# Do environmental fluctuations during development affect trait variation? An experimental test with salinity

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## Abstract

Climate change is a driver of extinction, with extreme events occurring more frequently. It increases both the amount and variability in environmental stress that organisms experience. In novel environments, greater intra-specific trait variation increases the opportunity for natural selection as individuals with fortuitously advantageous trait expression may thrive. Many studies focus on changes in trait means between novel and historic environments but overlook changes in trait variation. We tested how salinisation – which currently affects 20-50% of freshwaters worldwide – alters trait variation in a freshwater fish, *Gambusia holbrooki*. We reared newborn fish in freshwater, stable-salinity or fluctuating-salinity water until maturation, and then compared variation in fitness-related traits in each sex during early and late adulthood. Salinisation had stronger effects on young than old adults, with sex-specific impacts (female: gut development; male: age at maturity and body size). When we accounted for mean differences in trait expression between environments, salinisation also

affected relative variation in female fecundity (egg size, offspring number). Notably, fluctuations in salinity did not magnify the effect of stable salinity, but sometimes reversed its effect. Our findings suggest that researchers should pay closer attention to environmental fluctuations: changes in trait variation in a population can alter its adaptive potential.

**Keywords**

Intraspecific trait variation, life history, salinisation, poor start in life, climate variability

## 1. Introduction

Extreme events are now occurring more frequently globally [1, 2], with climate change affecting both the mean and variability of environmental stress that organisms experience. Variable environments activate numerous physiological pathways that animals use to maintain homeostasis [3]. Variability experienced early in life can expose hidden genetic variation, reduce canalisation and increase trait variation in a population [4]. Increased trait variation in a population can also result from greater individual differences in how resources are allocated when animals are stressed [5], leading to higher variation in life-history strategies. Greater trait variation implies that individuals with traits that deviate from the norm have reduced survival capacity [6]. However, the fitness consequences of greater trait variation can be context-dependent [7]. In novel environments, existing trait values are often sub-optimal [8] and increased trait variation creates a greater opportunity for selection: the subset of individuals with fortuitously advantageous trait expression are more likely to thrive [9]. This leads to trait evolution if there is genetic variation in developmental responses to novel environments [10, 11].

Females and males often differ in how trait expression changes in a more challenging environment [12]. This difference is partly due to sex-specific selection on allocation to reproduction and somatic maintenance [13]. Sexual selection is typically stronger on males [14], favouring greater investment in traits that increase mating and fertilization success, thereby diverting resources from self-maintenance [15]. In contrast, females rarely struggle to acquire a mate and often benefit from greater allocation to self-maintenance, which enhances fecundity [16]. Consequently, males seem to be more sensitive to environmental change [17] and may exhibit greater variability in fitness-related traits when stressed [18]. However, the

magnitude and direction of sex differences in trait variability can also depend on the trait's function. For example, males usually have greater variation than females in sexually selected traits [19], whereas females show greater variation than males in immunocompetence [20]. Greater variation in life-history traits (e.g., immunity, body size) implies that individuals vary in their performance of fitness-enhancing tasks (e.g., parasite resistance, resource utilisation), potentially lowering the risk of extinction when the environment fluctuates because at least some individuals will still survive and breed [9]. In addition, increased variation in male sexual traits can accelerate selection for males that confer more advantageous traits to offspring when females choose males based on sexual traits that signal offspring fitness [21]. To understand how population extinction risk changes when individuals are exposed to greater stress, and whether this risk is modified by stressors being stable or fluctuating, it is essential to test for sex differences in trait variability in different types of environments.

Here, we test how trait variation in males and females shift in response to changes in salinity during development in a freshwater fish. Salinity levels fluctuate naturally due to precipitation, evaporation, and events such as flooding and the isolation of water pools [22, 23], but these natural fluctuations are now exacerbated by human activities (e.g., irrigation, mining) [24, 25]. For example, wastewater from shale gas operations has caused salinity levels to become seven times higher than that of seawater in some places [26]. Salinisation currently affects 20-50% of freshwater globally [27]. Most freshwater fish species inhabit environments with relatively stable salinity and struggle to acclimate to rapid changes in salinity [28]. This underscores the direct threats that salinity fluctuations pose for fish [3]. Despite the crucial role of fish in freshwater ecosystems [29], only 16 of 193 recent studies on the effects of freshwater salinisation were on fish (review: [25]), and few (if any) of these studies investigated how trait variability changes when salinity fluctuates.

To test the effect of salinity during development on trait variation between individuals, we ran an experiment using the mosquitofish (*Gambusia holbrooki*) – an invasive freshwater fish with a global distribution [30]. Many established populations have ready access to saline waters in the wild [31-34], where they compete with endemic fish species [35]. In general, a salinity level of ~10‰ is considered to be an eco-physiological barrier that separates freshwater and marine fauna [36]. We reared *G. holbrooki* from birth to maturity in either freshwater (0‰), stable salinity (10‰) or fluctuating salinity (0-20‰; mean = 10‰). We measured key life-history traits (size and age at maturity, gut length, immunity) in both sexes and sex-specific traits (size of intromittent organ, sperm/egg production, total offspring number). These traits were measured twice (in early and late adulthood) to test for age-dependent effects. Trait variance can respond to environmental changes differently from trait means, potentially altering the environmental impact on population viability [37]. Here we focus on how salinisation affects trait variation, with trait means reported elsewhere [38].

