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Living on the edge: gamete release and subsequent fertilization in Fucus vesiculosus are weakened by climate change forced hyposaline conditions

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Living on the edge: gamete release and subsequent fertilization in *Fucus vesiculosus* (Phaeophyceae) are weakened by climate change forced hyposaline conditions

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ABSTRACT: Populations at range margins of marine organisms are at the front line of climate-induced changes in abiotic factors and are thus particularly susceptible. Especially, macroalgae that are externally reproducing rely on optimal environmental conditions for gamete release and subsequent fertilization. However, the effect of climate change forced decrease in salinity on these critical life stages has been largely overlooked. We tested the impact of forecasted hyposaline conditions on a marginal population of the rockweed *Fucus vesiculosus* growing at 5.8 PSU on the Finnish coast of the Baltic Sea. We incubated individuals with receptacles for at least 7 days at 2.5, 4.1, 5.8, and 7.2 PSU and determined their gamete release and subsequent fertilization success. We further tested for sperm performance at 3.5, 5.0, and 6.1 PSU. Salinity of 2.5 PSU, that is predicted to occur in the region by the end of this century, reduced egg release. In contrast, sperm and antheridia release were not consistently affected by the different salinities, but the size of the sperm swell up at 3.5 PSU. Since fertilization success was drastically reduced at 2.5 and 4.1 PSU, we suggest that sperm performance was compromised such that sperm dysfunction hampered the fertilization success. Our results demonstrate that the forecasted hyposalinity negatively affects egg release and sexual reproduction in *F. vesiculosus* at its northern distributional limit. This macroalga can probably only withstand the future decrease in salinity when populations proliferate via asexual reproduction.

KEY WORDS: Climate change, Fertilization success, Gametes, Hyposalinity, Macroalgae, Marginal populations, Zygote

The establishment and persistence of benthic macroalgae depend upon the production and release of their propagules and subsequent fertilization success. Both processes are directly influenced by a delicate interplay of environmental factors, especially when fertilization occurs externally (broadcasting-type macroalgae) (Santelices 2002). Propagule release is triggered by light (Serrão *et al.* 1996; Pearson *et al.* 1998), and usually occurs under calm water conditions to prevent their dilution (Brawley *et al.* 1999; Andersson *et al.* 1994). Fertilization success depends on the female-to-male ratio (Serrão *et al.* 1999; Berndt *et al.* 2002), the age (Serrão *et al.* 1999), the synchronous release of viable propagules (Pearson & Serrão 2006), chemical signals (Maier & Müller 1986), sperm motility (Serrão *et al.* 1996) and an effective polyspermy block (Serrão *et al.* 1999). Variation in the fertilization success can occur due to changes of the listed factors during the reproductive season (Serrão *et al.* 1999; Berger *et al.* 2001; Steen 2004). Ongoing climate change is recognized to alter both the environmental conditions and cues that are essential for reproductive assurance in externally fertilizing species. Climate-driven changes in abiotic factors are known to hamper the formation of reproductive structures in marginal populations (Rothäusler *et al.* accepted) but little is known about the performance of the propagules themselves under forecasted climate change scenarios.

