability, the differences occurred between the S-Tank group and one or both of the Cage groups ($F_{2,44} \geq 7.417$, $p \leq 0.002$ for 3, 6, 9 and 12°C; Figs. 5 & 6). The exception was at 10 s and 3°C where there was no significant difference among groups ($F_{2,44} = 0.810$, $p = 0.451$). Finally, there were no overall significant differences between 10 and 30 s ($p > 0.05$, results not shown).

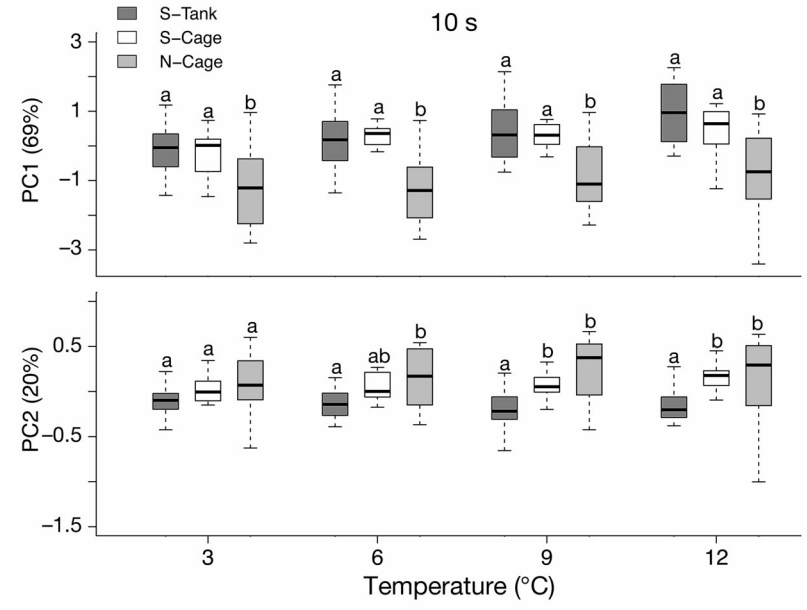

Fig. 5. Mean PC1 and PC2 values for the 3 groups at the different temperatures 10 s after motility activation. Value in parentheses on the *y*-axis is the percentage of variance explained by each component. Each box represents the 50% quartile and the dark line the median, the whiskers represent 1.5 times the inter-quartile range. Different letters represent significant differences between the groups for the same temperature. Significant differences detected with Tukey's HSD test ($p < 0.05$)

DISCUSSION

Several studies have shown that temperature plays a crucial role in fish reproductive biology (Donelson et al. 2010, Morgan et al. 2010, Pankhurst & Munday 2011), including sperm physiology (reviewed by Alavi & Cosson 2005). However, the way in which an individual's environmental history and genetic background shape the effect of temperature on reproductive performance has remained unclear. In the present work, our focus on population-level, in addition to individual-level temperature effects (cf. Pigliucci 2005) has shown that phenotypic plasticity in sperm swimming characteristics to different temperatures (i.e. mean thermal reaction norm) was driven more by genetic background than by environmental history. While the mean reaction norm for the percentage of spermatozoa in the high quality cluster was similar for the groups sharing a common genetic background (i.e. southern groups) at both 10 and 30 s post-activation, it differed for the groups sharing a common environmental history (i.e. the sea-cage groups) but different genetic background at both times. A similar pattern was observed for PC1, which accounted for 69% of the data variability. Only for PC2, which accounted for 20% of the

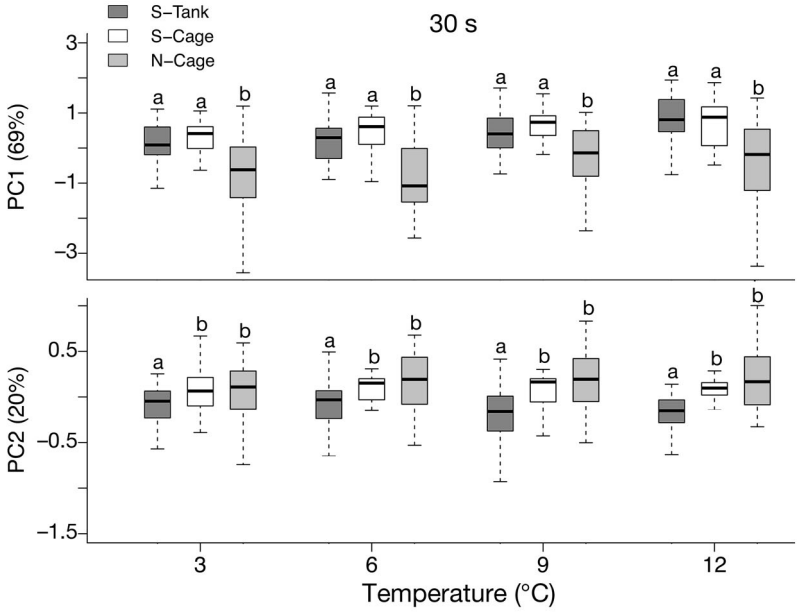

Fig. 6. Mean PC1 and PC2 values for the 3 groups at the different temperatures 30 s after motility activation. Value in parentheses on the *y*-axis is the percentage of variance explained by each component. Each box represents the 50% quartile and the dark line the median, the whiskers represent 1.5 times the inner quartile range. Different letters represent significant differences between the groups for the same temperature. Significant differences detected with Tukey's HSD test ($p < 0.05$).