## 2. Methods

### (a) Experimental overview

Adult mosquitofish were collected from a stream in Canberra, Australia in 2020-2021 and held in 90L freshwater tanks (ca. 50 fish/tank). Pregnant females were transferred to individual 1L freshwater tanks to give birth. Newborn fry from 118 broods were randomly assigned to three treatments (maximum 11 fry per brood/treatment): freshwater (0‰; $n$ = 343), stable salinity (10‰; $n$ = 397), or fluctuating salinity (0-20‰ with mean = 10‰; $n$ =392). Siblings in the same treatment were housed in communal tanks at a density of ≤1

individual/L. Three tanks (one per treatment) for each brood were placed near each other to minimise variation in rearing conditions (e.g., temperature; light) within temperature-controlled rooms. We accounted for potential differences among broods in our analyses (see below). All fish were kept under a 14 light:10 dark cycle at 28 ± 1°C and fed *Artemia* nauplii twice daily.

We manipulated salinity concentrations by dissolving aquarium salt (Aquaforest, Brzesko, Poland) with aged tap water and aquatic conditioner (Seachem, Madison, USA). We monitored salinity every two days using a refractometer and renewed the water weekly. Fish in the *freshwater* treatment were kept at 0‰ salinity. To minimise mortality due to initial salinity exposure, newborn fish in the two salinity treatments were first transferred to tanks with 2.5‰ saline water. After two days, fish in the *stable salinity* treatment were then kept at 10‰ salinity, while those in the *fluctuating* treatment were exposed to cyclically varying salinities (10‰ →20‰ →10‰ →0‰ →10‰). Fluctuating salinity levels were generated by rotating fish between tanks with different salinity levels every two days. To control for any effect of being transferred, fish in the freshwater and stable salinity treatments were also rotated between tanks with the appropriate water (0‰ or 10‰, respectively).

We inspected fish every two days to determine the date of maturation (gravid spot for females; a pointed anal fin for males). Newly matured adults were individually transferred to 1L tanks of freshwater. After one week in this common garden setting, we euthanised a random subset of females ($n$ =72 freshwater; 118 stable salinity; 100 fluctuating salinity) and males ($n$ =68 freshwater; 81 stable salinity; 92 fluctuating salinity) to test if the developmental environment affected life-history traits and reproductive traits (see below) of young adults. To test the long-term effect of the developmental environment, we paired each

of the remaining females ($n$ =99 freshwater; 98 stable salinity; 87 fluctuating salinity) and males ($n$ =104 freshwater; 100 stable salinity; 113 fluctuating salinity) with a stock fish of the opposite sex in a 4L freshwater tank to breed. We replaced the stock fish fortnightly to maintain the sexual interests of focal fish. After 12 weeks, focal fish were euthanised to again measure life-history and female reproductive traits. Male reproductive traits (see below) did not require euthanasia, so we measured these traits on the same males when young and old (i.e., before and after the mating period).

**(b) Life-history traits**

*Body length*

Fish were anaesthetised in ice-water, placed on a glass slide with a scale, and the side of their body was photographed to measure their standard length (snout tip to end of vertebral column) using *ImageJ*.

*Immune response*

We used an in vivo reaction to phytohemagglutinin to measure cell-mediated immunity. Once a fish was anaesthetised, we used a spessimeter (Mitutoyo 547-301; accuracy: 0.01mm) to measure the thickness of the posterior end of its dorsal fin five times. We then injected 0.01mg phytohemagglutinin dissolved in 0.01mL of PBS solution. After 24h, the thickness was again measured five times. The difference between the pre- and post-injection measures (i.e., swelling) is an index of immunity.

*Gut length*

Fish guts facilitate ion regulation and water uptake, which are critical for salinity acclimation [39]. After the immunity test, we dissected fish to extract and photograph their gut (the digestive tract from mouth to anus). We measured its length using *ImageJ*.

**(c) Female reproductive traits**

We dissected females to count how many eggs they held. For young females (still virgins), we photographed their unfertilised eggs and measured the diameter using *ImageJ*. We did not measure the eggs of old, mated females because they carried embryos (whose size varies with their developmental stages; [40]). Instead, we recorded (a) the number of embryos they held at the end of the experiment and (b) the number of offspring they had produced during the mating period. We considered (a)+(b) as the total offspring number.

**(d) Male reproductive traits**

We measured the length of the male intromittent organ (gonopodium) from photographs. We recorded sperm count and velocity following methods in [41]. Briefly, we pressed a male's abdomen to empty his sperm reserves. After seven days we repeated this process to obtain sperm with a standardised age. We collected the sperm with extender medium to dilute the concentration for subsequent sperm counting using a CEROS sperm tracker. For sperm velocity, we collected two separate samples of four sperm bundles in extender medium. Each sample was activated using 125mM KCl and 2mg/mL bovine serum albumin. We used the sperm tracker to record sperm velocity (see *electronic supplementary material* for details).

**(e) Statistical analysis**

We estimated covariances between traits across individuals using Bayesian Multivariate Mixed Effects Models with all traits modelled together for each of the four age/sex groups separately. We ran separate models for the life-history and reproductive traits of young males because these were measured on different males. We treated environment (3 levels) as a fixed factor and brood identity as a random factor to account for measurement of several fish from the same brood. 'Age at maturity', 'immunity', 'egg number of young females' and 'sperm count' were power transformed to meet the assumption of multivariate normality. In each model, we ran four MCMC chains (each with 5000 iterations and a warmup of 1000) and confirmed convergence ($R_{hat}$ =1). The effective sample size for each parameter always exceeded 1480, with all samples generated by iterations ($n$ =16000) retained.