Macroalgal populations inhabiting the distributional edge are at the front line of climate related environmental change and are therefore particularly susceptible. This makes them important early warning indicators of climate change related shifts. Populations of *Fucus vesiculosus* Linnaeus inhabiting the Northern Baltic Sea are at their distributional edge. This area, with a salinity gradient from ~3 to 7 PSU, represents a fragile environment for the release of viable gametes, which may be a problem for sexual reproduction and thus for the maintenance of genetic variation (Tatarenkov *et al.* 2005). *F. vesiculosus* is a dioecious species and releases male and female gametes from their receptacles into the water column before they fuse to form a zygote. For instance, Serrão *et al.* (1996) showed that sperms of the same species kept at 6 PSU still swam and fertilized eggs at ~4 PSU, whereas a very low fertilization success was observed at 3 PSU (Serrão *et al.* 1996). This was caused by an impairment of sperm motility as well as the loss of flagella (Serrão *et al.* 1996). Given these clear results already under prevailing conditions, future climate change scenarios for the Baltic Sea that predict a decrease in salinity of ~2.5 PSU by the end of this century (Meier & Kauker 2003; Meier *et al.* 2012) are expected to have drastic effects on macroalgal life cycles. To date, the strongest reduction in salinity is predicted for the Northern Baltic Sea (i.e. Bothnian Bay and Gulf of Finland, ranging from 61 to 65°N). These regions are foreseen to be a hotspot for climate change (Meier *et al.* 2012), where profound salinity decreases may have far-reaching implications for the marine communities, including rocky littoral habitats. Hence there is an urgent need to learn how gamete release and fertilization success of an important foundation species will be affected in the new environment. Thus, we determined gamete release and fertilization success of this brown alga from its distributional edge at ~6 PSU and hypothesized that both critical life cycle processes are affected by a climate-driven change in salinity decrease.

A total of 40 algae with receptacles were sampled from the shallow intertidal on the Orhisaari Island (60°16’N, 21° 59’E) in July 2016 (*in situ* water temperature ~17.5°C, 5.8 PSU). Algae were immediately transported to the University of Turku laboratories, and maintained in an indoor recirculating aquarium system (16°C, 5.8 PSU) for one week before further preparations. Sex of each alga was determined from receptacles by a transversal cut with a razor blade through their many spherical conceptacles containing antheridia and/or oogonia (male and female gametangia, respectively) and examination under a microscope. Each sexed alga was thereafter split into 4 parts and each algal part (hereafter individual) consisted of up to 20 receptacles of different size and shape, which were cleaned from epiphytes if necessary.

To test if salinity affects gamete release and fertilization success, four 20 L aquaria were set up at ~16°C and used to realize a gradient in salinity treatments, namely high 7.2 PSU, current 5.8 PSU, low 4.1 PSU, and future 2.5 PSU. The future salinity level is based on a model by Meier *et al.* (2012). Receptacles of each algal individual were thus represented in the four different salinity treatments and incubated for one to two weeks. The PAR intensity at the water surface was 130 µmol photons * m^{-2} * s^{-1} with a light:dark rhythm of 14:10 h. The four different salinities were produced artificially by using distilled water and adding the respective sea salt concentrations. Each of the aquaria received a combination of 2 µg phosphate, 0.1 ml vitamins and 0.1 ml trace metals per liter (Guillard 1962, 1975), resembling the sea surface nutrient concentrations at the sampling site during summer (SYKE, Finnish Environment Institute). Once a week, water in the aquaria was replaced and thereby also the position of the algae and of the aquaria.

After the incubation, receptacles of the same individual treated in the four salinities were cut off, wrapped in moistened paper tissue, and stored in a refrigerator at 5°C. Herewith we simulated the natural calm conditions that are required for Baltic *F. vesiculosus* to release gametes (Serrão *et al.* 1996). After four days, in the late afternoon, receptacles were unpacked and transferred to 100 ml vials containing their respective salinity treated water to allow gamete release for one hour. This was done at 16°C under the same PAR intensity as described above. If gametes were released, we determined their concentration (gamete / ml) by counting them under a microscope via a Buerker chamber (depth: 0.1 mm; square width: 0.2 mm). Female gamete release consisted of eggs and oogonia (one oogonium contains eight haploid eggs) from N = 12 individuals per salinity. Male gamete release was composed of sperms and antheridia (one antheridium contains 64 flagellated haploid sperms) from N = 15 individuals per salinity. Receptacles with released gametes were then dried in an oven at

50°C to a constant weight for a minimum of two days in order to determine their dry weight.

