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Size-dependent reproductive success of wild zebrafish *Danio rerio* in the laboratory

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Size-dependent reproductive success of wild zebrafish *Danio rerio* was studied under controlled conditions in the laboratory to further understand the influence of spawner body size on reproductive output and egg and larval traits. Three different spawner size categories attained by size-selective harvesting of the F_1-offspring of wild *D. rerio* were established and their reproductive performance compared during a 5 day period. As to be expected, large females spawned more frequently and had significantly greater clutch sizes than small females. Contrary to expectations, small females produced larger eggs when measured as egg diameter with similar amounts of yolk compared to eggs spawned by large spawners. Eggs from small fish, however, suffered from higher egg mortality than the eggs of large individuals. Embryos from small-sized spawners also hatched later than offspring from eggs laid by large females. Larval standard length (L_S)-at-hatch did not differ between the size categories, but the offspring of the large fish had significantly larger area-at-hatch and greater yolk-sac volume indicating better condition. Offspring growth rates were generally similar between offspring from all size categories, but they were significantly higher for offspring spawned by small females in terms of L_S between days 60 and 90 post-fertilization. Despite temporarily higher growth rates among the small fish offspring, the smaller energy reserves at hatching translated into lower condition later in ontogeny. It appeared that the influence of spawner body size on egg and larval traits was relatively pronounced early in development and seemed to remain in terms of condition, but not in growth, after the onset of exogenous feeding. Further studies are needed to explore the mechanisms behind the differences in offspring quality between large- and small-sized spawners by disentangling size-dependent maternal and paternal effects on reproductive variables in *D. rerio*.

Key words: egg size; offspring growth; parental effects; reproductive output; size-at-hatch.

INTRODUCTION

Body size is a fundamental trait affecting many aspects of fish performance, including individual reproductive output (Peters, 1983; Marshall *et al.*, 1998; Marteinsdottir & Begg, 2002; Persson & de Roos, 2007; Sogard *et al.*, 2008). For example, in many

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fish species, an allometric relationship between female body size and fecundity exists resulting in fecundity scaling linearly with body mass (Wootton, 1998). Additionally, in some species male body size and condition are important during competition for access to females during spawning (Fleming & Gross, 1994; Quinn & Foote, 1994). Small fishes may also face energetic constraints and low condition, which may result in delay, or even skipping, of reproduction (Rideout *et al.*, 2005) to increase future reproductive output and fitness by growing to a larger size (Houston & McNamara, 1999; Jørgensen & Fiksen, 2006). Alternatively, to compensate for reduced fecundity, small individuals may invest in gamete quality by increasing the size of gametes (Smith & Fretwell, 1974; Hendry *et al.*, 2001) or the amount of resources allocated to gametes (Murry *et al.*, 2008). These compensatory investments into gamete quality, however, have received little attention as much research has concentrated on studying positively size-dependent parental effects (*i.e.* parents' non-genetic contribution to offspring's phenotype; Bernardo, 1996) on reproductive variables. This literature usually reports that large spawners produce higher quality eggs and larvae than small spawners, which in turn can be more resistant to cope with various environmental challenges (Blaxter, 1986; Chambers & Leggett, 1996; Berkeley *et al.*, 2004; Gregersen *et al.*, 2009). These parental effects have been found to be pronounced early in development and to decay rapidly after the larval phase (Bernardo, 1996; Heath & Blouw, 1998). Limited field studies, however, are available on the long-term parental effects on offspring quality and performance (Heath & Blouw, 1998), presumably due to the difficulties in tracking individual reproductive success in field settings.

To improve basic understanding of the effects of spawner body size on reproductive traits such as egg production, egg traits and offspring characteristics, experimental studies with small-bodied model species conducted under controlled conditions are advisable. One of the most popular model species in developmental biology is the freshwater cyprinid zebrafish *Danio rerio* (Hamilton). Despite its small size and capability to produce eggs frequently, this species has only occasionally been used to investigate size-dependent reproductive variables (Skinner, 2004; Spence & Smith, 2006). Moreover, available studies concerning *D. rerio* egg production and reproductive success have used domesticated strains well habituated to laboratory conditions, and limited knowledge is available on the size-dependent reproductive success of wild *D. rerio*.

Danio rerio is a small-bodied species native to India, Bangladesh and Nepal (Spence *et al.*, 2008). Natural spawning is seasonal and reproduction may be cued by food availability (Spence *et al.*, 2006) and quality (Meinelt *et al.*, 1999). As a batch-spawner, *D. rerio* females spawn relatively large eggs in small clutches over a short period at dawn (Spence *et al.*, 2007). Domesticated strains spawn continuously at intervals of 1 to 6 days and clutch size is known to correlate positively with both female body size and inter-spawning interval (Spence & Smith, 2006). There is also some evidence that female reproductive success may be correlated with male size as females prefer (Pyron, 2003) and strategically allocate their eggs towards larger males if exposed to both small- and large-sized males in sequence (Skinner & Watt, 2007). Other studies, however, have shown that the female reproductive success in *D. rerio* does not correlate with male body size (Spence & Smith, 2006) but with other, yet unresolved, male characteristics, and olfactory cues or genetic compatibility may play a decisive role (Gerlach & Lysiak, 2006).

In addition to mate preferences, sex ratio and population density may have an effect on *D. rerio* mating behaviour and mating success (Spence & Smith, 2005). High density and male- or female-biased sex ratio may lead to increased levels of aggressive interactions, which can have a negative effect on the total number of eggs spawned per female (Pritchard, 2001; Spence & Smith, 2005; Paull *et al.*, 2008). Social systems in *D. rerio* are characterized by the establishment of dominance hierarchies, and dominant individuals generally behave aggressively towards the subordinate ones governing access to food resources (Pritchard, 2001). In addition to reproductive success, this may translate into differences in body size and condition between dominant and subordinate individuals, but these relationships are speculative and have not been studied so far.