To test if trait variances were affected by the developmental environment, we modelled both the mean and the standard deviation (SD) on a logarithmic scale for each trait (and trait covariances) as a function of environment. From the posterior distribution, we obtained the estimated trait mean and SD for each environment. We used the natural logarithm of the ratio between SDs from two environments (i.e., lnVR = ln [$SD_1/SD_2$]) to assess the environmental effect on raw variance of each trait. In addition, we used the natural logarithm of the ratio between coefficients of variation (i.e., CV = SD/mean) from two environments (i.e., lnCVR = ln [$CV_1/CV_2$]) to assess the environmental effect on *relative* trait variance while accounting for expected increases in variance with the mean. Notably, lnCVR assumes a linear relationship between ln(mean) and ln(SD), with a slope of 1 [42, 43]. lnCVR is particularly useful in when comparing or combining values across studies with a large absolute difference

in mean and SD (e.g., meta-analyses; [44, 45]). In our study, however, we compared variation of the same trait, where mean values were similar in the three environments. Nonetheless, we reported lnCVR in the supplementary material as a sensitivity analysis to assess *relative* trait variance. In most case (45 of 48 tests), the results using lnVR and lnCVR were the same. We compared specific pairs of environments to test the effect of salinity (*stable salinity* / *freshwater*) and the effect of fluctuations (*fluctuating* / *stable salinity*). A positive lnVR (or lnCVR) indicates an increase in trait variance with salinity or fluctuations in salinity respectively, and *vice versa* for negative values. The lnVR (or lnCVR) was significant when its 95% credible intervals excluded zero. We calculated the probability (pMCMC) of the observed effect (null hypothesis = no effect) using the posterior distribution from our models.

## 3. Results

There were significant differences in trait variance in young fish for 2 of 13 tests for an effect of salinity, and for 3 of 13 tests for an effect of fluctuations in salinity (Figure 1a,b). There were fewer differences in trait variance for older fish (Figure 1c,d): no significant differences in variance due to higher salinity (0 of 11 tests) and only one due to fluctuations in salinity (1 of 11 tests). Variance in traits had the same propensity to be affected by the environment in males (3 of 13 traits) and females (1 of 11 traits). Together, more traits (4 of 24 traits) than expected by chance alone (1.2 of 24 traits, if alpha = 0.05) showed a significant difference in variation when reared in different environments (Figure 1).

Female gut length when young (+88%) and male age at maturity (+41%) were significantly more variable for fish that developed in saline rather than freshwater. Young females that developed in water with fluctuating rather than stable salinity had less variation in gut length

(-30%). For males, age and size at maturity (-34%, -35%) were significantly less variable when they developed in water with fluctuating rather than stable salinity. Neither salinity nor its fluctuations affected trait variation for older females, but older males that developed in water with fluctuating rather than stable salinity varied significantly less in body size (-25%) (Figure 1).

When accounting for differences between environments in mean trait values using lnCVR, the environmental effects were consistent with those based on lnCV for 45 of the 48 tests (*supplementary material*, Figure S1). The exceptions were egg size and offspring number in females, and body size in older males. Fluctuations in salinity (fluctuating versus stable salinity) significantly increased the *relative* variation in egg size (+27%, pMCMC =0.028), while higher salinity (stable salinity versus freshwater) decreased the *relative* variation in offspring number (-31%; pMCMC =0.013), and there was no significant difference in *relative* variation in body size between older males from the fluctuating and stable salinity environments (pMCMC =0.107).

## 4. Discussion

Species with a broad niche and strong resilience to environmental change often have high geographic variation in life-history traits [46]. It is assumed that this variation is partly due to adaptively plastic responses to local environmental conditions, especially those experienced during development [47]. In contrast, variation among individuals experiencing the same local environmental conditions is more likely to reflect disruption of adaptive responses. Variation could, however, also reflect genetic or stochastic variation among individuals that determines optimal allocation to different life history traits. For example, it is well

established that sexually selected traits show high phenotypic variation because they tend to be condition-dependent [48], and males vary in their luck or genetically influenced ability to accrue resources [49].

In our study, there were several important changes in trait variation when mosquitofish, *G. holbrooki*, were developed in an environment with either higher than usual salinity, or both higher and more fluctuating salinity. Importantly, these changes were always sex-specific. Shortly after maturation, higher salinity (*stable salinity* vs *freshwater*) significantly increased (both raw and relative) variation in female relative gut length, while fluctuations in salinity (*fluctuating* vs *stable salinity*) reduced the variation. No such changes occurred for males. Greater deviation in gut allometry at higher salinity might have a negative effect on female osmoregulation [50]. It might also affect the ability of individual females to utilise different food sources by affecting their capacities for food retention [51]. Greater variation in female relative gut length might therefore broaden the dietary range of a population of females because they can digest a greater variety of prey types [52]. This has potential ecological consequence because *G. holbrooki* are highly invasive and their aggressiveness can lead to the local extinction of endemic fish species [53]. Future research should test if changes in salinity, and its fluctuations, generate dietary shifts in females that affect competition with other fish species. Another future challenge is to explain why salinity did not affect male gut length.