Immediately after the gamete density was determined, male and female gametes released in the same salinity from N = 11 individuals were transferred into 100 ml vials to test for the fertilization success. The crossings were stored overnight at 16°C for a minimum of 9 and a maximum of 14 hours. To determine fertilization success, eggs were stained on a microscope slide with 10^{-4} % calcofluor white solution, which binds to the cell wall when zygotes are formed and can be detected via fluorescent microscopy (Serrão *et al.* 1999). Approximately 500 female gametes (including lysed and non-fertilized eggs) per crossing were counted to determine the fertilization success.

In an extra experiment, we measured the morphology of the sperm. Therefore, male receptacles of different *F. vesiculosus* individuals (N = 12) collected at the same sampling site were put separately in three different salinities (3.5, 5.0, and 6.1 PSU). Prior to gamete release, receptacles were treated as described above. After male receptacles had been kept for 5 min in their respective salinities in a 100 ml vial, a gamete sample was pipetted from the bottom of the vial and carefully dropped on a glass slide. From each sperm encountered under the microscope we measured its vertical projection area by using Computer Assisted Sperm Analysis, CASA (Integrated Semen Analysis System, ISAS v1: Proiser, Valencia, Spain) with B/W CCD camera (capture rate 60 frames/s) and negative phase contrast microscope (100 x magnification).

The differences in gamete release as well as in the subsequent fertilization success were determined by using generalized linear mixed models with a negative binomial distribution of residual variation and the four salinity treatments as a fixed factor (SAS software, SAS Institute Inc). Receptacle dry weight was used as a covariate for gamete release, while the total female gametes counted were used as a covariate for the fertilization success. When possible, we ran all models of male and female gametes with the algal individual as a random factor. The covariate alone and with all its interactions with the fixed factor was tested. We then simplified the model by removing non-significant effects, starting from the higher-order interactions, with the aid of the Akaike Information Criterion (AIC) (Littell *et al*. 2006).

The release of male gametes (sperm and antheridia) did not vary among the different salinities tested (Figs. 1, 2); neither did the receptacle dry weight influence the male gamete release (Table S1). The egg release was significantly reduced in future salinity conditions at 2.5 PSU compared to 5.8 and 7.2 PSU (Table S2, Fig. 3). The oogonia release was not dependent on salinity and receptacle dry weight (Fig. 4). Concurrent with the egg release, the subsequent fertilization success showed lowest zygote formation in the 2.5 PSU and the 4.1 PSU treatments (Table S3). Highest fertilization success was found in the ambient (5.8 PSU) and high (7.2 PSU) salinities (Fig. 5).

The lowest salinity of 3.5 PSU provoked that the sperm significantly swelled up in size ($F_{(2, 22.6)} = 6.58$, $P < 0.006$) (Fig. 6), a phenomenon that did not differ among individuals tested ($X^2 = 0.06$, $P = 0.41$). Smallest sperm size was detected for the control salinity (6.1 PSU), which significantly increased from the 5.0 to the 3.5 PSU treatments. The change in sperm morphology in hyposaline conditions most probably implies osmotic problems and is likely to indicate a negative effect on fertilization ability of sperm at low salinities.

Predicted future salinity negatively affected sexual reproduction of *F. vesiculosus* from a marginal population of the Finnish Archipelago Sea. We showed that two mechanisms were involved as both the egg release and the subsequent fertilization success of the released eggs were hampered at the lowest salinities tested. Connecting the drop in egg release to about one forth in the lowest salinities with only about one tenth of these eggs being fertilized at 2.5 PSU, the total decrease in fertilization success can be immense. Furthermore, the latter drop may be related to a reduced sperm performance as sperm swell up at 3.5 PSU, implying malfunctioning. Thus, future salinities of 2.5 PSU as well as naturally prevailing low salinities of ~3.5 PSU have the potential to hamper the sexual reproduction of *F. vesiculosus* in the Northern Baltic Sea. This is likely to be accompanied by a loss of genetic diversity due to a trend towards increased asexual reproduction (Tatarenkov *et al.* 2005; Ardehed *et al.* 2016).