The objective of the present study was to compare the reproductive performance of differently sized wild *D. rerio* under controlled laboratory conditions. Three different size categories were established by selecting spawners from large laboratory-held wild *D. rerio* populations, and these size-matched spawner populations were compared in terms of their reproductive success, egg and larval traits and offspring growth. It was hypothesized that 1) large fish would perform better in terms of egg output than small fish, 2) large fish would produce larger and better conditioned egg and larvae than small fish and 3) the offspring of the large fish would develop earlier and grow faster in consecutive months than offspring from small fish due to provisioning effects associated with parental body size on egg and larval quality.

MATERIALS AND METHODS

FISH AND HOLDING CONDITIONS

The fish used in this experiment were first-generation offspring from a wild *D. rerio* population captured from a river system 70 km west of Coochbihar (West Bengal, India, 22°56′ N; 87°67′ E) and reared under laboratory conditions for 6 months. Fish were raised in six round, grey tanks (material: glass fibre–polyester, diameter: 79 cm, height: 135 cm, volume: 320 l) in a light (14L:10D) and temperature-controlled (mean ± S.D. 25·3 ± 0·2° C) recirculation facility with an inflow rate of 0·25 l s^{-1}. The recirculation system was run with insipid tap water, and the water quality was controlled weekly for pH (mean ± S.D. 8·4 ± 0·1), nitrite (N-NO_2^-; <0·3 mg l^{-1}) and ammonium (N-NH_4^+; <0·05 mg l^{-1}) and daily for oxygen levels (mean ± S.D. 8·3 ± 0·3 mg l^{-1}). The stocking density per holding tank was 0·9 ± 0·2 individuals l^{-1} (mean ± S.D.). All fish were reared in identical densities to control for density effects and to minimize the potential bias of dominance hierarchies on feeding success. Fish were fed *ad libitum* five times a day with freshly hatched *Artemia* sp. nauplii (Inve Aquaculture NV; www.inve.com) and a commercial flake food (TetraMin, Tetra GmbH; www.tetra.net; 47·0% protein, 10·0% fat). Multiple feedings of small amounts of food allow fishes to better utilize the food and also spread the food more equally among individuals reducing the size heterogeneity within a tank (Steffens, 1985). Due to the similar stocking densities and feeding rates among the tanks, the potential of social hierarchies differentially influencing the stocks in the six tanks was minimized.

Danio rerio start reaching maturation at *c.* 90 days post-fertilization (dpf; Schilling, 2002), and once all experimental fish had certainly matured at 180 dpf, spawners were selected based on their body length. Fish were caught using a dip-net, and standard length (L_S) was measured to the nearest mm and wet mass (M_W) to the nearest 0·1 g. In addition to L_S and M_W, the Fulton's condition factor (K; Ricker, 1975) was estimated as a proxy for the nutritional state of the three size-matched treatment groups. In two of the tanks, 25% of the largest fish were retained. Fish from these two tanks were pooled into a spawning stock constituting large males and females (L_S range, 28–35 mm; $n = 191$). In two other tanks, 25% of the smallest fish were retained. Fish selected from these two tanks formed a spawning stock with small-sized spawners (L_S range, 15–24 mm; $n = 162$). Two additional tanks served as controls and were randomly harvested for fish body size. These fish were used as a random-sized spawner treatment (L_S range, 18–35 mm; $n = 210$). The mean ± S.D. K values between large- ($0{\cdot}50 \pm 0{\cdot}08$), random- ($0{\cdot}52 \pm 0{\cdot}09$) and small-sized spawners ($0{\cdot}52 \pm 0{\cdot}18$) did not differ significantly (nested ANOVA, d.f. = 5 and 539, $P > 0{\cdot}05$).

The reproductive performance of the three size categories (large, random and small) was compared under a controlled laboratory environment that exposed all individuals to similar conditions and densities in terms of number of fish per volume of water, water quality, illumination and type of tank. Before the experiment, males and females from each of the three size categories were kept together within their respective size treatment for 14 days in six (fish selected from one tank per aquarium) 126 l glass aquaria (length: 60 cm, width: 60 cm, height: 35 cm). By the time the experiment was initiated, spawners (*i.e.* spawning females and males) were transferred into a standalone spawning facility (Aquarien-Bau Schwarz; www.aquaschwarz.com; mean ± S.D. temperature: $26{\cdot}1 \pm 0{\cdot}1^\circ$ C; mean ± S.D. pH $8{\cdot}4 \pm 0{\cdot}1$; N-NO_2^-; $<0{\cdot}3$ mg l^{-1}; N-NH_4^+; $<0{\cdot}05$ mg l^{-1}; mean ± S.D. oxygen level $8{\cdot}3 \pm 0{\cdot}4$ mg l^{-1}) in spawning boxes specifically designed to prevent egg cannibalism by separating adults from eggs. This was achieved by inserting a grid with a mesh-size of 2 × 2 mm inside each spawning box. Each spawning box was equipped with green plastic filter material serving as a spawning substratum. Fish were allowed to spawn in two differently sized boxes: spawning boxes of 5 l volume (length: 29 cm, width: 12 cm, height: 10 cm) were stocked with two females and four males and 3 l boxes (length: 21 cm, width: 11 cm, height: 13 cm) were stocked with one female and two males. Preliminary trials testing the effect of box size (with twenty-two 5 l and twelve 3 l boxes) on reproductive metrics revealed no significant effects of box type on reproductive variables (spawning frequency: two-way ANOVA, d.f. = 1 and 32, $P > 0{\cdot}05$; clutch size: repeated-measures ANOVA, d.f. = 1 and 28, $P > 0{\cdot}05$; fertilization rate: mixed-model GLM, d.f. = 1 and 54, $P > 0{\cdot}05$), thus the data from differently sized boxes were pooled to compare the reproductive performance of the three size categories. All female-related data were expressed on a per-female basis by dividing the spawning frequency and the daily egg number with the number of females per box following Paull *et al.* (2008). Due to limited number of boxes for spawning trials and slightly different number of spawners in each size category (due to unequal sex ratios), the final number of boxes used varied among the large ($n = 34$), small ($n = 54$) and random size categories ($n = 25$). Each box of each size category was treated as a replicate. The reproductive output of the fish from the three different size categories was assessed for five consecutive days (29 November to 3 December 2007). Domesticated zebrafish are known to spawn every 1 to 6 days (Spence & Smith, 2006), thus a 5 day spawning period was expected to allow each fish to spawn at least once. Five days was expected to be sufficient time to establish the spawning pattern because *D. rerio* have been shown to spawn for 20 consecutive days without significant changes in egg output as a function of increasing study time (Paull *et al.*, 2008).