variability, were the scores similar between groups sharing a common environmental history and different for groups of a common genetic background (exception being 10 s at 3°C). Thus, plasticity in sperm swimming was more affected by the origin of the sperm donor's parents — population genetic background — than by their environmental history. Furthermore, there was a significant interaction between group and temperature for the proportion of spermatozoa in the high quality cluster, meaning that differences between groups were variable according to the temperature. Even so, groups whose parents originated from historically warmer environments (S-Cage and S-Tank groups) had a higher percentage of spermatozoa in the high quality cluster than those from historically cooler parental environments (N-Cage). All groups showed a positive mean reaction norm, though the slopes appeared steeper for the S groups than the N-Cage group. In other words, unequal slopes amplified differences among groups with increasing temperature. These results are similar to the observations by Harrald et al. (2010) and Purchase & Brown (2000) that juvenile growth rates in Atlantic cod populations are higher at 12°C than at 7 to 8°C, and to Harrald et al. (2010) where differences in growth rates are more apparent at higher temperatures. Interestingly, the Newfoundland and Labrador and southern populations are known to exhibit differences in the frequency of temperature-associated SNPs (Bradbury et al. 2010).

Sperm velocity, explained by PC1, appears to be the main source of variation between the different groups in the present study. As explained in the introduction, an increase in sperm velocity with temperature was expected (see Alavi & Cosson 2005) and so too should an increase in the percentage of spermatozoa in the high quality cluster be expected. All groups exhibited positive mean thermal reaction norms, average plastic responses, with a higher percentage of spermatozoa in the high quality cluster at 12°C than at 3°C. Nonetheless, this variation with temperature was more evident in the southern groups. The positive plastic response to increasing water temperature could be attributed to both a decrease in water viscosity and an increase in sperm metabolism. Seawater viscosity decreases with increasing temperature such that for the same amount of effort the sperm cells displace farther (Larsen & Riisgard 2009). Moreover, higher temperatures also cause increased metabolic rates that are expected to affect flagellar beating and consequently the swimming velocity (e.g. Mansour et al. 2002). While a decrease of motility duration at higher temperatures caused by a higher rate of ATP consumption (Alavi & Cosson 2005) might also be expected, we did not measure this. However, there was no decrease in speed between 10 and 30 s after motility activation in any of the groups, which could be because Atlantic cod sperm can remain motile for more than 1 h (Trippel & Morgan 1994a). Nonetheless, Purchase et al. (2010), also working with Atlantic cod sperm, measured sperm swimming for 180 s at 3, 6, 11 and 21°C, and found a decrease in sperm velocity (VCL) for all temperatures between all time intervals starting at 30 to 60 s. This decrease in VCL observed by Purchase et al. (2010) was higher at 11 than at 6°C and higher at 21 than at 11°C, supporting the hypothesis of a decrease in motility duration at higher temperatures.

In contrast to the southern groups, the mean sperm swimming phenotype of the N-Cage group showed little thermal plasticity, perhaps related with the large variation in reaction norms among males for the CASA parameters. The N-Cage group showed

greater inter-individual variation than both southern groups. Nonetheless, while some N-Cage males had positive thermal plastic responses, most of them showed flat (canalized) reaction norms. If the average reaction norms are representative of what happens in the wild, according to Valen's (1965) 'niche variation hypothesis', the Newfoundland and Labrador population has a wider reproductive niche than that of the southern population. In species such as cod that reproduce under sperm competition, sperm swimming velocities should always be high, and plasticity to decreasing temperatures would be maladaptive. In addition, the present results are an example of uncommon CoGV as colder water and northern genotypes produce slower sperm. Most studies reporting gradient variation in Atlantic cod populations have found CnGV (Purchase & Brown 2000, Salvanes et al. 2004, Marcil et al. 2006), which is biologically more intuitive since it counteracts the phenotypic plasticity along an environmental gradient, and is usually considered to be adaptive (Conover et al. 2009). Nonetheless, the observation made by Harrald et al. (2010) of 2 northeast Atlantic cod populations showing that the one from the warmer environment has higher juvenile growth rates no matter the test temperature is indicative of CoGV, a pattern similar to ours. The cause of the CoGV with temperature in our study and that of Harrald et al. (2010) remains unclear. Nonetheless, unless these 2 populations come in contact in the future, and there is sperm competition between males of the 2 populations, the magnitude of sperm swimming variation is not enough to jeopardize fertilization.

