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Letter to the Editor

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Male sexual signaling and expected effects of hatchery-induced sperm competition vary with water depth at which whitefish are caught

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Salmonids like whitefish (*Coregonus* spp.) are often propagated in supportive breeding. Spawners are caught from their spawning locations, their gametes mixed, and the resulting offspring reared in a protected environment before being released into the wild. This procedure can affect sexual selection, for example, by enhancing the importance of sperm competition or by reducing the relevance of sexual signals. While it is often unclear how sperm competitiveness is affected by a male's overall genetic quality, there is accumulating evidence that sexual signals reveal good genes and that mate choice based on such signals can increase offspring viability (Auld et al. 2019). Therefore, supportive breeding may affect the genetic variance and the mean genetic quality of next generations. We sampled whitefish from various locations along a depth gradient to test how male characteristics that are likely to affect sexual selection under natural conditions correlate with characteristics that affect hatchery-induced sperm competition. Whitefish are external fertilizers, and multi-male spawning and hence sperm competition is common under natural conditions. Mate choice is not sufficiently understood but could be based on breeding tubercles. These are small conical structures that grow on scales before the breeding season and fall off shortly afterwards. The size of breeding tubercles varies much among males and has repeatedly been found to correlate positively with offspring viability (Wedekind et al. 2001; Kekäläinen et al. 2010). Male–male dominance is typically dependent on body size (Auld et al. 2019) and could also be relevant in whitefish. Body size itself can reflect individual inbreeding coefficients (Su et al. 1996) and be an indicator of heritable genetic quality in small or structured populations (Neff and Pitcher 2008). In another fish with a somewhat comparable mating system, the size of breeding tubercles and male size was not correlated but could both be used to predict male reproductive success under close to natural conditions (Jacob et al. 2009). We study whitefish from Lake Hallwil (Switzerland). This lake has suffered so much from anthropogenic eutrophication that it is being artificially aerated since 1985. Three hatcheries around the lake are likely to have played a key role in maintaining the whitefish population, as concluded also from a recent mark–recapture experiment (Vonlanthen 2015). However, eutrophication combined with possible hybridization in hatcheries can have led to a speciation reversal (Vonlanthen et al. 2012) and may thereby have destroyed any genetic structure linked to water depth. Hatchery protocols now focus on maintaining overall genetic variance by pooling milt of many males before adding the mix to eggs of multiple females. Milt volume varies among sires, for example, because males often loose milt when being pulled up from deep locations (Figure 1), an effect that likely depends on how much the swim bladder is inflated by the change in pressure. This variance in milt volume is likely to affect the genetic variance that, in combination with the average genetic quality, may then affect the long-term survival of a population. The extent to which hatchery protocols affect genetic quality can be estimated by the correlations between male quality indicators and traits that affect hatchery-induced sperm competition, that is, sperm number, velocity, and longevity (summarized here as "milt potency," see also Supplementary Material). Many breeding protocols are likely to promote genetic quality if male attractiveness or dominance are positively correlated to milt potency. If there are no such correlations or negative ones because of life-history trade-offs, hatchery-induced sperm competition is likely to reduce the average genetic quality in future generations. We sampled fish from various depths and determined their age, size, breeding ornamentation, and milt potency (see methods in the Supplementary Material) to test whether and how different male characteristics affect reproductive success in supportive breeding in a heavily managed population.