Salinity differed in how it affected (both raw and relative) variation in the age and size at maturity between the sexes. Age and size at maturity were less variable for males from the fluctuating rather than stable salinity environment, while those from the stable salinity rather than freshwater environment showed greater variation in age at maturity. No such effects

occurred for females. Mating competition among males is intense in *G. holbrooki*, with size-dependent mating strategies [54]. Larger males typically monopolise females by chasing away smaller rivals [54]. Less variation in male age and size at maturity is likely to increase male-male competition: a greater number of similarly sized males will mature at the same time, and male-male contests will take longer to resolve because body size is a major determinant of fight outcome [55]. As males barely grow after maturation, fluctuations in salinity also reduced the variation in body size of older males. If a fluctuating salinity environment increases male-male competition, this might benefit females. Male aggression towards other males reduces the time available to harass females, which in turn enhance females' foraging efficiency [56]. In addition, males tend to senesce more rapidly when involved in mating activities [15], and females take longer than males to mature in a fluctuating salinity environment [38]. Consequently, when salinity fluctuates, the adult sex ratio may become more female-biased later in the breeding season. This could further increase female fecundity if the benefits of reduced sexual conflict outweigh any costs arising from a lack of suitable mates. Future research should compare seasonal changes in sexual conflict and female reproduction between environments with stable and fluctuating salinity.

Although salinity did not affect raw variation in reproductive traits in either sex, there were sex-specific effects of salinity on relative variation when attempting to control for the relationship between mean and SD [42]. Fluctuations in salinity led to greater *relative* variation in egg size, while higher salinity reduced *relative* variation in how many offspring females produced. There were no equivalent effects on male sperm traits. The greater relative variation in egg size was driven by the reduction in average egg size in a fluctuating salinity environment [38]. Smaller eggs may increase vulnerability to external stresses [57] as a larger surface-to-volume ratio is likely to elevate the demands of osmoregulation [58]. Moreover,

greater relative variation in egg size may heighten differences in offspring relative competitiveness ([59] but see [60, 61]). Consequently, a proportion of the offspring not only become more susceptible to external environmental pressures but also have to compete with larger conspecifics.

Lower relative variation in offspring number was driven by more offspring being produced by females that developed in the stable salinity than freshwater environment [38]. Lower relative differences in female fecundity have several implications. First, a more equal reproductive contribution of females is likely to maintain a higher level of genetic diversity (e.g., [62]). Second, there is less opportunity for net selection on females if our count of offspring is a good proxy for total fitness. This should slow the rate of local adaptation that might improve the ability to survive in a more saline environment (e.g., more gill rakers [63] and larger body size [10], as seen in three-spine stickleback). To determine how relative or absolute differences in fecundity affect net selection on females, future studies could compare the genetic diversity of *G. holbrooki* populations from high and low salinity environments.

In sum, changes in salinity affected variation in reproductive and life history traits in mosquitofish, thereby altering the opportunity for natural and sexual selection. This will affect the rate of local adaptation when there is genetic variation associated with these changes in trait expression. Subsequent evolution of these fitness-related traits could then alter the outcome of interspecific competition, with potential implications for the local persistence of endemism species if species differ in the rates of adaptation to changes in salinity.

**Data accessibility**

Data and code can be downloaded from Dryad

https://datadryad.org/stash/share/kDVffTZzYC9Ju9Aby0SVq_kHA9A9B323N40_xuVCfeY

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**Author contributions**

MHJC, DWAN and MDJ conceived the study and designed the experiment. MHJC collected all data. All authors contributed to data analysis and interpretation. MHJC wrote the first draft, with DWAN and MDJ providing critical revisions. All authors gave final approval for publication.

**Ethic statement**

The project received approval from the Australian National University's Animal Ethics Committee (Ethics Protocol: A2021/04).

**Conflict of interest**

The authors declare no competing interests.

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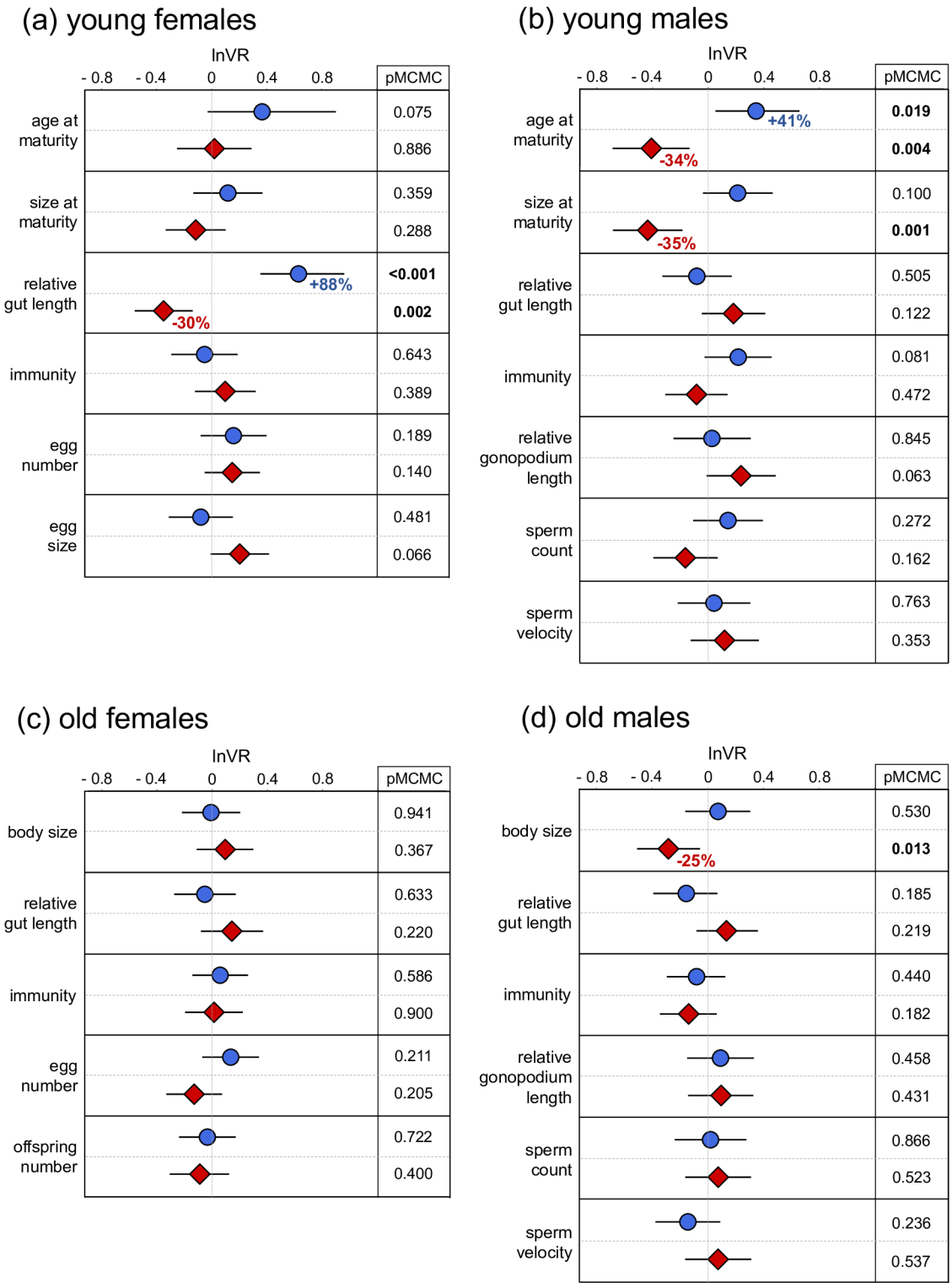