Our findings are consistent with the observations by Serrão *et al.* (1999). They found that egg release was impeded in Baltic *F. vesiculosus* from the Swedish coast at ≤ 4 PSU, and the fertilization success was shown to be close to 0% below 4 PSU and between 70% to 100% in their natural seawater (6 to 7 PSU). Similarly, Steen (2004) found that Norwegian *Sargassum muticum* extruded oogonia from receptacles at salinities from 10 to 30 PSU, but fertilization was only seen at 20 to 30 PSU. Herein, we demonstrated for the first time negative effects of extreme hyposaline conditions, namely of 2.5 PSU, on egg release and subsequent fertilization. These extreme hyposaline conditions are predicted to occur in the Northern Baltic Sea by the end of this century, yet its effect on the life cycle of *F. vesiculosus* has been largely overlooked.

The release of the male gametes (sperms and antheridia) was not dependent on the tested salinities and sperms were motile during the counting. It seems likely that these sperms lost their fertilization ability because hyposaline conditions of 3.5 PSU caused a substantial increase in sperm size, similar as reported by Wright & Reed (1990). Hence, hyposaline conditions affected sperm velocity and linearity such as observed by Serrão *et al.* (1996) at 3 to 4 PSU. Probably, salinities of 3.5 PSU provoked a higher solute concentration within the sperm cells, so that water molecules could passively move along the osmotic gradient through the cell membranes. This resulted in an expansion of the sperm size, which may impede egg penetration and thus successful fertilization. Indeed, Serrão *et al.* (1996) showed evidence that only one out of three tested males released sperms at 3 PSU that could actually swim. These restricted motilities of sperms may also explain the low fertilization success at 2.5 and

4.1 PSU.

In summary, hyposalinity of 2.5 PSU compromised egg release as well as the subsequent fertilization success in one population of *F. vesiculosus* situated at its northern distributional limit in the Baltic Sea. Assuming that these results are generalizable to field conditions, future salinity has the potential to impact the sexual reproduction by reducing egg release as well as by sperm dysfunction (Wright & Reed 1990; Serrão *et al.* 1996, and this study). If life cycle processes of *F. vesiculosus* are affected, a low fertilization success will cause a decline in populations along the shores of the species range margin. In fact, growth and survival (Rugiu *et al.* 2018) as well as receptacle formation (Rothäusler *et al.* accepted) were compromised under hyposaline (2.5 PSU) and warming conditions (~16°C) in three range marginal populations of *F. vesiculosus* from the Baltic Sea. Nevertheless, our results highlight that the only chance for *F. vesiculosus* to persist within the northern and eastern margins of the Baltic Sea by the end of this century is likely to be via asexual reproduction.

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Figures

Figures 1-4. Density of released gametes (gametes/ml) of *F. vesiculosus* in the four salinity treatments (2.5, 4.1, 5.8, and 7.2 PSU) for sperms (1), antheridia (2), eggs (3) and oogonia (4). Data are means ± SE of N = 15 male thalli with receptacles and N = 12 female thalli with receptacles. Different letters above bars indicate significant differences.

Figure 5. Fertilization success (number of counted zygotes) of *F. vesiculosus* in the four salinity treatments (2.5, 4.1, 5.8, and 7.2 PSU). Data are means ± SE of N = 11 crossings. Different letters above bars indicate significant differences.

Figure 6. Area of vertical projection of *F. vesiculosus* sperms (μm^2) after their release in three different salinities (3.5, 5.0, and 6.1 PSU). Data are means ± SE of N = 12 male thalli with receptacles. Different letters above bars indicate significant differences.