REPRODUCTIVE PERFORMANCE

Reproductive output

Danio rerio spawn within the first few hours after sunrise (Hisaoka & Firlit, 1962), and all trait measurements took place between 0800 and 1200 hours to ensure complete spawning and assessment of egg production. To estimate reproductive performance, daily spawning frequency (estimated through identification of eggs spawned) and clutch sizes (*i.e.* the number of eggs spawned per female per spawning event) were assessed for every box over the 5 day spawning period. Accordingly, each morning the spawning boxes were cleaned, the occurrence of a spawning event (*i.e.* spawning frequency) assessed and the total number of eggs counted. For assessing the fertilization rate per spawning event, fertilized eggs were separated from unfertilized eggs. The eggs are translucent and fertilized eggs can be identified by the presence of a multi-cellular blastodisc, which is not present in unfertilized eggs (Kimmel *et al.*, 1995).

Egg traits

Egg trait measurements for the three size classes included the assessment of egg mortality rate, egg diameter and egg yolk diameter. To estimate post-fertilization egg mortality rate in each of the five spawning days, a pooled sample of 50 fertilized eggs from each of the size categories were placed in five Petri dishes, but only for spawning events with at least $n = 50$ eggs. Petri dishes were transferred into a rearing incubator (Tintometer GmbH; www.tintometer.de) at 27° C. Embryos are known to develop faster in slightly higher temperatures than the 26° C spawning temperature (Westerfield, 1994) and because *D. rerio* embryos are not particularly sensitive to small differences in temperature (Westerfield, 1994), eggs were incubated in a temperature of one degree higher than the spawning temperature. The egg mortality rate for each size category was estimated by counting the number of dead eggs 48 h post-fertilization from each of the Petri dishes. The egg size was measured both as egg diameter and as egg yolk diameter from randomly selected and pooled eggs within size categories. Yolk is the main energy component of an egg, thus egg yolk diameter is relevant in indicating the nutrient resources supplied to an embryo (Kamler, 2005). The eggs were photographed and the diameter was measured from these photographs under a profile projector with an accuracy of 0·0001 mm (Quick Scope; AT112-220F; www.mitutoyo.co.jp). The egg diameter measurements were conducted for three spawning days (the first, second and the fifth spawning day). Yolk diameter was measured from eggs at the gastrulation stage. Because not all the eggs from every spawning day were in this developmental stage by the time they were photographed, measuring the same eggs for egg diameter and egg yolk diameter was not possible. Thus, the egg yolk diameter measurements included eggs from three different days.

Larval traits

Larval traits for the three size categories were assessed as larval hatching time, larval length-at-hatch, larval area and larval yolk-sac volume. Larval trait measurements were treated as pooled samples for the three size categories. Larvae hatched in the rearing incubator in several Petri dishes. The L_S of a random sample of newly hatched larvae from each size category (large: $n = 63$, random: $n = 72$, small: $n = 45$) was measured under the dissection scope, and the hatching time was recorded. The same aged larvae (4 dpf) from three spawning days were used to compare the larval L_S-at-hatch between the size categories. Measurements based on larval length, however, may not be a reliable size-related morphological indicator due to variability in body shape (Kamler, 2008). Therefore, individual larvae were photographed to allow the subsequent measurements of larval area and yolk-sac volume as a more precise indicator of the dimension of the larvae (Heyer *et al*., 2001; Probst *et al*., 2006). Larval area and height and width of the yolk sac were measured from photographs using the digitizing software Image Tool for Windows (version 3.0; http://ddsdx.uthscsa.edu/dig/itdesc.html). The yolk-sac volume (V) was then estimated using the following formula (Chambers *et al*., 1989): $V = \pi(6LH^2)^{-1}$, where L represents the length (horizontal measurement; mm) and H the height (vertical measurement; mm) of the yolk sac. The yolk-sac volume and larval area-at-hatch were estimated over 3 days.

Offspring growth

Two days after hatching, all larvae were transferred into 5 l plastic boxes in the standalone unit and external feeding was initiated. Larvae from each size category were reared separately in replicate boxes in which density varied from 20 to 30 individuals l^{-1}. Larval densities in the boxes were kept well below recommended levels (60 individuals l^{-1}; Brand *et al*., 2002) to avoid too high densities and stressing the larvae by transferring them into larger rearing boxes after they would have reached larger body sizes surpassing the limits of biomass per box. Fish were fed eight times per day with larval dry food (Sera micron Staubfutter; 50·2% protein, 8·1% fat; www.sera.de) and hatched *Artemia* sp. nauplii. The water inflow rate to the larval boxes was 3 ml s^{-1}, and the photoperiod was 14L:10D. At age 30 days, $n = 10$ fish from 10 rearing boxes per size class were photographed and measured for L_S (mm) and M_W (g). Thereafter, the density was standardized and the fish were further reared in equal densities (two individuals l^{-1}) in 10 rearing boxes per size category. Body size measurements for a sub-sample of 50 fish per size category were repeated at ages 60 and 90 dpf, and to determine growth rates, growth of individual offspring was related to the mean L_S and M_W of the previous time period. In addition to L_S and M_W, the K of the offspring was estimated at 90 dpf.

