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Stress Reduces the Quality of Gametes Produced by Rainbow Trout[1]

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ABSTRACT

In this study we have used the rainbow trout as a model animal to study the biological consequences of stress in terms of gamete quality and quantity. Groups of 30 mature male and female rainbow trout were subjected to repeated acute stress during the 9 mo prior to spawning. Time of ovulation, fecundity, and egg size were recorded in mature females, and sperm counts were carried out on the milt from the male fish, from both the stressed and control groups. Eggs from ovulated females were fertilized with milt from males subjected to the same treatment regime. Approximately 300 eggs from each female were fertilized with a sperm dilution of 10^{-3} in diluent. Subsequent development of the fertilized eggs was then monitored. There were no differences in somatic weight or length between the two groups at the end of the experiment, but exposure of rainbow trout to repeated acute stress during reproductive development resulted in a significant delay in ovulation and reduced egg size in females, significantly lower sperm counts in males, and, perhaps most importantly, significantly lower survival rates for progeny from stressed fish compared to progeny from unstressed control fish. Hence, stress reduces the quality of gametes produced by rainbow trout.

INTRODUCTION

Stress is a ubiquitous feature of life and reproduction is one physiological process that is particularly sensitive to its disruptive effects. Acute and chronic stress have been shown to adversely affect a range of reproductive indices, including a suppressive effect on reproductive endocrinology, in humans [1, 2], mammals [3–7], birds [8], reptiles [9–11], amphibians [12–14], and fish [15–18]. There is considerable evidence that corticosteroids, acting at the level of the hypothalamus [3, 13, 19], pituitary gland [20, 21], and the gonads [5, 22, 23] mediate the suppressive effects of stress on reproduction. A great deal of work has been directed at elucidating the mechanisms believed to mediate stress-induced inhibition of reproductive functions and the anatomical sites at which these effects take place [for review, see 24]. The ultimate consequences of stress in terms of overall reproductive success have, however, received much less attention. It is possible to measure the concentrations of a wide variety of hormones and neurotransmitters involved in coordinating the reproductive process, and other reproductive parameters such as sperm counts and motility, egg size, and chemical composition, etc., in an attempt to assess the inhibitory influence of acute and chronic stress on reproductive function. However, it is the successful production of viable offspring which attests to any animal's full reproductive capability, and it is the effect of stress on this ultimate reproductive parameter which is most important.

The successful fertilization of eggs and subsequent development of offspring will depend greatly on the quality of gametes produced by the parents. In humans, female fertility declines with advancing age and it has been established that this age-related reproductive failure results from diminished oocyte quality rather endometrial inadequacy and implantation failure [25]. The parameters that determine egg and sperm quality are still not well defined and thus the study of the factors that affect gamete quality are of considerable interest, not only from a fundamental point of view, but also because a better understanding would undoubtedly improve the success of the many different assisted reproduction techniques (such as artificial insemination and in vitro fertilization). However, the identification of gamete parameters that correlate best with fertility and offspring survivorship and the factors that affect gamete quality are difficult to achieve in most animals, especially in species such as mammals and humans where there is little possibility of performing the large scale studies required to establish these relationships. The limiting factor for studying gamete quality in these species is egg production; only a small number of tiny eggs, often at different stages of maturation, can be obtained, and these must often be removed from a number of different individuals. Furthermore, there are subsequent difficulties in monitoring embryonic survival and development, because this usually takes place in utero or within an opaque egg. In this study we have used the rainbow trout as a model animal to study the biological consequences of stress in terms of gamete quality and quantity, because its mode of reproduction offers many advantages.