Figure 1-4

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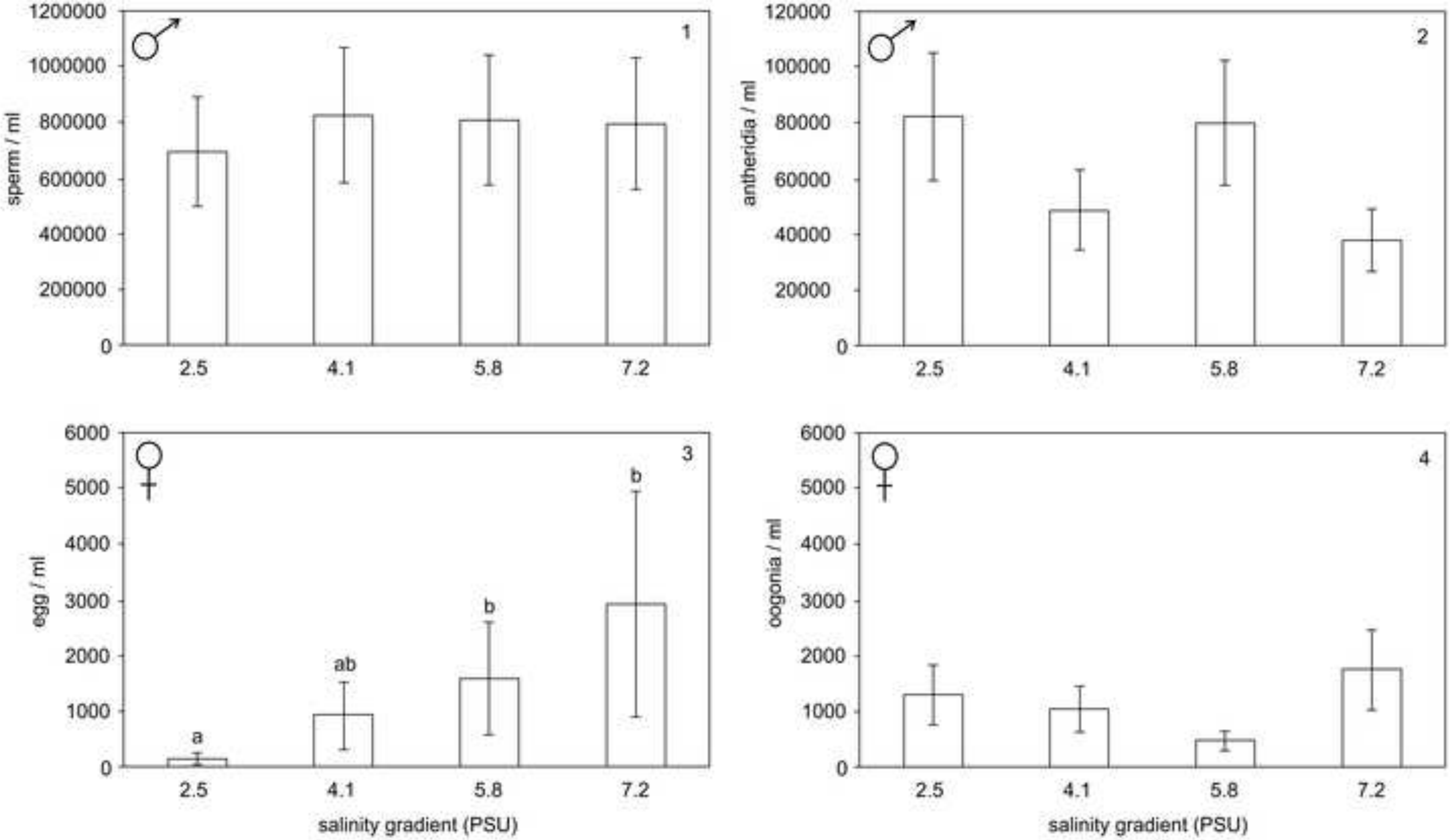