STATISTICAL ANALYSIS

The data were checked for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene's test). In cases of significant ($P < 0{\cdot}05$) non-normality and heteroscedasticity, data were ln-transformed before parametric analysis and appropriate *post hoc* tests were used that accounted for the nature of the between-sample variance. Logistic regression was used to estimate the probability of spawning with size category as a categorical variable and spawning day as covariate. Repeated-measures ANOVA were used to estimate the effect of the spawner body size on clutch size. Size category was treated as fixed effect and spawning day as a repeated variable. Due to unequal sample sizes, a mixed-model GLM was used to compare egg numbers from successful spawnings and fertilization rates between size classes. Size category was treated as fixed effect and spawning day as repeated variable. The coefficient of variation (C.V.) was calculated in daily egg production and egg size, and one-way ANOVA was used to compare the C.V. of these traits between the size categories. Egg mortality rate, egg size, hatching time and larval traits were analysed using repeated-measures ANOVA, with size category as fixed effect and spawning day as repeated variable. Additionally, the Petri dishes where the larvae hatched were treated as a random effect in repeated-measures ANOVA of hatching time, larval length-at-hatch, larval area-at-hatch and yolk-sac volume, with egg size as a covariate. To analyse differences in offspring body size and K at age 90 dpf, a two-way ANOVA was used with size category treated as fixed effect and rearing box as a random effect. To estimate growth rate in two rearing periods (30–60 and 60–90 dpf), a mean value per rearing box was calculated, and the growth rates were compared between the size categories using one-way ANOVA. All analyses were followed by Sidak's multiple comparison *post hoc* tests or alternatively Dunnett-T3 if variances were still heterogeneous after transformation. In the results, mean values are presented with standard errors (S.E.). All data were considered statistically significant at $P < 0{\cdot}05$. All statistical analyses were performed with SPSS for Windows (Release 14.0.1, SPSS Inc.; www.spss.com).

RESULTS

REPRODUCTIVE OUTPUT

Large- and random-sized fish established a fairly regular spawning activity during the 5 day experiment, while small fish spawned more irregularly. Accordingly, the spawning frequency was more variable among small spawners (C.V. = 33·7%) compared to large (19·5%) and random (21·3%) fish. Over the 5 day spawning period, spawning occurred in 82·4 and 76·0% of all large and random fish spawning trials, respectively, but only in 37·0% of the spawning trials of the small fish. On average, each large (1·0 ± 0·1) and random female (1·1 ± 0·2) spawned at least once per 5 days, whereas each small female spawned only 0·7 ± 0·2 times per 5 days. Thus, the inter-spawning interval was on average 5 days for large and random sized and 7 days for small females. Logistic regression analysis revealed a significantly higher likelihood to spawn for large (logistic binary regression, $\beta = 4{\cdot}643$, d.f. = 1, $P < 0{\cdot}001$) and random-sized females ($\beta = 3{\cdot}595$, d.f. = 1, $P < 0{\cdot}001$) compared to small females.

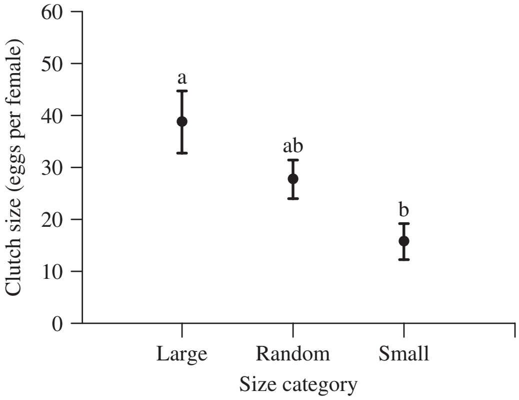

FIG. 1. The effect of *Danio rerio* body size on mean ± S.E. clutch size (repeated-measures ANOVA, d.f. = 2 and 110, $P < 0{\cdot}01$). Large fish: $n = 34$, random-sized fish: $n = 25$ and small fish: $n = 54$. Different lowercase letters indicate significant differences.

The number of eggs produced by wild *D. rerio* correlated significantly with the spawner's body size (repeated-measures ANOVA, d.f. = 2 and 110, $P < 0{\cdot}01$). The mean ± S.D. clutch size (normalized on a per-female basis) per day was higher among large fish (38·7 ± 6·0 eggs per day) compared to small (15·7 ± 3·5 eggs per day), while random-sized fish spawned intermediate clutch sizes (30·0 ± 3·7 eggs per day; Fig. 1). The mean clutch size per day did not vary significantly between the spawning days (d.f. = 4 and 107, $P > 0{\cdot}05$), and there was no size group × spawning day interaction (d.f. = 8 and 214, $P > 0{\cdot}05$). When comparing the number of eggs released in single successful spawnings instead of the average daily clutch sizes, small females (111·6 ± 18·2) produced the same number of eggs per successful spawning as large females (104·5 ± 12·3), and the random-sized females produced slightly fewer eggs (64·6 ± 5·5). These differences were not statistically significant (mixed-model GLM, d.f. = 2 and 69, $P > 0{\cdot}05$). The variance in daily egg production was significantly higher among small (C.V. = 238·8%) compared to random-sized fish (151·5%; one-way ANOVA, d.f. = 2 and 110, $P < 0{\cdot}01$), but there was no significant difference between small and large fish (189·5%; d.f. = 2 and 110, $P > 0{\cdot}05$).