Variation in reaction norm slopes among individuals (I) is referred to as an individual-by-environment interaction (I × E; Pigliucci 2005, Nussey et al. 2007, Purchase & Moreau 2012). In the present study, we tested the hypothesis that both genotype and the environmental history would affect individual reaction norms. The groups with the same environmental history but different genotypic background, N-Cage and S-Cage, presented much more significant differences than the groups with the same genotypic background but different environmental history (S-Cage, S-Tank). However, both factors were shown to affect phenotypes, and for this reason, when averaging individual reaction norms, it is important to understand the origin of variation before making conclusions. Nonetheless, any extrapolation of these results to wild populations should be done cautiously. The present study compared F_1 individuals reared in a single common environment (sea cages) that allowed us to control environmental history; however, patterns could be different in a different common environment. Moreover, the N-Cage group was one year older than the S groups, and while some studies in Atlantic cod have indicated that sperm quality is not affected by the individual's age (Trippel & Morgan 1994b, Rakitin et al. 1999), there is the possibility of some influence. In addition, southern groups had a higher condition factor than the N-Cage group, a pattern that is similar to that found by Purchase & Brown (2001) where fish from the Grand Banks (corresponding to our Newfoundland and Labrador population) always developed a lower condition factor, no matter the temperature, than individuals from the Gulf of Maine (corresponding to our southern population). In cod, condition factor seems to influence sperm quality, but patterns are inconsistent. Tuset et al. (2008) found a negative correlation between condition factor and sperm swimming straightness while Rakitin et al. (1999) found a positive relation between condition factor and male fertilization success. Also, there may have been other non-genetic parental effects that we did not control. Finally, climate change is expected to occur over several generations, yet we know little about the evolutionary potential of these populations to respond to such change.

Our different population responses to temperature show that adaptations in traits related to reproduction that are likely to mediate responses to climate change can be driven by males, as was also demonstrated by Donelson et al. (2010) in a tropical reef fish. Under the predicted increase in sea surface temperatures, our results indicate that the ability of the sperm to achieve fertilization should not be a limiting factor in reproductive performance, even when considering individual variation in responses. Nevertheless, our data show that when in sperm competition, a male's genotype and environmental history will affect fertilization success and, ultimately, selection. Thermal plasticity in gonadal maturation, embryo development and viability, larval survival and mismatch between spawn timing and food availability will also affect Atlantic cod resilience to climate change. As an example, Purchase & Brown (2000) detected a greater positive plasticity to temperature in terms of growth, for a northern population of cod from the western Atlantic compared to a more southerly population. Moreover, sperm thermal reaction norms should increase with temperature up to a critical temperature at which point sperm swimming characteristics are likely to decrease, resulting in a dome shape as observed by Purchase et al. (2010). It is expected that we would have observed this effect if we had tested higher

temperatures. For example, in the case of the reef fish *Acanthochromis polyacanthus* an increase of only 3°C in the water temperature caused a reduction in the proportion of the spermatozoa in the testis (Donelson et al. 2010). In this context, although not reflected in our study, extreme temperatures, such as 12°C or higher, will most likely have a negative effect on Atlantic cod gametogenesis. Indeed, to counteract effects of higher temperatures, southern Atlantic cod populations migrate to areas with a maximum water temperature of 8°C during the spawning season (Righton et al. 2010). Thus, rather than remain in their current locations, cod populations will likely move northward to new areas looking for their preferred temperature range.

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Appendix

Table A1. Parameters used to set the ImageJ CASA plugin

Plugin parameter	Value used
Minimum sperm size (pixels)	3.0
Maximum sperm size (pixels)	12.0
Minimum track length (frames)	50.0
Maximum sperm velocity between frames (pixels)	10.0
Minimum VSL for motile (μm s^{-1})	1.0
Minimum VAP for motile (μm s^{-1})	2.0
Minimum VCL for motile (μm s^{-1})	4.0
Low VAP speed (μm s^{-1})	2.0
Maximum percentage of path with zero VAP	1.0
Maximum percentage of path with low VAP	25.0
Low VAP speed 2 (μm s^{-1})	10.0
Low VCL speed (μm s^{-1})	15.0
High WOB (percent VAP/VCL)	80.0
High LIN (percent VSL/VAP)	80.0
High WOB 2 (percent VAP/VCL)	50.0
High LIN 2 (percent VSL/VAP)	60.0
Frame Rate (frames per second)	100.0
Microns per 1000 pixels	956.0

Table A2. Summary results of the 2 principal components (PC) analyses. PC1 and PC2 are the first 2 PCA extracted from the analysis. The table shows, for each PC, the comparison of factor loadings for each starting variable, the variance explained by the PC and its cumulative proportion

Parameter	PC 1	PC 2
VCL	0.420	−0.335
VAP	0.532	−0.140
VSL	0.535	0.024
LIN	0.172	0.927
WOB	0.473	0.089
Proportion of variance	0.687	0.197
Cumulative proportion	0.687	0.884

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