Female rainbow trout undergo a very pronounced annual reproductive cycle, during which two large ovaries develop. Immediately prior to spawning, the ovaries together comprise approximately 20% of the body weight. At ovulation 2000–3000 eggs per kg body weight, each measuring approximately 5 mm, are synchronously released into the body cavity [26]. Following ovulation, the eggs can be man-

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ually recovered from females by routine stripping procedures and fertilized externally with milt similarly removed from the males. Eggs are then placed in incubation trays, and it is possible to monitor fertilization success and subsequent development of the eggs by the naked eye. Thus the rainbow trout constitutes an excellent model system for the study of the factors that determine gamete quality, since a very large number of eggs—all at exactly the same stage of maturation—can be obtained from each female, the external fertilization stage is readily manipulated, and the subsequent development of the eggs can be very easily monitored.

Previous work using this animal model, the trout, has shown that acute and chronic stress and plasma cortisol elevation have a suppressive effect on reproductive endocrinology, resulting in significantly reduced plasma testosterone levels in males and significantly reduced plasma sex steroids and vitellogenin levels in females [17]. The aim of this study was to establish the consequences of this stress-induced endocrine dysfunction on gamete quality and quantity.

MATERIALS AND METHODS

Animals and Treatment

Three-year-old rainbow trout (Annan strain) were maintained at a density of 30 fish (mean weight 1462 ± 55.8 g, n = 90)/tank in 1500-L outdoor fiberglass tanks, each supplied with a constant flow of lake water (35 L/min). The fish were fed once daily with commercial feed at a rate of 1% body weight per day. To determine the effects of repeated acute stress on a variety of reproductive parameters in both male and female rainbow trout, two groups of 30 maturing females, all of which had spawned in the previous year, and two groups of 30 maturing males were subjected to repeated acute stress during the 9 mo prior to spawning. A similar number of control fish were maintained in identical tanks but were not subjected to the stress. The fish were stressed by exposure to a brief period of emersion; this involved allowing the water to drain completely from the tanks, leaving the fish exposed to the air for about 3 min before the tanks were slowly allowed to refill. Trout have been shown to acclimate to repeated exposures to a stressful stimulus [27] as have sheep [28], rats [29], cattle [30], and birds [31]; hence the emersion stress was applied at random intervals to prevent acclimation (see Fig. 1).

To assess whether emersion continued to be a stressful procedure, a subsample of fish was taken from each tank and blood was sampled approximately halfway through the experiment, 4 mo prior to spawning. During sampling, the fish were removed with as little disturbance as possible to the fish remaining in the tanks, and anesthetized in 2-phenoxyethanol (1:2000; Sigma Chemical Company Ltd., Poole, Dorset, UK). Blood samples were taken from the sinus venosus by means of heparinized syringes, kept on ice for < 30 min, and then centrifuged at 4°C. The resultant blood plasma was stored at −20°C until assayed for cortisol, using a previously validated radioimmunoassay [32].

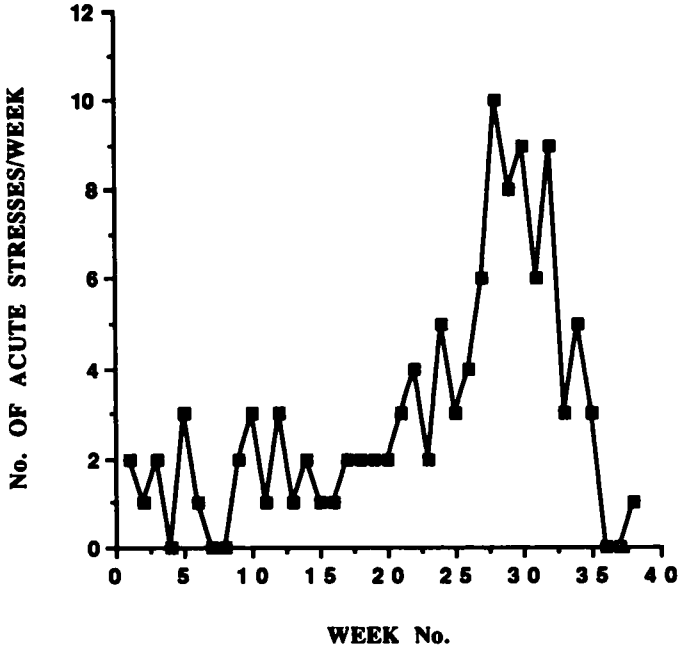

FIG. 1. Experimental design. The experiment began in mid-April and ended in January 1991. The stress was applied randomly throughout the 38 wk. At the beginning, the stress was applied, on average, once a week; later in the experiment, the fish were stressed more frequently. The interval between each stress and the time of day the stress was applied varied randomly.