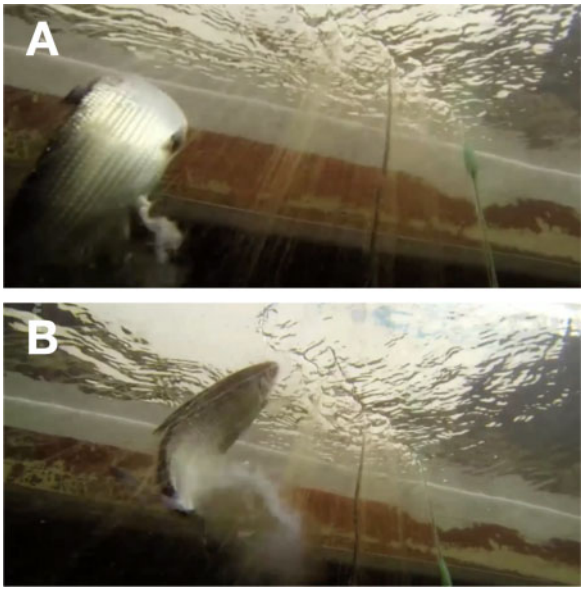

Figure 1. Male being pulled up from deep water and loosing milt just before being lifted into the boat. Photograph **A** also reveals the breeding tubercles on the male's skin. Photograph **B** was taken about half a second after photograph A (©arte tv; reproduced with permission).

The samples taken at 25, 12, and 4 m did not differ in age (Fisher's exact test, $N_1 = 54$, $N_2 = 30$, $N_3 = 14$, $P = 0.58$) nor in sex ratios ($P = 0.28$). The males caught at different depths did also not differ in size (Supplementary Figure S1A). However, the mean size of their breeding tubercles varied with depth (Supplementary Table S1 and Supplementary Figure S1B). The tubercles were significantly larger in males caught at 25 m depth than at 12 m depth (*post hoc* Tukey's HSD test, $P = 0.03$), while no such differences were observed in the other possible comparisons (P always > 0.12). Overall, larger males had larger breeding tubercles (Supplementary Table S1), but this relationship varied with location and was strongest in males caught at the deepest location (Supplementary Table S1 and Supplementary Figure S1C). Sperm velocity did not vary with water depth (measured at 12 versus 4 m for sperm characteristics), increased with body length (but not with age), and was not significantly revealed by the size of the breeding tubercles (Figure 2A–C and Supplementary Tables S2A and S3A). Mean sperm concentration did also not seem to vary with water depth, body length, or breeding tubercles (Figure 2D–G and Supplementary Tables S2 and S3B). Milt potency was significantly reduced in males caught at larger depth (Figure 2G and Supplementary Table S3B) and was overall not significantly linked to body length and breeding tubercles. However, there was a significant interaction between the effects of depth and breeding tubercles on milt potency (Figure 2H–J and Supplementary Table S2B).