**Figure 1. lnVR with 95% credible intervals showing environmental effects on SD for each trait in (a) young females, (b) young males, (c) old females and (d) old males.** Blue circle = salinity effect ($lnSD_{stable\ salinity} - lnSD_{freshwater}$). Red diamond = fluctuation effect

($lnSD_{fluctuating\ salinity} - lnSD_{stable\ salinity}$). Positive values indicate that greater salinity (blue) or fluctuation in salinity (red) 'increases' trait variation, while negative values indicate the opposite. Significant differences in trait variation (pMCMC < 0.05; see Methods) are shown with the percentage difference, calculated as *[exp(lnVR) – 1]*100%*.

**Electronic supplementary material**

*Part 1. Method for sperm measurements*

## Sperm count

A male was anesthetised in icy water and placed on a glass slide covered with a 1% polyvinyl alcohol solution under a dissecting microscope. We swung his gonopodium forward and gently pressed his abdomen to expel his sperm reserves. The male was then returned to his 1L tank for sperm replenishment. After seven days, we re-stripped the male to collect the sperm into an Eppendorf tube with 400-1200 uL of extender medium (207 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 0.49 mM $MgCl_2$, 0.41 mM $MgSO_4$, 10 mM Tris (Cl); pH 7.5). We vortexed the sperm solution for 30s and mixed it using a 10-µl pipette. 3µL of the solution was then placed on a 20-micron capillary slide (Leja) to count the sperm number using a CEROS Sperm Tracker (Hamilton Thorne Research, Beverly, MA, USA) under 100× magnification. The sperm count was calculated using the mean value of five randomly selected subsamples per male (repeatability: $r = 0.829 \pm 0.010$ SE, $p = 0.001$, $n = 525$ ejaculates). We were unable to analyse 28 samples due to the shortage of capillary slides during the Covid-19 lockdown.

## Sperm velocity

When stripping the sperm reserves after 7 days, we also collected four sperm bundles into each of two Eppendorf tubes containing 2uL of extender medium. Each sample was analysed separately. We placed 3uL of the sperm sample into a cell in a 12-cell multi-test slide (MP Biomedicals, USA) coated with 1% polyvinyl alcohol solution, activated the sample using a 3µL solution of 125 mM KCl and 2 mg/ml bovine serum albumin for 30s, and then covered it with a coverslip. We measured the actual velocity along the sperm trajectory (i.e., curvilinear velocity) using a CEROS Sperm Tracker. The weighted average of the motile sperm tracks in both samples (66.66 ± 1.45 SE tracks; $n = 553$ ejaculates) was considered as sperm velocity.