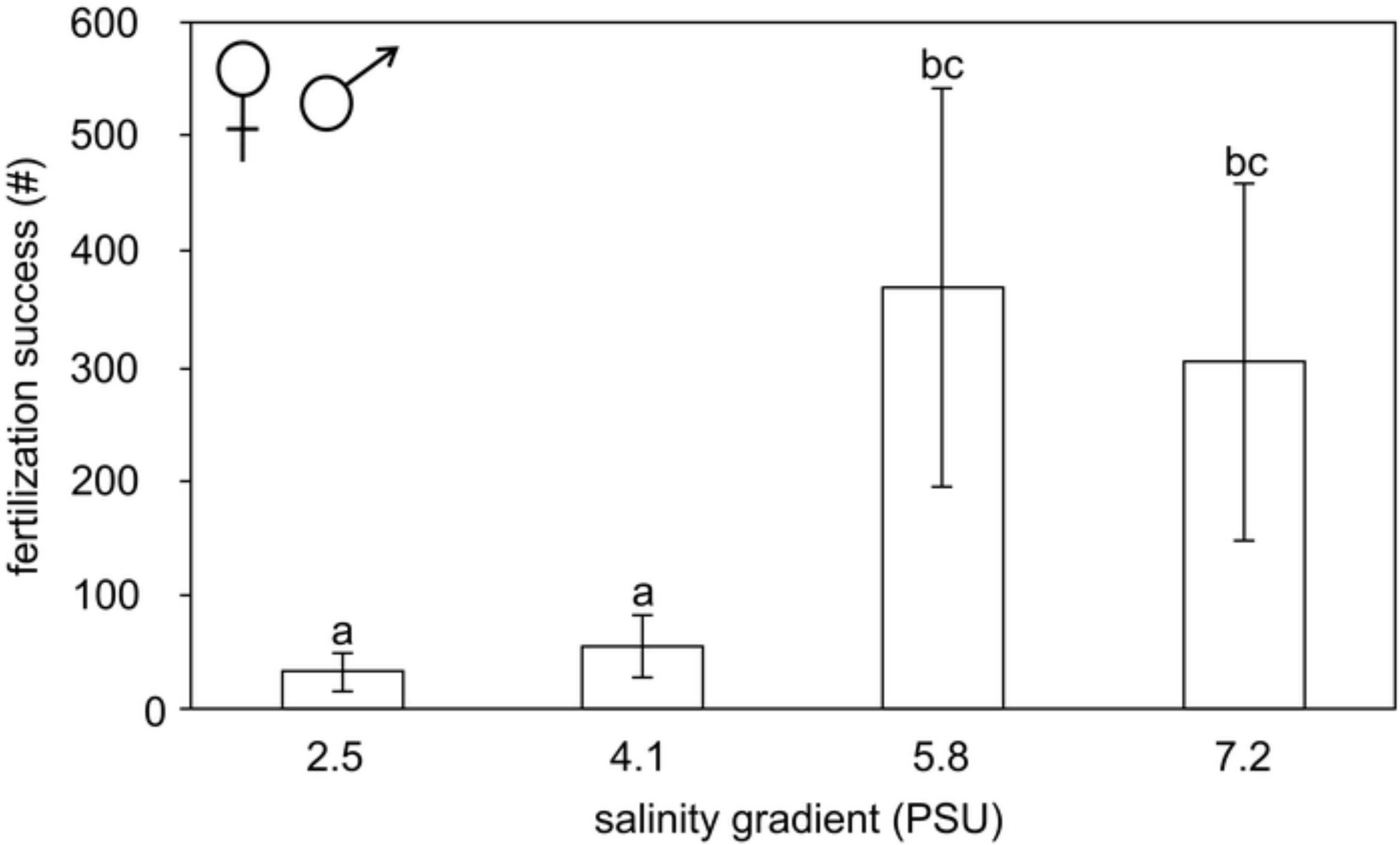
600
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200
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fertilization success (#)
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2.5
4.1
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7.2
salinity gradient (PSU)