Collection of Gametes

Time of ovulation, fecundity, and egg size were recorded in mature females, and sperm counts were estimated for mature males from both stress and control groups.

The first female ovulated on 15 January 1991, which was thereafter termed Day 0. Every 1–2 days the fish were examined, under 2-phenoxyethanol anesthesia, to check for ovulation. This was done by applying gentle pressure to the abdomen in an anterior-to-posterior direction; mature eggs are easily extruded from the genital pore by this method, following ovulation. The mature eggs were stripped from each ovulated female into a dry plastic bowl, the coelomic fluid was drained and discarded, the total number of eggs was weighed, and a subsample of 300 eggs was taken for fertilization. A dissecting microscope fitted with an eyepiece micrometer was used to measure egg diameters; 10 eggs from each female were measured on a random orientation basis (to the nearest 0.1 mm) and individual egg volumes were calculated. Ten eggs from each female were also weighed (to the nearest 0.001 g). The fecundity of each female was estimated by dividing the total weight of eggs collected by the mean individual egg weight.

When required for the fertilization stage of the experiment, mature males were rapidly netted from the appro-

priate stress or control groups and anesthetised. Milt (sperm + seminal fluid) was then stripped from the males by gentle abdominal massage and collected into dry plastic tubes. An aliquot of milt from each male was diluted 1:1000 in 0.5% NaCl (Sigma Chemical Company Ltd.) and used for estimating sperm counts using a hemocytometer (chamber volume 0.00025 mm^3) under a phase-contrast microscope at 250× magnification. Ten squares were counted per fish. The following formula was used:

$$\frac{\text{sperm}}{\text{mm}^3 \text{ milt}} = \frac{D \times N}{S \times K}$$

where D = dilution (1000)
N = total number of sperm counted
S = number of squares counted
K = cubic capacity of one square in mm^3 (0.00025 mm^3)

Weights and lengths of all fish were recorded following stripping.

Fertilization and Progeny Survival

An in vitro fertilization technique was used for this stage of the experiment. The gametes, collected separately from each fish, were held separately for fertilization and rearing. The eggs obtained from stressed females were fertilized with milt from stressed males and the eggs from control females were fertilized with milt from control males. The males were selected randomly and each male was used only once, to fertilize a batch of 300 eggs from a single female. Thirty experimental crosses were set up using the first 15 females to ovulate in both the stress and control groups.

It was important not to use a large excess of sperm, because doing so might mask any reduction in overall sperm quality. Previous work [33] has shown that 10 μl of milt, once appropriately diluted, is sufficient to fertilize a few hundred eggs. Thus the 300 eggs from each female were fertilized with 10 μl of milt. Each milt sample was first prediluted 1:10 in a nonactivating solution of 40 mM KCl (Sigma Chemical Company Ltd.). The inhibitory effect of this diluent on trout sperm motility was checked before any sperm dilutions were carried out; it was found to completely inhibit motility. This was then further diluted 1:100 in 125 mM NaCl buffered to pH 9 with Tris [33]. This solution activates the sperm, which are then motile for 30 sec. The 1:1000 dilution of milt, final volume 10 ml, was added immediately to a batch of 300 eggs that were then gently stirred before being left undisturbed for 10 min. The fertilized eggs were placed in separate compartments within egg trays in incubators supplied with a constant flow of filtered lake water. Subsequent survival of the progeny was monitored through egg development, hatching, and up to 28 days after hatching.