The protocols of supportive breeding at Lake Hallwil and most (if not all) other Swiss lakes promote sperm competition (Wedekind et al. 2007). We studied what kind of males would be expected to profit from such hatchery-induced sperm competition. Our samples had similar size and age distributions. Nevertheless, fish caught at the deepest location had the largest breeding tubercles. It remains to be shown whether this difference in breeding ornamentation is due to genetics, environmental factors, or a combination of both. Intralacustrine diversification may have largely vanished during the eutrophication crisis (Vonlanthen et al. 2012) but could now be rebuilding itself so that phenotypic differences reflect genetic differences. Alternatively, if breeding tubercles are generally valid indicators of good genetic quality, fish caught in deeper locations could be on average of better genetic quality than fish caught in more shallow regions. However, the link between body length and breeding ornaments changed also with water depth, suggesting that either the information content of breeding tubercles does not depend on genetic quality alone, or that variance in genetic quality has location-specific effects on growth. We found that larger males had faster sperm, and that sperm velocity could not be predicted by breeding tubercles or water depth. There was no link between male size and milt potency, but we found an interaction between breeding tubercles and depth on milt potency. These observations suggest that there were no trade-offs between male dominance or mate attractiveness and investment into milt. On the contrary, males that are predicted to be successful under natural conditions, because they are large and/or well ornamented, seemed still able to invest more into high-quality sperm than small or less ornamented males, that is, there seemed to be positive correlations between the various fitness-relevant traits (Reznick et al. 2000). It remains to be tested whether such correlations are to be expected in non-managed populations or can be a consequence of hatchery-induced selection in previous generations. Our measure of milt potency was based on sperm number, velocity, and lifespan, but fertilization success can also be affected by the composition of the fertilization media (pH, ovarian fluids, etc.) and possibly further factors that may influence sperm motility. Based on our measurements, we conclude that the current protocol used in supportive breeding (mixing the milt of many males, then adding the mix to the eggs) may give large males and males caught from more shallow regions a reproductive advantage. Alternative breeding protocols would then affect hatchery-induced sperm competition differently: (i) When equalizing milt volume, hatchery-induced sperm competition would be driven by sperm velocity and sperm concentration. (ii) When equalizing cell counts, that is, taking sperm concentration into account, male reproductive success would be largely determined by sperm velocity. (iii) When equalizing milt potency, no type of male would be favored. However, minimizing the effects of milt potency, for example, in full-factorial crossings, would only minimize the loss of genetic variation over time. Genetic quality would not be promoted by such a breeding design. If breeding tubercles and body size are indeed indicators of heritable genetic quality, genetic quality could be promoted in hatcheries by giving large males and/or males with large breeding tubercles a reproductive advantage over small and/or poorly ornamented males. However, with declining effective population sizes (N_e), minimizing the loss of genetic variance become increasingly important. Small N_e therefore require an optimization between minimizing the loss of genetic variance and minimizing the loss of genetic quality in order to maximize a population's long-term survival probability. In conclusion, the consequences of hatchery-induced sperm competition are different for whitefish males caught at different water depths and at different body sizes. If variance in milt potency is ignored in the breeding protocols (as currently in the study population), males caught from more shallow locations are favored over males caught from deeper locations. Breeding protocols that would be based on equalized milt volume or even equalized sperm counts per male would give males with high sperm velocity a reproductive advantage. In our study population, these would be the large males. Any hatchery-induced variance in male reproductive success could be avoided, for example, in full-factorial breeding designs that minimize mean kinship within the next generation. However, minimizing the loss of genetic variance can reduce average genetic quality, because elevating the reproductive success of large and/or well