EGG TRAITS

The fertilization rate was unrelated to body size (mixed-model GLM, d.f. = 2 and 69, $P > 0{\cdot}05$). It was slightly, but not significantly, higher among large- (52·3 ± 3·1%; $n = 60$) and random-sized fish (51·5 ± 3·4%; $n = 52$) compared to small fish (42·2 ± 4·0%; $n = 36$). Small females (0·899 ± 0·002 mm) produced significantly larger eggs than random-sized (0·879 ± 0·002 mm) and large females (0·862 ± 0·003) when egg size was measured as egg diameter [Fig. 2(a)]. The trend remained but the difference was no longer significant when egg size was measured as egg yolk diameter: small (0·525 ± 0·002 mm), random-sized (0·523 ± 0·001 mm) and large fish (0·521 ± 0·001 mm) produced eggs with similar yolk sizes [Fig. 2(b)]. The egg size varied significantly between the spawning days in terms of both

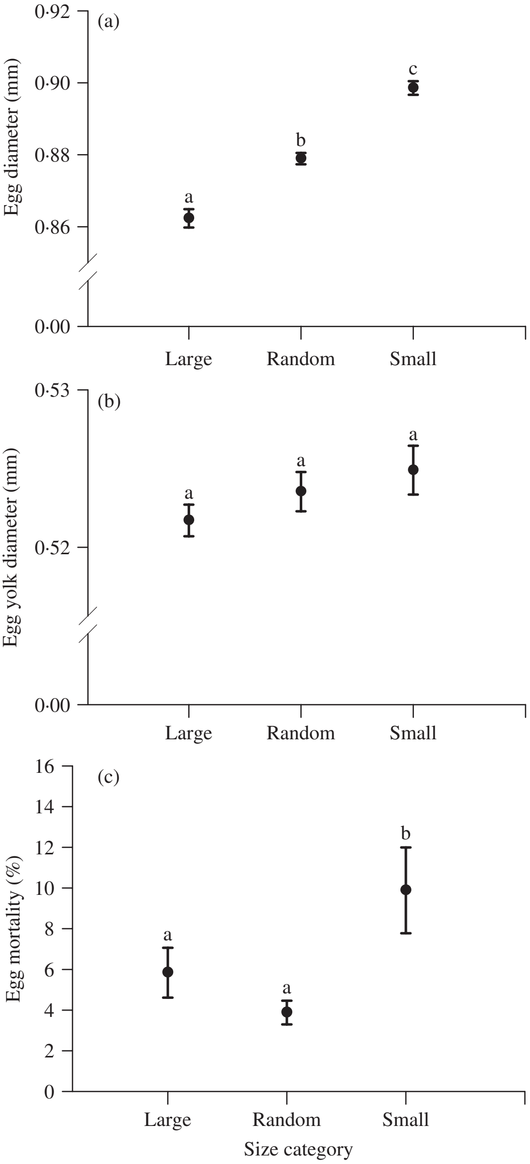

FIG. 2. The effect of *Danio rerio* body size on mean ± S.E. (a) egg diameter (repeated-measures ANOVA, d.f. = 2 and 227, $P < 0{\cdot}001$; large fish: $n = 48$, random-sized fish: $n = 93$ and small fish: $n = 89$), (b) egg yolk diameter (d.f. = 2 and 171, $P > 0{\cdot}05$; large fish: $n = 64$, random-sized fish: $n = 61$ and small fish: $n = 49$) and (c) egg mortality (d.f. = 2 and 24, $P < 0{\cdot}05$; large fish: $n = 10$, random-sized fish: $n = 10$ and small fish: $n = 7$). Different lowercase letters indicate significant differences.

egg diameter (repeated-measures ANOVA, d.f. = 2 and 226, $P < 0{\cdot}01$) and egg yolk diameter (d.f. = 2 and 170, $P < 0{\cdot}001$). There was also a significant interaction between size category and spawning day in egg diameter (d.f. = 4 and 452, $P < 0{\cdot}001$) [Fig. 3(a)] but not in egg yolk size (d.f. = 4 and 340, $P > 0{\cdot}05$). In neither of the egg size analyses were the C.V. between size classes significantly different.

Eggs from small fish exhibited significantly higher mortality (9·9 ± 2·1%) than eggs spawned by large (5·8 ± 1·2%) and random-sized fish (3·9 ± 0·6%) [Fig. 2(c)]. Spawning day had a significant effect on egg mortality rate (repeated-measures ANOVA, d.f. = 4 and 21, $P < 0{\cdot}001$) and also the interaction between size category and spawning day was significant (d.f. = 8 and 42, $P < 0{\cdot}05$) [Fig. 3(b)].

LARVAL TRAITS

Larvae from large (4·9 ± 10·1 days) and random fish (5·2 ± 0·1 days) hatched significantly earlier than larvae from small fish (5·6 ± 0·1 days; Fig. 4). L_S-at-hatch did not differ significantly between large (3·10 ± 0·03 mm) and small fish (3·04 ± 0·03 mm), while random-sized fish produced significantly larger larvae (3·19 ± 0·03 mm; repeated-measures ANOVA, d.f. = 2 and 55, $P < 0{\cdot}01$). Spawning day did not have a significant effect on larval L_S (d.f. = 2 and 55, $P > 0{\cdot}05$) neither did egg size (d.f. = 1 and 57, $P > 0{\cdot}05$). The interaction between size category and spawning day, however, was significant in terms of larval L_S-at-hatch (d.f. = 4 and 110, $P < 0{\cdot}001$) [Fig. 3(c)]. The larvae of small fish had significantly lower area-at-hatch (0·95 ± 0·02 mm^2) than the offspring of random-sized (1·04 ± 0·01 mm^2) and large fish (1·01 ± 0·01 mm^2) [Fig. 5(a)]. The spawning day had a significant effect on larval area-at-hatch when a potential Petri dish effect was controlled (repeated-measures ANOVA, d.f. = 2 and 46, $P < 0{\cdot}05$), but no interaction between size category and spawning day was found (d.f. = 2 and 92, $P > 0{\cdot}05$). Egg size did not have a significant effect on larval area-at-hatch either (d.f. = 1 and 47, $P > 0{\cdot}05$). The yolk-sac volume of the newly hatched larvae was significantly higher among the offspring of large (0·025 ± 0·001 mm^3) than random-sized (0·023 ± 0·001 mm^3) and small fish (0·019 ± 0·001 mm^3) [Fig. 5(b)]. The sampling day had a significant effect on yolk-sac volume (repeated-measures ANOVA, d.f. = 2 and 53, $P < 0{\cdot}01$) but no interaction between the size category and the sampling day was found (repeated-measures ANOVA, d.f. = 6 and 106, $P > 0{\cdot}05$). Egg size was not found to have an effect on larval yolk-sac volume (d.f. = 1 and 54, $P > 0{\cdot}05$). The effect of the Petri dish was not significant in any of these analyses.