Data Analysis

Student's *t*-test for unpaired comparisons was used to determine the significance of the observed effects. Statistical difference was indicated when the p value was less than 0.05. The results of replicate treatments were tested for similarity, shown to be similar, and then combined. The females began to ovulate on 15 January 1991, designated Day 0, and all ovulated over the following 65 days. The mean number of days to ovulation were compared between the stressed and control females using the Student's *t*-test for unpaired comparisons. The progeny survival data were transformed, by conversion to proportions and by arcsin, before statistical analysis was performed. Possible relationships between (1) egg size and progeny survival, and (2) sperm counts and progeny survival were tested by simple regression analysis. The data from both treatment groups were combined to generate the regression line. All calculations were carried out using MINITAB (Minitab Inc., State College, PA).

RESULTS

Plasma Cortisol Levels

Approximately half way through the experiment, 1 h after an emersion stress, plasma cortisol levels in the stressed and control fish were compared (Fig. 2). The results showed that plasma cortisol levels in the fish subjected to the acute stress were significantly higher than plasma cortisol levels in the unstressed control fish (stressed males = 26.4 ± 3.2 ng cortisol/ml compared to control males = 13.5 ± 4.7 ng cortisol/ml; stressed females = 33.8 ± 5.9 ng cortisol/ml compared to control females = 10.8 ± 1.5 ng cortisol/ml). These results show that the regimen of repeated emersion stress was effective in provoking a stress response and thus that the fish had not acclimated to this form of stress.

Weights and Lengths

There were no significant differences in either somatic weights or lengths between the stressed and control fish at the end of the experiment (Fig. 3). The condition factors ((weight (g) ÷ (length^3 (cm))) × 100) of these fish were also calculated and found not to differ significantly between the two groups (stressed males = 1.56 ± 0.03 compared to control males = 1.62 ± 0.04; stressed females = 1.46 ± 0.03 compared to control females = 1.41 ± 0.03). The fish exposed to the regimen of repeated acute stress were observed to recover rapidly from the stress and were fed normally throughout the course of the experiment.

Ovulation and Egg Parameters

The first reproductive parameter observed to be affected by stress was timing of ovulation. The females began to ovulate on 15 January 1991, designated Day 0, and the majority ovulated over the following 65 days. Those females