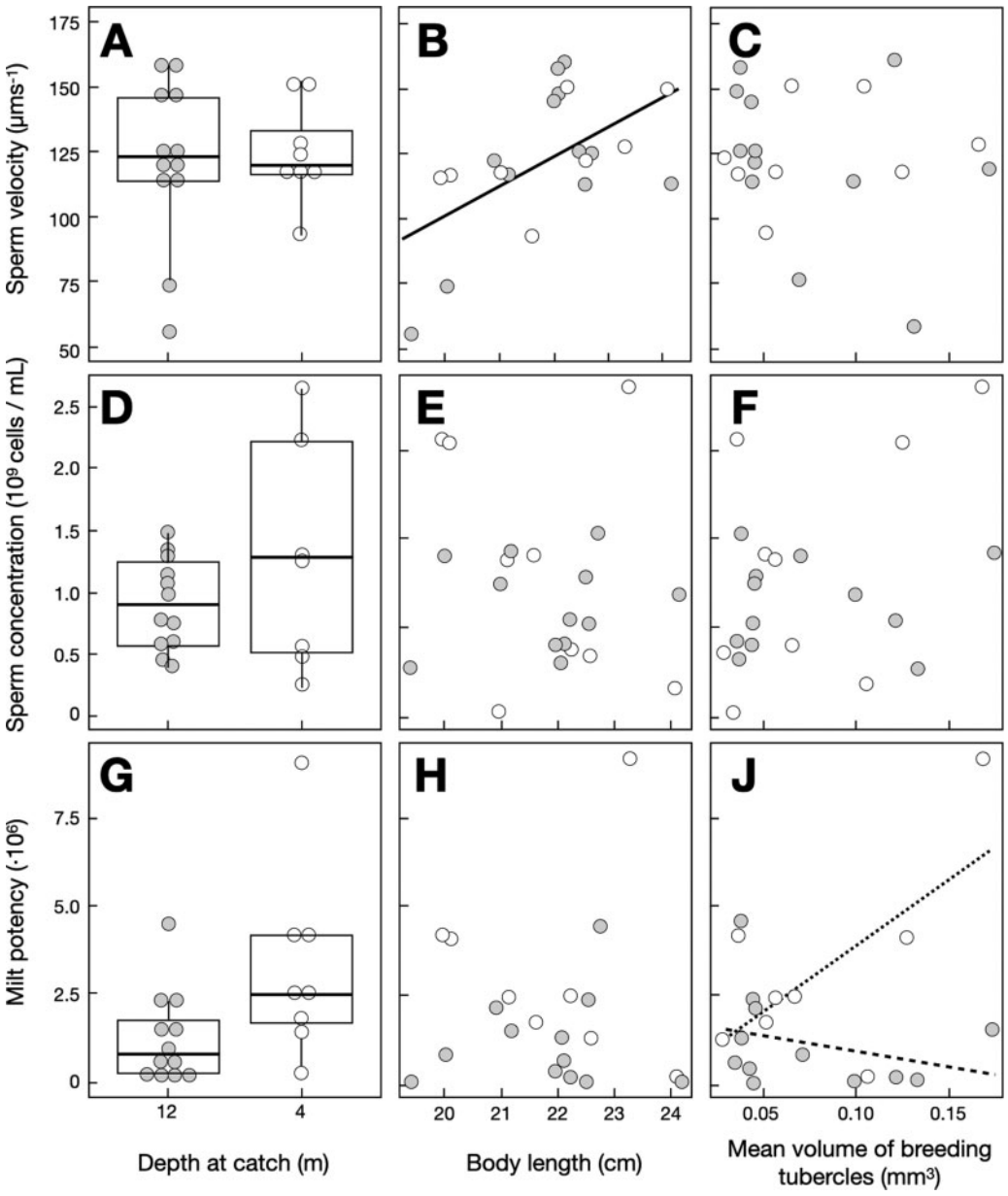

Figure 2. Sperm velocity (**A**–**C**), sperm concentration (**D**–**F**), and milt potency (**G**–**J**) versus water depth, body length, and mean breeding tubercles volume for fish caught at 12 m (gray symbols, dashed regression line) and fish caught at 4 m (open symbols, dotted line). Panels **A**, **D**, and **G** show Tukey boxplots with quartiles and whiskers. The regression line in panel **B** shows the significant relationship between body length and sperm velocity at both depths. The regression lines in panel **J** illustrate the significant interaction between depth and mean breeding tubercles volume on milt potency. No significant links could be observed in panels **C**, **E**, **F**, and **H**. See text for statistics.

ornamented males can positively affect the mean viability of the next generation.

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Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

Conflict of Interest

The authors declare no conflicts of interest.

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Supplementary Material

Male sexual signalling and expected effects of hatchery-induced sperm competition vary with water depth at which whitefish are caught

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Methods

Adult whitefish (*Coregonus suidteri*, n=104) were caught from Lake Hallwil (Switzerland; 47.2772° N, 8.2173° E) during their breeding season in January 2020 using gill nets set at 40, 25, 12, or 4 m depth along a line perpendicular to the bank (at decreasing distance to the shore, following the natural depth gradient). The nets were separated by distances of about 230, 50, and 40 m, respectively. At each depth, gill nets of 25, 27, 30, and 35 mm mesh size were used to ensure that adult whitefish of all size could be caught. Fish were immediately killed and kept in ice water until further processing. Standard body length (i.e. excluding the caudal fin) was determined, and scales were sampled above the lateral line from the caudal half of the body to later estimate fish age based on the number of annuli, a method offering same age estimates as methods based on otoliths or fin ray for fish younger than 5 years (Muir et al. 2008). Milt was collected by applying gentle bilateral abdominal pressure (see below). Fish were then scanned with a 3D optical scanner to determine mean breeding tubercles volume (see below).