OFFSPRING GROWTH

In general, small differences in offspring growth rate between size categories were observed, but the offspring of small fish exhibited significantly higher growth rate in terms of L_S during 60–90 dpf than random-sized or large fish offspring (Table I). Moreover, random-sized fish offspring grew significantly less in terms of M_W than those of large and small fish during the rearing period of 30–60 dpf, but this difference was no longer evident in the rearing period of 60–90 dpf (Table I). At age 90 dpf, offspring of small fish were significantly larger in terms of L_S than the offspring of large and random-sized fish (Table I). Differences in M_W between small, large and random fish offspring, however, were not significant (Table I). At age 90 dpf, the offspring of small fish had significantly lower K than the offspring of random-sized or large fish (Table I and Fig. 6).

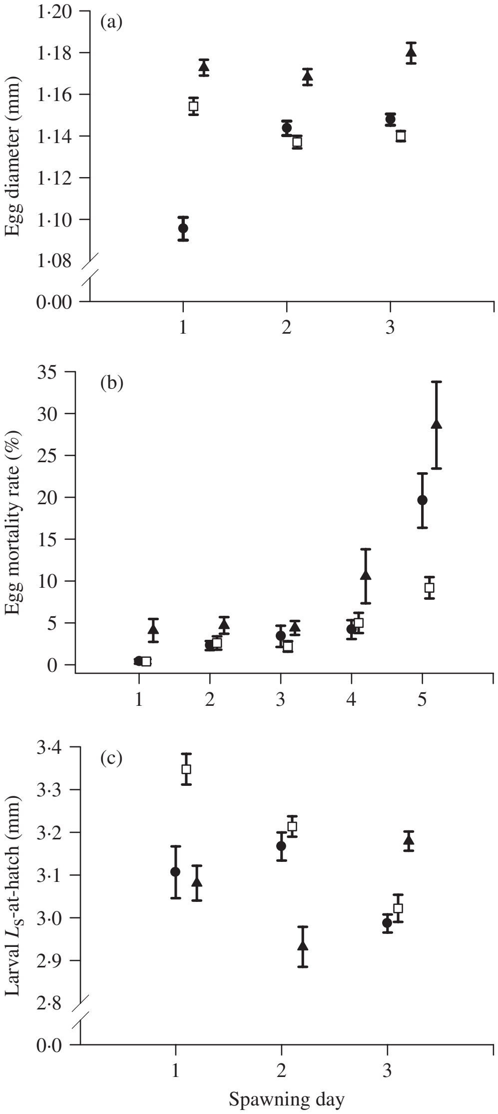

FIG. 3. The effect of spawning day of *Danio rerio* and body size [large (●), random (☐) and small (▲)] on mean ± S.E. (a) egg diameter (repeated-measures ANOVA, d.f. = 2 and 226, $P < 0{\cdot}001$; large fish: $n = 48$, random-sized fish: $n = 93$ and small fish: $n = 89$), (b) egg mortality rate (d.f. = 2 and 21, $P < 0{\cdot}05$; large fish: $n = 21$, random-sized fish: $n = 24$ and small fish: $n = 15$) and (c) larval standard length (L_S)-at-hatch (d.f. = 2 and 110, $P < 0{\cdot}05$; large fish: $n = 63$, random-sized fish: $n = 72$ and small fish: $n = 45$).

DISCUSSION

The present study revealed an unambiguous effect of spawner body size on reproductive success in wild *D. rerio* held in the laboratory. Results supported the first hypothesis according to which large females were expected to spawn more

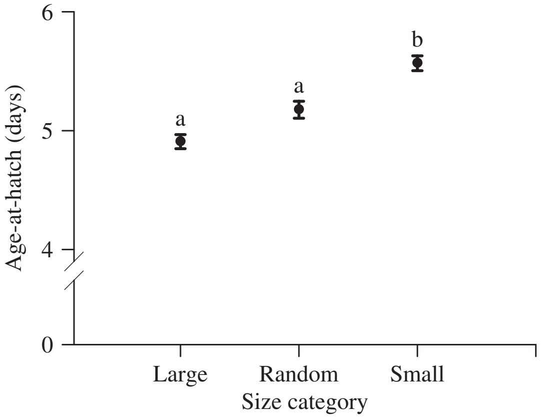

FIG. 4. The effect of *Danio rerio* body size on mean ± S.E. larval age-at-hatch (repeated-measures ANOVA, d.f. = 2 and 238, $P < 0{\cdot}01$). Large fish: $n = 72$, random-sized fish: $n = 55$ and small fish: $n = 114$. Different lowercase letters indicate significant differences.

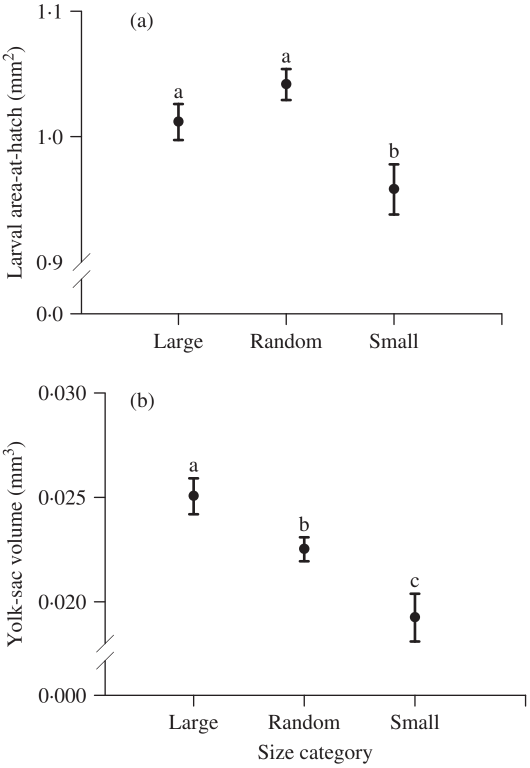

FIG. 5. The effect of *Danio rerio* body size on mean ± S.E. (a) larval area-at-hatch (repeated-measures ANOVA, d.f. = 2 and 47, $P < 0{\cdot}05$; large fish: $n = 14$, random-sized fish: $n = 28$ and small fish: $n = 9$) and (b) larval yolk-sac volume (d.f. = 2 and 54, $P < 0{\cdot}05$; large fish: $n = 15$, random-sized fish: $n = 29$ and small fish: $n = 13$). Different lowercase letters indicate significant differences.