*Part 2. Full statistical results*

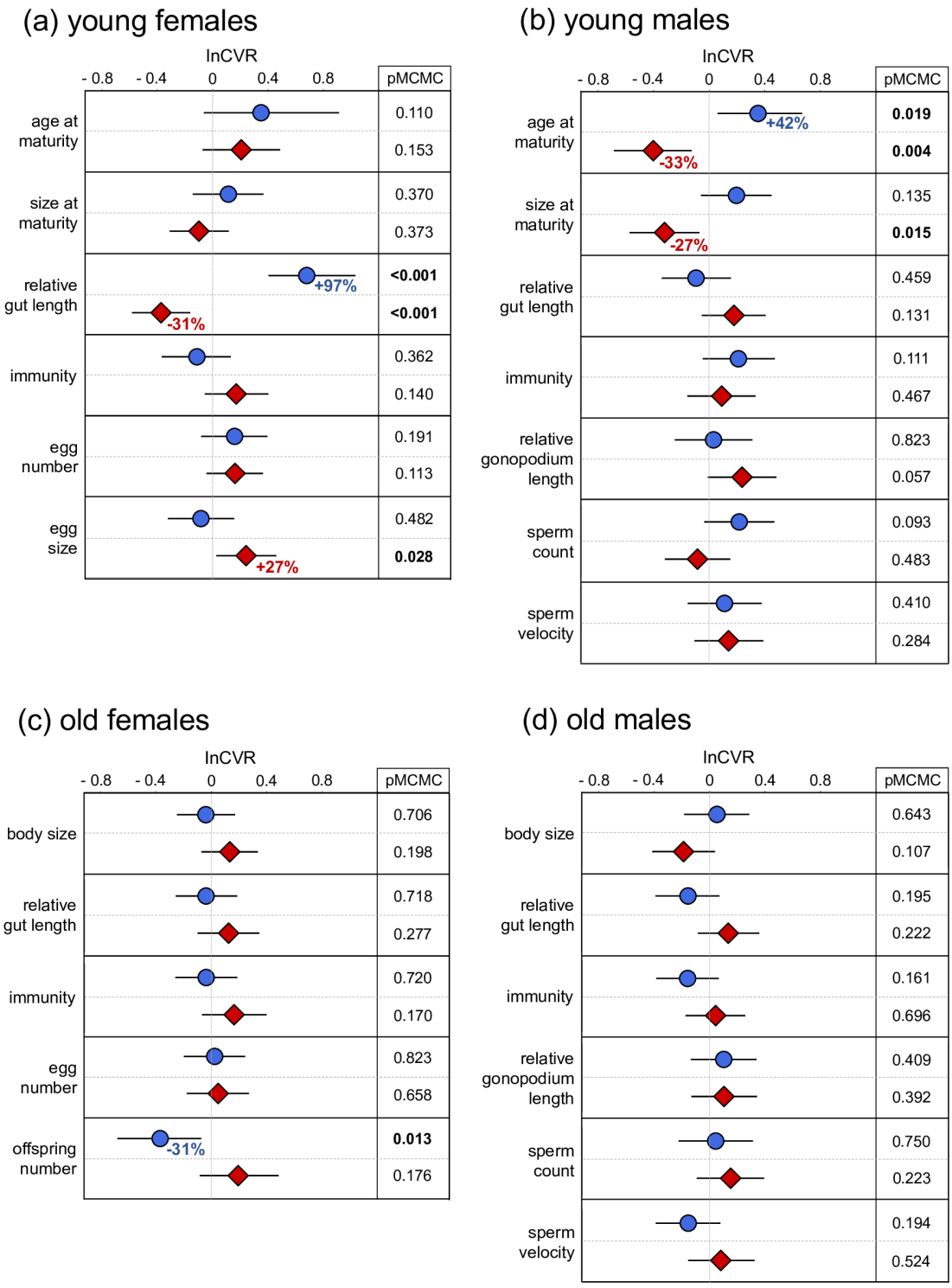


**Figure S1. lnCVR with 95% credible intervals showing environmental effects on CV for each trait in young females (a) and males (b); and old females (c) and males (d).** Blue circle = salinity effect ($lnCV_{stable\ salinity} - lnCV_{freshwater}$). Red diamond = fluctuation effect ($lnCV_{fluctuating\ salinity} - lnCV_{stable\ salinity}$). Positive values indicate that higher salinity (blue) or fluctuation in salinity (red) 'increase' relative variance, while negative values indicate the opposite. Significant differences in relative variance (pMCMC < 0.05; see Methods) are shown with the percentage difference, calculated as *[exp(lnCVR) – 1]*100%.*

**Table S1. The natural logarithm of the ratio of posterior standard deviations (lnVR) and posterior coefficients of variation (lnCVR) from two environments, along with its 95% credible intervals. (A) Young females, (B) young males, (C) old females and (D) old males.** The effect of salinity represents ($lnSD_{stable\ salinity} - lnSD_{freshwater}$) for lnVR, and ($lnCV_{stable\ salinity} - lnCV_{freshwater}$) for lnCVR. The effect of fluctuations represents ($lnSD_{fluctuating\ salinity} - lnSD_{stable\ salinity}$) for lnVR, and ($lnCV_{fluctuating\ salinity} - lnCV_{stable\ salinity}$) for lnCVR.

**(A) Young females**

(i) lnVR

| Trait | Term | Estimate (lnVR) | 2.5% | 97.5% | pMCMC | Difference (%) |
|---|---|---|---|---|---|---|
| Age at maturity | Effect of salinity | 0.367 | -0.029 | 0.903 | 0.075 | 44 |
| | Effect of fluctuations | 0.020 | -0.251 | 0.290 | 0.886 | 2 |
| Size at maturity | Effect of salinity | 0.117 | -0.134 | 0.368 | 0.359 | 12 |
| | Effect of fluctuations | -0.117 | -0.335 | 0.100 | 0.288 | -11 |
| Relative gut length | Effect of salinity | 0.630 | 0.353 | 0.963 | **<0.001** | 88 |
| | Effect of fluctuations | -0.351 | -0.561 | -0.140 | **0.002** | -30 |
| Immunity | Effect of salinity | -0.055 | -0.297 | 0.185 | 0.643 | -5 |
| | Effect of fluctuations | 0.095 | -0.125 | 0.317 | 0.389 | 10 |
| Egg number | Effect of salinity | 0.159 | -0.079 | 0.399 | 0.189 | 17 |
| | Effect of fluctuations | 0.150 | -0.050 | 0.351 | 0.140 | 16 |
| Egg size | Effect of salinity | -0.084 | -0.317 | 0.149 | 0.481 | -8 |
| | Effect of fluctuations | 0.199 | -0.012 | 0.412 | 0.066 | 22 |