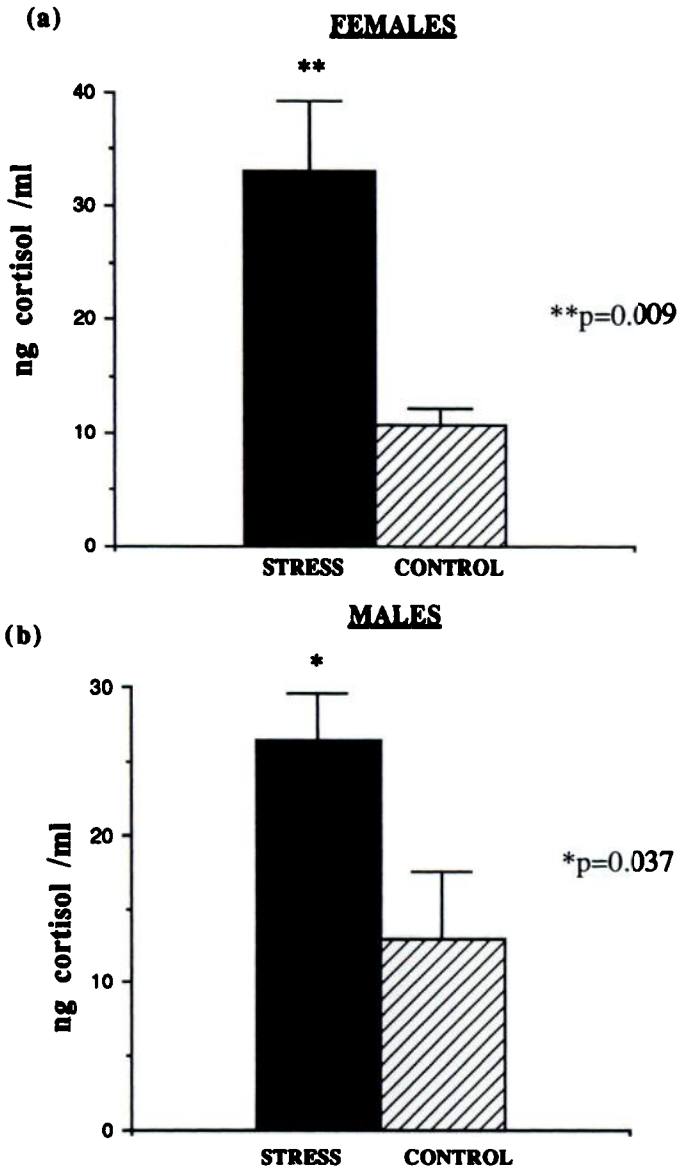

FIG. 2. Plasma cortisol concentrations in female (a) and male (b) rainbow trout 1 h after an emersion stress performed mid-way through the 9 mo of the experiment. Values are mean ± SEM (n = 10).

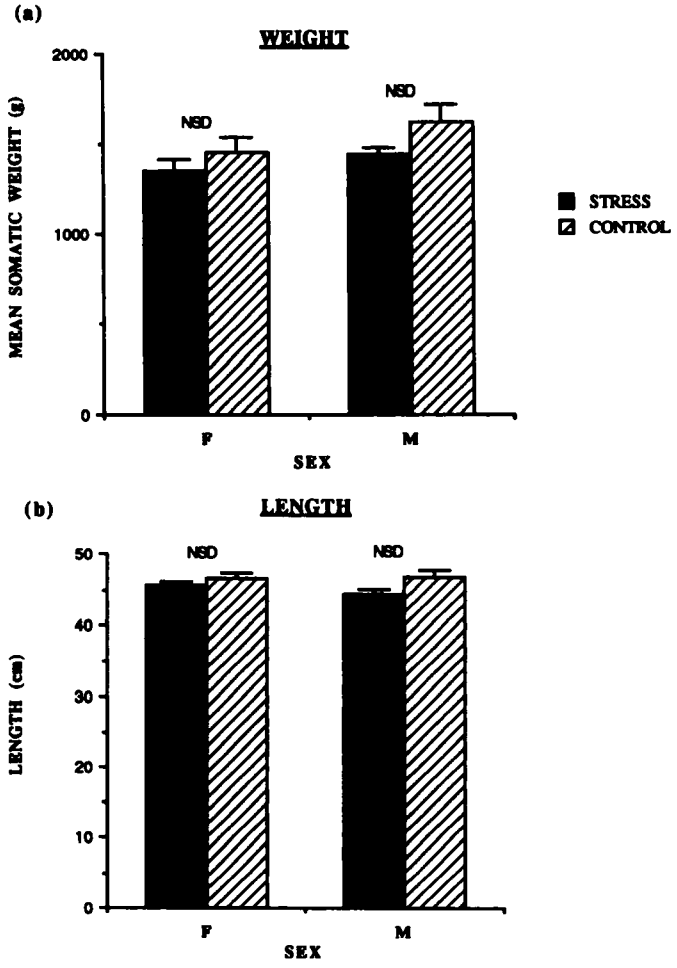

FIG. 3. Mean weight (a) and mean length (b) of stressed and control rainbow trout at the end of the experiment. Values are means ± SEM (n = 15).

that did not ovulate during this period, which was an equal number of fish in both the stressed and control groups, were found to be immature at the end of the experiment. The mean number of days to ovulation were compared between the stressed and control females and ovulation was found to be delayed significantly ($p = 0.031$) in the females exposed to the regimen of repeated acute stress (Fig. 4).