TABLE I. Mean ± S.E. growth rate, standard length (L_S), wet mass (M_W) and condition factor (K) of offspring from three differently sized *Danio rerio* spawners (d.f. = 2) at age 90 days post-fertilization with results of ANOVA tests

Trait	Size group	Mean ± S.E.	F	P
Growth rate 30–60 days (mm day^{-1})	Large	0·24 ± 0·01	0·527	>0·05
	Random	0·24 ± 0·01		
	Small	0·22 ± 0·02		
Growth rate 30–60 days (mg day^{-1})	Large	1·83 ± 0·12^a	3·383	<0·05
	Random	1·50 ± 0·09^b		
	Small	2·17 ± 0·26^a		
Growth rate 60–90 days (mm day^{-1})	Large	0·14 ± 0·01^a	5·544	<0·05
	Random	0·13 ± 0·01^a		
	Small	0·21 ± 0·01^b		
Growth rate 60–90 days (mg day^{-1})	Large	3·39 ± 0·31	0·173	>0·05
	Random	3·57 ± 0·30		
	Small	3·36 ± 0·43		
L_S (mm)	Large	19·09 ± 0·31^a	6·161	0·01
	Random	18·74 ± 0·34^a		
	Small	20·84 ± 0·54^b		
M_W (mg)	Large	167·04 ± 7·80	2·629	>0·05
	Random	158·85 ± 9·38		
	Small	186·13 ± 15·73		
K	Large	2·24 ± 0·06^a	3·619	<0·05
	Random	2·23 ± 0·05^a		
	Small	2·00 ± 0·17^b		

Different lowercase superscript letters indicate significant differences ($P < 0·05$).

frequently and produce larger egg clutches than small fish. The general notion of higher fecundity exhibited by large individuals compared to small spawners is in agreement not only with previous studies in *D. rerio* (Spence & Smith, 2006; Paull *et al.*, 2008) but also with studies on a number of other fish species (Marteinsdottir

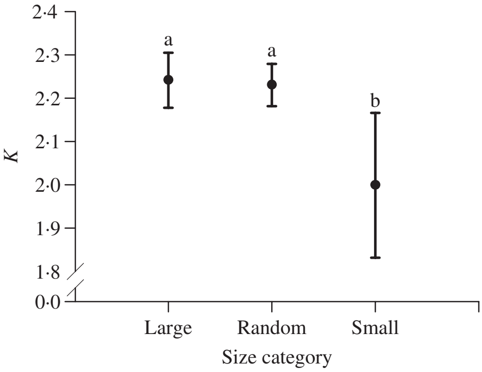

FIG. 6. The effect of *Danio rerio* body size on the offsprings' mean ± S.E. condition factor (K) at age 90 days post-fertilization (two-way ANOVA, d.f. = 2 and 143, $P < 0·05$; large fish: $n = 50$, random-sized fish: $n = 50$ and small fish: $n = 50$). Different lowercase letters indicate significant differences.

& Begg, 2002; Berkeley *et al*., 2004; Trippel & Neil, 2004). When small *D. rerio* succeeded to spawn, they produced egg numbers per female equal to large females, suggesting that small *D. rerio* may need longer time to accumulate enough energy to produce the same amount of eggs as large females. The average spawning interval was 5 days for large and random-sized fish, which agrees with an earlier study by Hisaoka & Firlit (1962), but it was on average 7 days for small fish with substantial between-day variability. Variance in daily egg production was high among all size groups, which was previously reported from domesticated *D. rerio* strains (Paull *et al*., 2008). The reasons for the variability in daily egg production can be due to differences in female spawning strategies (Hisaoka & Firlit, 1962; Spence & Smith, 2006) affected by behavioural interactions (Gerlach, 2006).

The second hypothesis, which stressed that large *D. rerio* produce better quality eggs, received mixed empirical support. Contrary to expectations, it was found that small fish produced larger eggs in terms of egg diameter. Small females might compensate for reduced fecundity and spawning frequency by increasing the size of the eggs (Hendry *et al*., 2001; Murry *et al*., 2008). Alternatively, small females may produce larger eggs compared to large females due to greater inter-spawning interval (Wootton, 1998), allowing accumulation of energy resources across time before the actual spawning (Kamler, 2005). In the present study, however, changes in egg size and larval L_S-at-hatch did not exhibit a clear decreasing trend over time among large or random-sized spawners (Fig. 3). Thus, the exact mechanism for the greater egg diameter of the eggs spawned by small *D. rerio* remains unclear. Irrespective, data from this study indicate that small females produced eggs with significantly larger perivitelline space (*e.g.* due to swelling; Kamler & Kato, 1983) or thicker chorion (Craik & Harvey, 1984) rather than larger yolk material. Egg yolk is the energy source of a developing embryo and is a better indicator of egg quality than mere egg size (Kamler, 2005). Because the egg yolk did not differ significantly between size categories in the present study, there is no convincing evidence that the larger egg size of small spawners represents a biologically relevant compensatory response.