* The percentage difference for the ratio is shown using *[exp (lnVR) – 1]*100*

(ii) lnCVR

| Trait | Term | Estimate (lnCVR) | 2.5% | 97.5% | pMCMC | Difference (%) |
|---|---|---|---|---|---|---|
| Age at maturity | Effect of salinity | 0.349 | -0.064 | 0.912 | 0.110 | 42 |
| | Effect of fluctuations | 0.206 | -0.073 | 0.487 | 0.153 | 23 |
| Size at maturity | Effect of salinity | 0.115 | -0.142 | 0.369 | 0.370 | 12 |
| | Effect of fluctuations | -0.098 | -0.309 | 0.118 | 0.373 | -9 |
| Relative gut length | Effect of salinity | 0.679 | 0.403 | 1.031 | **<0.001** | 97 |
| | Effect of fluctuations | -0.373 | -0.583 | -0.162 | **<0.001** | -31 |
| Immunity | Effect of salinity | -0.114 | -0.368 | 0.131 | 0.362 | -11 |
| | Effect of fluctuations | 0.170 | -0.057 | 0.402 | 0.140 | 18 |
| Egg number | Effect of salinity | 0.160 | -0.082 | 0.397 | 0.191 | 17 |
| | Effect of fluctuations | 0.163 | -0.043 | 0.365 | 0.113 | 18 |
| Egg size | Effect of salinity | -0.085 | -0.324 | 0.155 | 0.482 | -8 |
| | Effect of fluctuations | 0.241 | 0.026 | 0.460 | **0.028** | 27 |

* The percentage difference for the ratio is shown using *[exp (lnCVR) – 1]*100*

**(B) Young males**

(i) lnVR

| Trait | Term | Estimate (lnVR) | 2.5% | 97.5% | pMCMC | Difference (%) |
|---|---|---|---|---|---|---|
| Age at maturity | Effect of salinity | 0.345 | 0.053 | 0.657 | **0.019** | 41 |
| | Effect of fluctuations | -0.408 | -0.686 | -0.134 | **0.004** | -34 |
| Size at maturity | Effect of salinity | 0.210 | -0.040 | 0.462 | 0.100 | 23 |
| | Effect of fluctuations | -0.437 | -0.687 | -0.187 | **0.001** | -35 |
| Relative gut length | Effect of salinity | -0.083 | -0.332 | 0.168 | 0.505 | -8 |
| | Effect of fluctuations | 0.180 | -0.047 | 0.409 | 0.122 | 20 |
| Immunity | Effect of salinity | 0.218 | -0.024 | 0.459 | 0.081 | 24 |
| | Effect of fluctuations | -0.082 | -0.307 | 0.141 | 0.472 | -8 |
| Relative gonopodium length | Effect of salinity | 0.027 | -0.249 | 0.306 | 0.845 | 3 |
| | Effect of fluctuations | 0.236 | -0.011 | 0.486 | 0.063 | 27 |
| Sperm count | Effect of salinity | 0.142 | -0.109 | 0.393 | 0.272 | 15 |
| | Effect of fluctuations | -0.165 | -0.395 | 0.068 | 0.162 | -15 |
| Sperm velocity | Effect of salinity | 0.041 | -0.220 | 0.303 | 0.763 | 4 |
| | Effect of fluctuations | 0.117 | -0.127 | 0.364 | 0.353 | 12 |

* The percentage difference for the ratio is shown using *[exp (lnVR) – 1]*100*

(ii) lnCVR

| Trait | Term | Estimate (lnCVR) | 2.5% | 97.5% | pMCMC | Difference (%) |
|---|---|---|---|---|---|---|
| Age at maturity | Effect of salinity | 0.351 | 0.060 | 0.668 | **0.019** | 42 |
| | Effect of fluctuations | -0.402 | -0.686 | -0.126 | **0.004** | -33 |
| Size at maturity | Effect of salinity | 0.196 | -0.059 | 0.449 | 0.135 | 22 |
| | Effect of fluctuations | -0.321 | -0.573 | -0.070 | **0.015** | -27 |
| Relative gut length | Effect of salinity | -0.093 | -0.339 | 0.158 | 0.459 | -9 |
| | Effect of fluctuations | 0.180 | -0.052 | 0.408 | 0.131 | 20 |
| Immunity | Effect of salinity | 0.210 | -0.047 | 0.472 | 0.111 | 23 |
| | Effect of fluctuations | 0.090 | -0.157 | 0.333 | 0.467 | 9 |
| Relative gonopodium length | Effect of salinity | 0.033 | -0.246 | 0.313 | 0.823 | 3 |
| | Effect of fluctuations | 0.238 | -0.008 | 0.485 | 0.057 | 27 |
| Sperm count | Effect of salinity | 0.216 | -0.036 | 0.469 | 0.093 | 24 |
| | Effect of fluctuations | -0.084 | -0.319 | 0.151 | 0.483 | -8 |
| Sperm velocity | Effect of salinity | 0.110 | -0.154 | 0.378 | 0.410 | 12 |
| | Effect of fluctuations | 0.139 | -0.106 | 0.389 | 0.284 | 15 |