Eggs obtained from females subjected to repeated acute stress were significantly smaller than eggs obtained from control females (Fig. 5). Ten eggs from each female were weighed and measured (this is a sufficiently large sample size because eggs from an individual female are of a very similar size [26]). The weight was significantly lower ($p < 0.05$) for eggs from stressed females compared to eggs from

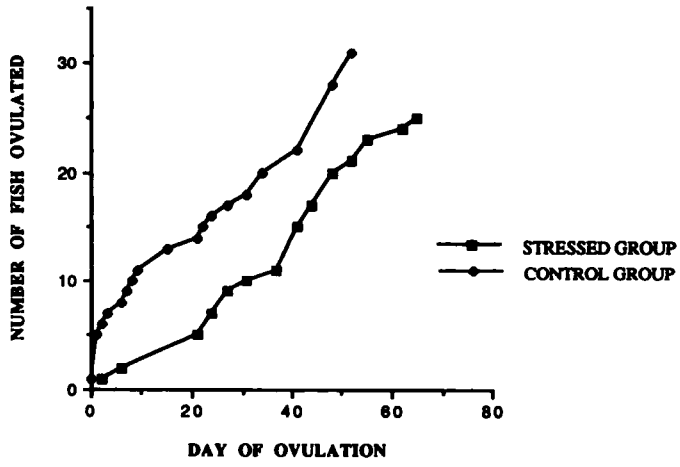

FIG. 4. Effect of repeated acute stress on the timing of ovulation in female rainbow trout. The day on which the first female ovulated (15 January 1991) was designated Day 0. The last mature fish ovulated 65 days later (on 21 March 1991). The difference in numbers between the two groups is due to the fact that 5 more stressed than control females died during the course of the experiment.