For age estimation, scales were first cleaned using a cloth and 60% ethanol and then rinsed in water. Three scales by fish were mounted onto microscope slides and photographed under a 1.6x magnification. Fish were then aged based on the number of annuli, except for 1 female caught at 25 m depth for which we had no readable scales.

The milt of 22 males caught at 12 m and 4 m depths was stripped into large Petri dishes (145 × 20 mm, Greiner Bio-one, Frickenhausen, Germany; the discrepancy between this sample size and total males sample size (n=71) was due to organizational problems in the field). Care was taken to strip the milt drop by drop and to separate theses drop on the Petri dish, so that all drops of milt that were non-contaminated with urine or faeces (i.e. usually all except the last drop) could be collected and individually pooled, 20 µL was stored at a dilution ratio of 1:9 in Storfish (IMV Technologies, l'Aigle, France), an isotonic inactivating medium, and kept on ice. The remaining milt was centrifugated to collect seminal plasma. Because the weight of the seminal plasma turned out to be not correlated to the sperm concentration ($r = 0.18$, d.f. = 18, $p = 0.46$), it was used to calculate the "milt potency", here defined as

$$\text{milt potency} = \text{sperm concentration} \times \text{weight of seminal plasma} \times \text{average sperm velocity} \times \text{maximum sperm longevity} \quad (1)$$

The milt stored in Storfish were transported to the laboratory where sperm velocity

and concentration were analysed within 48 h with CASA using the Qualisperm software (AKYmed AG, Cheseaux-sur-Lausanne, Switzerland) as in Nusbaumer et al. (2019), except that milt traits could mostly be summarised as a mean over 4 trials rather than over 2 trials. Briefly, 20 µL of each sample were activated in standardized water (OECD, 1992) at a 1:500 dilution ratio and measured at 20x magnification under phase contrast, at 6.5°C, 20s post-activation. Sperm longevity was measured as the time by which no more sperm motion could be observed. Our final sample size consisted of 20 males because the within sample measurement repeatability was too low in 2 samples (i.e. the standard error of sperm velocity and/or concentration exceeded 50% of the mean measurement; in the accepted samples, the standard errors of sperm velocity and concentration were on average 9 and 18 % of the means, respectively). The software also provided estimates for sperm motilities, i.e. percentage of activated sperm, but these estimates were not considered here because they showed low repeatability and included several unexplained outliers, i.e. motilities of < 30% while values >80% are usually expected (Sarosiek et al. 2016; Kowalski and Cejko 2019).

The average size of breeding tubercles per fish was determined with a 3D optical scanner (VR-5000, Keyence, Itasca, IL, USA). Each fish was first carefully dried before applying a thin layer of baby powder (Millette Baby powder, Migros, Zurich, Switzerland) on the skin to make the otherwise transparent breeding tubercles detectable by the optical scanner. The fish was then placed on a slightly angled plate so that its lateral line was approximately perpendicular to the laser beams in order to facilitate correction of the fish curvature in later analysis. Scans were then analysed using Keyence analysis software 3.1.0.56, with *curvature correction strength* set at 15, *reference plane* set as *continuous*, and measurements extracted using the *conplane method* to obtain the volume of individual breeding tubercles. All scans were carefully checked for anything that could potentially be wrongly identified as a breeding tubercle by the software.