* The percentage difference for the ratio is shown using *[exp (lnCVR) – 1]*100*

**(C) Old females**

(i) lnVR

| Trait | Term | Estimate (lnVR) | 2.5% | 97.5% | pMCMC | Difference (%) |
|---|---|---|---|---|---|---|
| Body size | Effect of salinity | -0.009 | -0.219 | 0.203 | 0.941 | -1 |
| | Effect of fluctuations | 0.094 | -0.112 | 0.298 | 0.367 | 10 |
| Relative gut length | Effect of salinity | -0.055 | -0.277 | 0.169 | 0.633 | -5 |
| | Effect of fluctuations | 0.141 | -0.083 | 0.368 | 0.220 | 15 |
| Immunity | Effect of salinity | 0.057 | -0.144 | 0.259 | 0.586 | 6 |
| | Effect of fluctuations | 0.013 | -0.197 | 0.221 | 0.900 | 1 |
| Egg number | Effect of salinity | 0.133 | -0.073 | 0.338 | 0.211 | 14 |
| | Effect of fluctuations | -0.132 | -0.333 | 0.071 | 0.205 | -12 |
| Offspring number | Effect of salinity | -0.037 | -0.242 | 0.169 | 0.722 | -4 |
| | Effect of fluctuations | -0.092 | -0.310 | 0.121 | 0.400 | -9 |

* The percentage difference for the ratio is shown using *[exp (lnVR) – 1]*100*

(ii) lnCVR

| Trait | Term | Estimate (lnCVR) | 2.5% | 97.5% | pMCMC | Difference (%) |
|---|---|---|---|---|---|---|
| Body size | Effect of salinity | -0.039 | -0.248 | 0.173 | 0.706 | -4 |
| | Effect of fluctuations | 0.134 | -0.071 | 0.337 | 0.198 | 14 |
| Relative gut length | Effect of salinity | -0.041 | -0.260 | 0.184 | 0.718 | -4 |
| | Effect of fluctuations | 0.122 | -0.102 | 0.344 | 0.277 | 13 |
| Immunity | Effect of salinity | -0.040 | -0.263 | 0.185 | 0.720 | -4 |
| | Effect of fluctuations | 0.162 | -0.071 | 0.397 | 0.170 | 18 |
| Egg number | Effect of salinity | 0.025 | -0.198 | 0.245 | 0.823 | 3 |
| | Effect of fluctuations | 0.050 | -0.177 | 0.274 | 0.658 | 5 |
| Offspring number | Effect of salinity | -0.371 | -0.680 | -0.074 | **0.013** | -31 |
| | Effect of fluctuations | 0.193 | -0.085 | 0.486 | 0.176 | 21 |

* The percentage difference for the ratio is shown using *[exp (lnCVR) – 1]*100*

**(D) Old males**

(i) lnVR

| Trait | Term | Estimate (lnVR) | 2.5% | 97.5% | pMCMC | Difference (%) |
|---|---|---|---|---|---|---|
| Body size | Effect of salinity | 0.074 | -0.161 | 0.305 | 0.530 | 8 |
| | Effect of fluctuations | -0.283 | -0.508 | -0.056 | **0.013** | -25 |
| Relative gut length | Effect of salinity | -0.154 | -0.391 | 0.070 | 0.185 | -14 |
| | Effect of fluctuations | 0.135 | -0.081 | 0.361 | 0.219 | 14 |
| Immunity | Effect of salinity | -0.082 | -0.295 | 0.124 | 0.440 | -8 |
| | Effect of fluctuations | -0.139 | -0.346 | 0.063 | 0.182 | -13 |
| Relative gonopodium length | Effect of salinity | 0.090 | -0.150 | 0.330 | 0.458 | 9 |
| | Effect of fluctuations | 0.094 | -0.143 | 0.326 | 0.431 | 10 |
| Sperm count | Effect of salinity | 0.021 | -0.236 | 0.279 | 0.866 | 2 |
| | Effect of fluctuations | 0.076 | -0.161 | 0.312 | 0.523 | 8 |
| Sperm velocity | Effect of salinity | -0.141 | -0.374 | 0.092 | 0.236 | -13 |
| | Effect of fluctuations | 0.076 | -0.161 | 0.315 | 0.537 | 8 |

* The percentage difference for the ratio is shown using *[exp (lnVR) – 1]*100*

(ii) lnCVR

| Trait | Term | Estimate (lnCVR) | 2.5% | 97.5% | pMCMC | Difference (%) |
|---|---|---|---|---|---|---|
| Body size | Effect of salinity | 0.054 | -0.183 | 0.287 | 0.643 | 6 |
| | Effect of fluctuations | -0.187 | -0.413 | 0.040 | 0.107 | -17 |
| Relative gut length | Effect of salinity | -0.151 | -0.386 | 0.076 | 0.195 | -14 |
| | Effect of fluctuations | 0.139 | -0.080 | 0.363 | 0.222 | 15 |
| Immunity | Effect of salinity | -0.160 | -0.386 | 0.065 | 0.161 | -15 |
| | Effect of fluctuations | 0.042 | -0.175 | 0.255 | 0.696 | 4 |
| Relative gonopodium length | Effect of salinity | 0.101 | -0.136 | 0.337 | 0.409 | 11 |
| | Effect of fluctuations | 0.103 | -0.132 | 0.341 | 0.392 | 11 |
| Sperm count | Effect of salinity | 0.043 | -0.225 | 0.311 | 0.750 | 4 |
| | Effect of fluctuations | 0.151 | -0.093 | 0.392 | 0.223 | 16 |
| Sperm velocity | Effect of salinity | -0.155 | -0.389 | 0.077 | 0.194 | -14 |
| | Effect of fluctuations | 0.080 | -0.156 | 0.323 | 0.524 | 8 |

* The percentage difference for the ratio is shown using *[exp (lnCVR) – 1]*100*