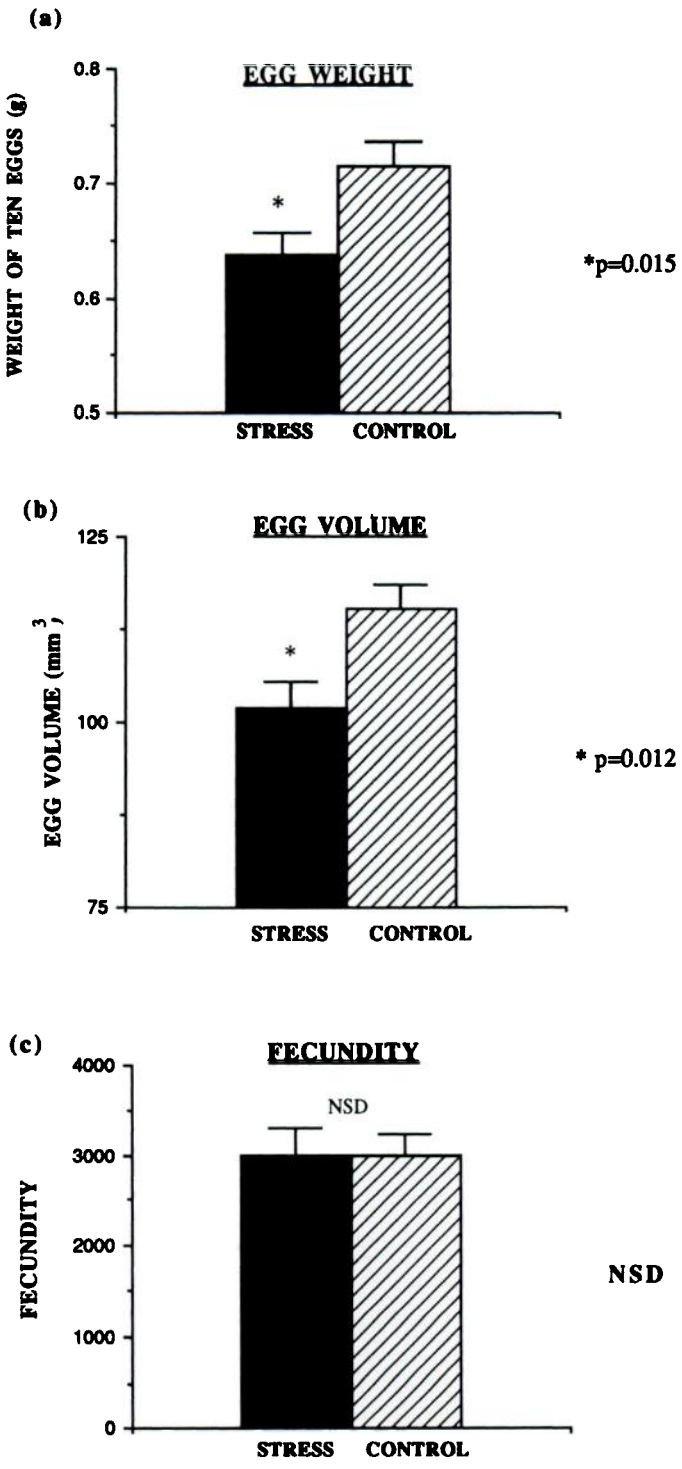

FIG. 5. Effect of repeated acute stress on egg weight (a), egg volume (b), and fecundity (c) of female rainbow trout. Values are means ± SEM (n = 15).

control females (0.638 ± 0.02 g for eggs from stressed females compared to 0.712 ± 0.02 g for eggs from controls). The mean volume of eggs obtained from stressed females was also significantly less ($p < 0.05$) than that of eggs from control females (101.9 ± 3.6 mm^3 for stressed females compared to 115.1 ± 3.4 mm^3 for controls). The fecundity of the fish was unaffected by the regimen of repeated acute stress (Fig. 5).

Sperm Counts

The sperm counts of the males used in this experiment varied between 3.4 and 24.0 × 10^9 sperm/ml of milt. The sperm counts of the males subjected to repeated acute stress were significantly lower ($p < 0.05$) than the sperm counts of the control males (10.4 ± 0.7 × 10^9 sperm/ml of milt for stressed males compared to 13.6 ± 1.2 × 10^9 sperm/ml for control males; Fig. 6).

Progeny Survival

There was no significant difference in survival rates between the eggs from the stressed and control crosses up to the eyed stage of development, when the eyes of the developing embryo are visible to the naked eye through the chorion (Fig. 7a). Over 90% of eggs from both the stressed and the control crosses became eyed, indicating a very good fertilization rate. A significant difference in survival rates between the progeny from the stressed and control crosses was, however, detected by the time the eggs hatched (% survival of progeny from stressed crosses to hatch = 76.5 ± 5.6% compared to 94.3 ± 1.41% for progeny from controls). This reduced survival of the progeny from stressed fish became only very slightly more pronounced as development continued (Fig. 7a). Thus, at the swim-up stage—a critical time when the fry start to feed—67.0 ± 6.1% of the progeny from the stressed fish were still alive, compared to 86.6 ± 2.4% of the progeny from the controls ($p < 0.01$). At the end of the monitoring period, 28 days post-hatch, 63.4 ± 7.3% of the progeny from the stressed crosses were still alive, compared to 84.8 ± 2.5% of the progeny from the control crosses.

The timing of the deaths of the progeny during development is probably more clearly illustrated by observing

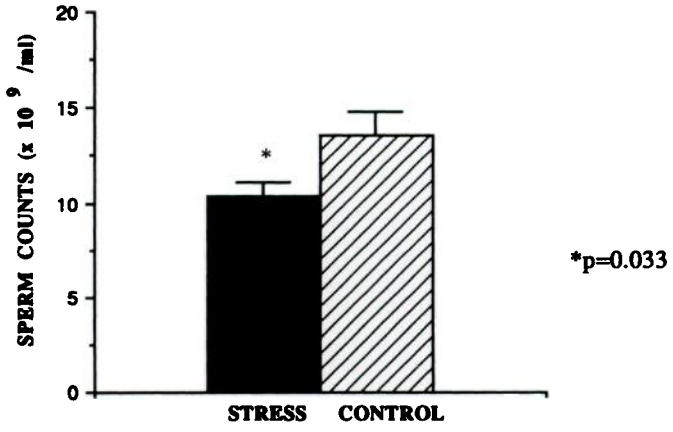

FIG. 6. Effect of repeated acute stress during the preceding 9 mo on sperm density in milt of male rainbow trout. Values are means ± SEM (n = 15).