Figure 6

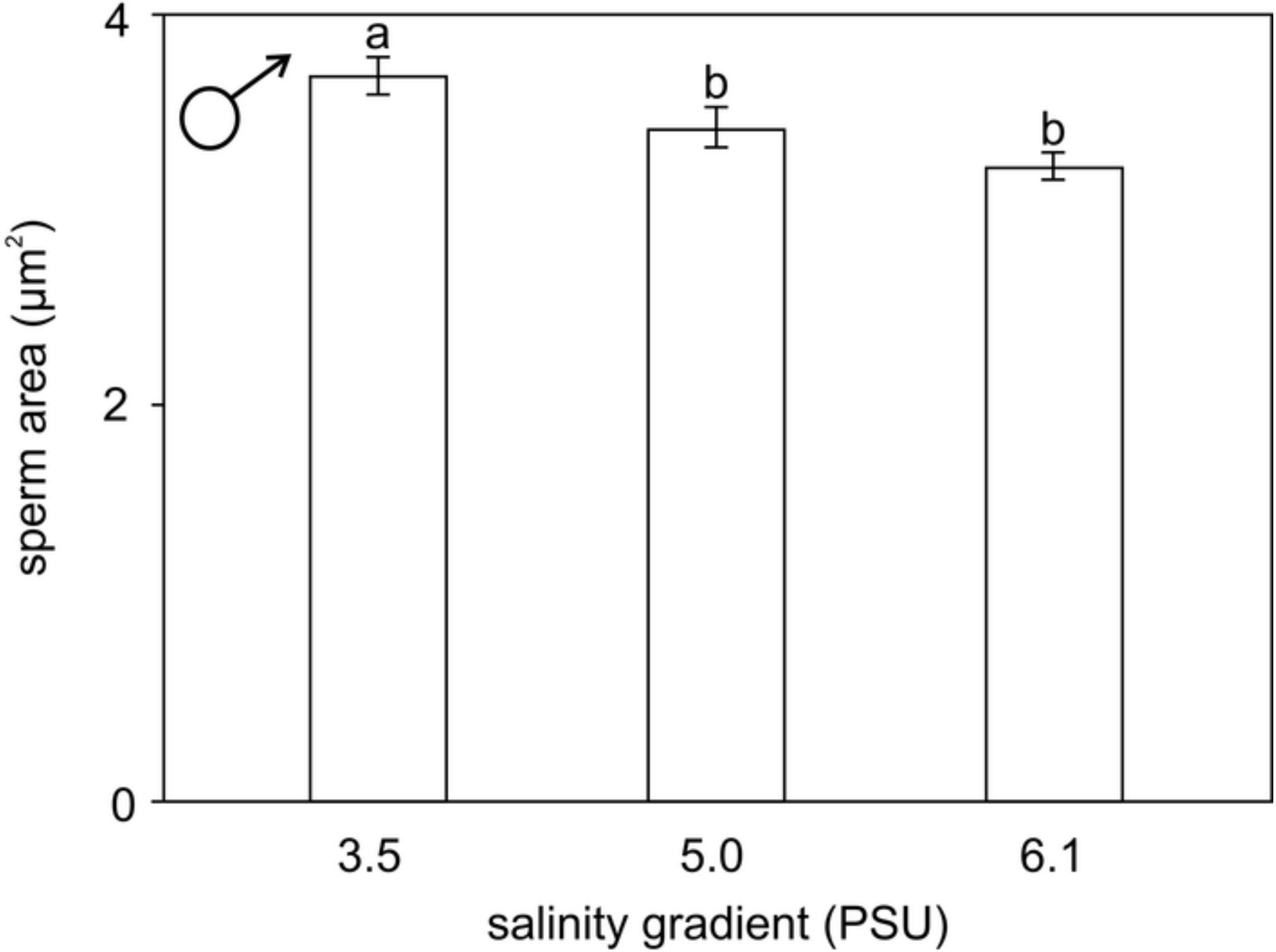

Table S1-S3

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Supplemental Material

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