Only 2 males and 4 females could be caught at 40 m depth. This depth category was therefore excluded from all statistical analyses. Also, because age classes varied from 1+ to 4+, but 89.5% of all fish where either 2+ or 3+, the fish were categorized as “young” (1+ or 2+, n=51) and “older” (3+ or 4+, n=44). Fisher’s exact test were then used for frequency analyses. An ANOVA was used to test for differences in body length between the catches. Because no differences in body length was found among the catches, a Gaussian generalised multiple regression analysis (GLM) was then used on the mean breeding tubercles volume as response variable to test the effects of depth at catch (specified as factor), body length, and the interaction of these two potential predictors (after graphically verifying that the model assumptions were not significantly violated). Analogous models were used to test whether depth at catch, body length, mean breeding tubercles volume, or any interaction between these factors would explain variance in mean sperm velocity and milt potency. The AIC of full models was compared to the AIC of models lacking a variable or an interaction and models with the lowest AIC were retained as final models. Wilcoxon rank sum tests were used on milt potency and components of milt potency to test whether they would vary between samples. The same method was used to test whether milt potency or components would vary between age categories. Analyses were done in RStudio 4.0.2 (R Development Core Team 2015) and JMP14.0.0 (SAS Institute Inc., Cary, NC).

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Table S1. Gaussian GLM on the effects of body length, depth at catch, and the interaction between the predictors on mean breeding tubercles volume. The full model had a lowest AIC, i.e. no factor or interaction was dropped during model selection. The tables give the estimated regression parameters, standard errors (SE), t-values, and p-values (N = 68).

Effect tested	Estimate	SE	*t*	*p*
Intercept	-0.48	0.1	- 4.3	< 0.001
Body length	0.28	0.3	1.0	< 0.001
Depth at catch = 12 m	0.03	0.0	5.3	< 0.001
Depth at catch = 4 m	0.52	0.1	3.6	< 0.001
Body length: depth at catch = 12 m	-0.03	0.0	-3.8	< 0.001
Body length: depth at catch = 4 m	-0.02	0.0	-1.2	0.32

Table S2. Gaussian GLM on the effects of depth at catch, mean breeding tubercles (BT) volume, body length, and potential interactions between the predictors on (a) sperm velocity and (b) milt potency. Model selection was based on AICs. The tables give the estimated regression parameters, standard errors (SE), t-values, and p-values (N = 20). The analogous model on sperm concentration could not be calculated because of significant differences of within-group variances (Bartlett $F_1 = 8.0$, $p < 0.01$). However, a graphical inspection of the data did not suggest significant effects of depth, body length, or breeding tubercles.

Effect tested	Estimate	SE	*t*	*p*
(a) Sperm velocity				
Intercept	-126.6	86.3	-1.5	0.16
Body length	11.4	3.9	2.9	0.01
(b) Milt potency				
Intercept	$1.8 \cdot 10^6$	$0.9 \cdot 10^6$	1.9	0.07
Depth at catch	$-1.5 \cdot 10^6$	$1.6 \cdot 10^6$	-1.0	0.34
BT mean volume	$-8.7 \cdot 10^6$	$11.1 \cdot 10^6$	-0.8	0.45
Depth at catch : BT mean volume	$45.2 \cdot 10^6$	$17.4 \cdot 10^6$	2.6	0.02

Table S3. Wilcoxon rank sum tests of difference in milt potency (in bold) and components of milt potency for (a) the two age groups, and (b) for fish caught at 12 m or 4 m depths.

Variable tested	W	*p*
(a) Fish age		
Milt potency	**31**	**0.61**
Sperm cells concentration	20	0.14
Mean sperm velocity	42	0.74
Weight of seminal plasma	40	0.87
Mean sperm longevity	35	0.83
(b) Depth		
Milt potency	**76**	**0.03**
Sperm cells concentration	59	0.43
Mean sperm velocity	48	1
Weight of seminal plasma	69	0.11
Mean sperm longevity	61	0.35