Despite small fish producing larger eggs, the eggs appeared to be of lower quality, as shown by increased egg mortality rate, compared to large and random-sized spawners. In terms of egg mortality, the quality of eggs appeared to decrease over time in all the size categories [Fig. 3(b)] indicating decreased reproductive quality over the 5 day spawning period. This might be explained by the depletion of energy over time, which may have reduced the amount of energy available for oocyte development (Bagamian *et al*., 2004). The significantly different egg mortality rate by small and large *D. rerio* spawners suggests size-dependent parental effects on egg quality. Indeed, size-dependent maternal effects have been shown to contribute to embryonic viability *via* egg matter composition rather than egg size (Kamler, 2005) and large females from a number of fish species have been reported to produce higher quality eggs than small females (Zastrow *et al*., 1989; Brooks *et al*., 1997; Marteinsdottir & Steinarsson, 1998). Therefore, factors other than egg size alone are likely to contribute to embryo quality and larger egg size of small *D. rerio* is possibly not an indicator of higher quality (see Keckeis *et al*., 2000). In addition, hormones (Brooks *et al*., 1997; Nguyen & Zhu, 2009) and sperm quality (Rideout *et al*., 2004) may influence embryo development and affect egg viability. In *D. rerio*, however, male size and sperm quality do not seem to correlate (Skinner, 2004; Paull *et al*., 2008), and this is indirectly supported also by the similar fertilization success between small and large males. Further studies following a factorial breeding design are required to disentangle the relative importance of maternal and paternal effects on egg quality variables in *D. rerio*.

Larvae of large fish hatched earlier and were in a better nutritional condition compared to larvae produced by small fish. Hatching time depends upon the lysis of egg chorion, which is usually thicker in late hatching embryos (Kimmel *et al.*, 1995) and in larger eggs (Craik & Harvey, 1984). In the present study, small fish produced larger eggs, in terms of egg diameter, possibly with thicker chorion than large fish. The strength of the chorion has been reported to vary individually in *D. rerio* (Kimmel *et al.*, 1995), but no correlation with maternal body size has been reported. Hatching time is also known to depend on muscular activity of the embryo, and early hatching larvae have generally higher muscular activity during embryogenesis than late hatching larvae (Kimmel *et al.*, 1995). Thus, early hatching time, which was exhibited in the present study by large fish offspring, is probably an indicator of better larval condition and faster development rate.

Despite egg size being commonly considered to correlate with larval size-at-hatch (Chambers & Leggett, 1996; Pepin *et al.*, 1997; Marteinsdottir & Begg, 2002), such a relationship was not evident in the present data. Large fish offspring had identical L_S-at-hatch but significantly higher area-at-hatch and yolk-sac volume than small fish offspring. This suggests that factors other than egg size may be decisive in embryogenesis defining larval size-at-hatch (Kamler, 2005). In fact, larvae vary in their developmental and yolk absorption rates (Kamler, 2008); thus embryonic growth rate and efficiency at which yolk energy is converted to body tissues during the embryogenesis may result in different hatching time, larval area-at-hatch and yolk-sac volume despite similar egg yolk volumes. Furthermore, differences in yolk energy content, which results not only from the yolk size but also from the caloric value of yolk, can lead to different quality eggs and larvae (Kamler, 2005). These mechanisms may explain the higher area-at-hatch and yolk-sac volume of larvae produced by large fish, suggesting that they were in better condition than offspring of small *D. rerio*.

Larger larvae in terms of body area and yolk-sac volume produced by large fish had a higher K value at age 90 dpf, which supported the third hypothesis of the present study. Contrary to expectations, however, offspring of large fish did not express higher somatic growth rates during early ontogeny compared to larvae produced by small fish. This finding contradicts other studies according to which initial size differences of the offspring from differently sized parents may be maintained for several weeks into the juvenile life stage (Chambers *et al.*, 1988, 1989; Einum & Fleming, 1999; Lindholm *et al.*, 2006). In fact, smaller larvae produced by small *D. rerio* exhibited higher growth rates (in terms of L_S) in a particular juvenile phase (60–90 dpf) than larvae originating from large spawners. This might be indicative of compensatory growth, as offspring of small fish may have compensated the lower energy reserves of the yolk sac by growing relatively fast during the early juvenile phase (Ali *et al.*, 2003).

Population density and skewed sex ratio have been shown to influence *D. rerio* mating behaviour and success through increased aggression (Pritchard, 2001; Spence & Smith, 2005) potentially confounding the effect of body size on reproductive variables. The present study controlled for these effects by keeping the density and sex ratio similar in all trials for all size categories. The establishment of dominance hierarchies might suppress the resource utilization of subdominant fish, and it may be argued that small fish might have been undernourished; hence, nutritional state rather than body size explains the study results. This is, however, unlikely because *K* of small, random and large fish did not differ after the holding period. *K* has been shown to be an unreliable measure of the fish energy stores in the wild because it may fluctuate seasonally stemming from reproductive cycles and varying food availability (Le Cren, 1951; Fechhelm *et al*., 1995). In *D. rerio*, however, *K* has been shown to reflect differences in feeding regimes and thus in feeding opportunities and nutritional state (S. Uusi-Heikkilä, A. Kuparinen, C. Wolter, T. Meinelt, A. C. O'Toole & R. Arlinghaus, unpubl. data). The potential existence of dominance hierarchies during the holding phase should therefore not have affected the general size effect on reproductive traits reported here unless new dominance hierarchies were established during the reproductive trials influencing the reproductive output differently between size categories.

To conclude, a spawning stock composed of large *D. rerio* had higher reproductive output compared to small individuals. Furthermore, eggs produced by large females appeared to be of higher quality by exhibiting significantly lower mortality rates than eggs produced by small females. It can thus be concluded that there are size-dependent parental effects in laboratory-held wild *D. rerio*, which are reflected in egg, larval and offspring quality but not necessarily in juvenile growth rate. Further studies are needed to investigate the mechanisms behind the differences in egg and larval quality between large- and small-sized parents. In particular, there is a need to disentangle the relative importance of maternal and paternal effects on reproductive output and egg and larval traits in *D. rerio*. Such studies are needed to inform fisheries models about the impact of size-selective mortality on recruitment dynamics and also to help guide laboratory protocols for studies on *D. rerio*, which depend on unequivocal assessment of reproductive variables.

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