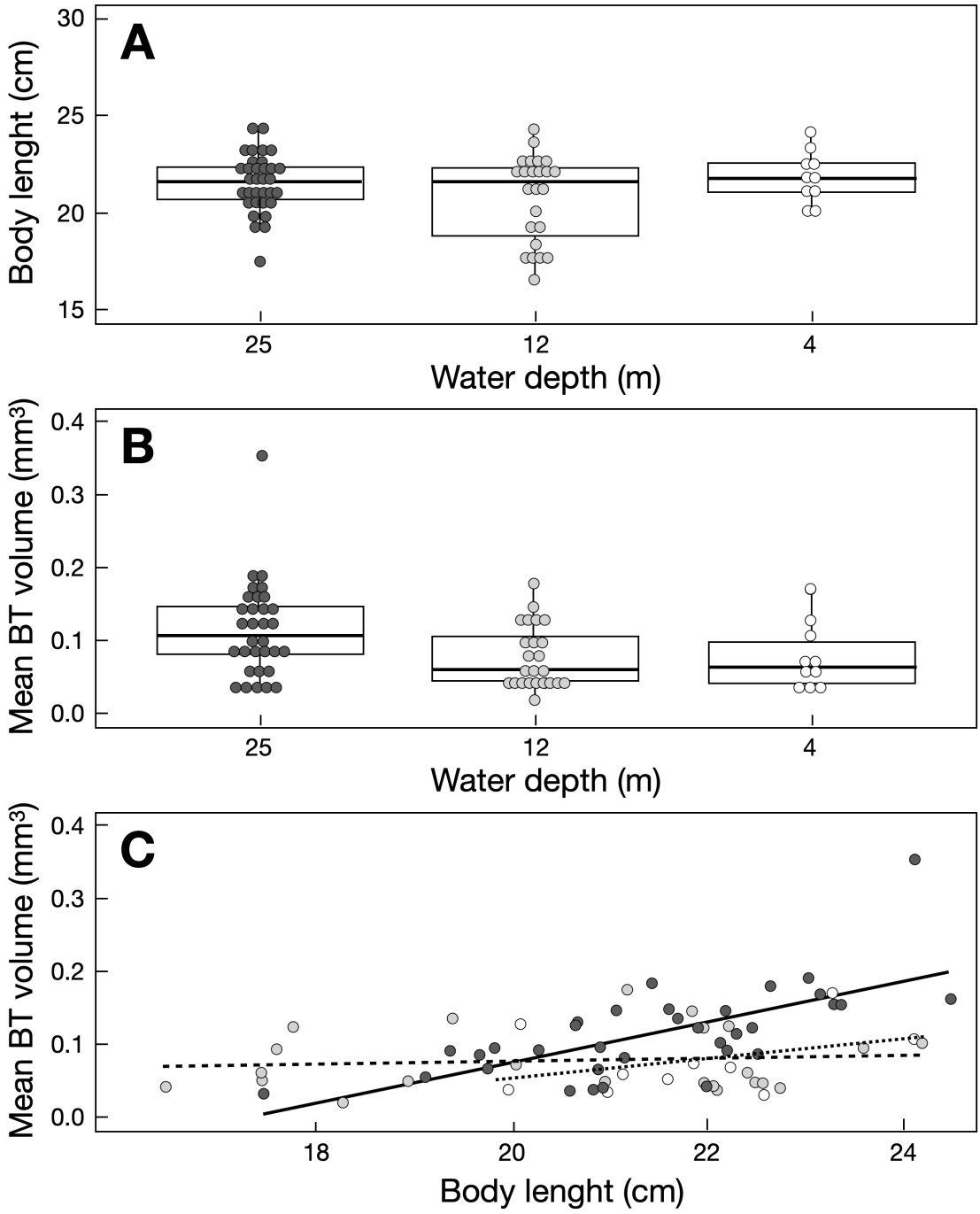

Figure S1. Phenotypes of males caught at different depths. (A) Body length (Tukey boxplots with quartiles and whiskers; ANOVA, F = 1.8, d.f. = 2, p = 0.17), (B) mean breeding tubercles volume, and (C) relation between body length and breeding tubercles for fish caught at 25 m (dark grey symbols, solid regression line), at 12 m (grey symbols, dashed regression line) and at 4 m (open symbols, dotted line). BT: breeding tubercles. See Table S1 for statistics.