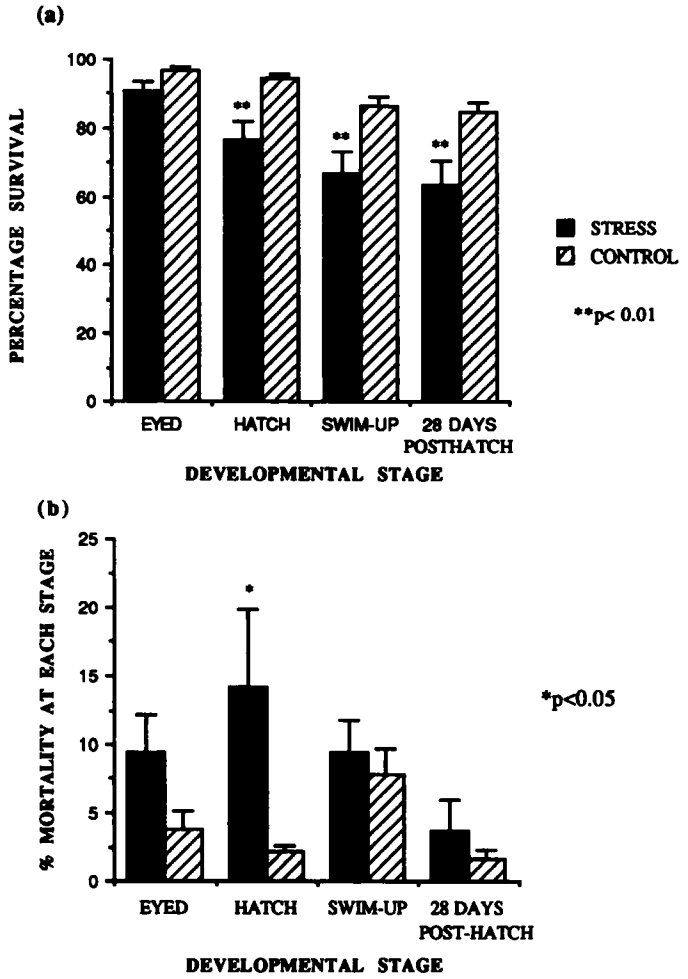

FIG. 7. Survival (a) and mortality (b) rates of eggs and embryos obtained from stressed and control rainbow trout. The rate of survival (and hence mortality) was assessed at four developmental stages: the eyed-egg stage, hatch, swim-up, and 28 days post-hatch.

the degree of mortality at each of the stages of development we assessed (Fig. 7b). This shows that the increased mortality observed in the progeny from the stressed fish occurred primarily around the time of hatch. There was a difference in the mortality rate at the eyed stage, but this was not significant (p = 0.069). However, at the time of hatch there was a significant difference in the mortality rate between the progeny of the stressed and control fish. Subsequently, there was no significant difference in the rate of mortality of the progeny from the two groups of fish. There was no significant correlation between egg size and progeny survival (Fig. 8).

DISCUSSION

This study shows that exposure of rainbow trout to incidences of acute stress during reproductive development results in delayed ovulation and reduced egg size in females, significantly lower sperm counts in males, and, perhaps most importantly, significantly lower survival rates for progeny from stressed fish compared to progeny from unstressed control fish.

Following exposure to the repeated acute stress regime for a period of 20 wk, blood samples were taken from a subsample of fish 1 h after an emersion stress. The significantly higher cortisol levels in the stressed fish compared to the controls indicated that acclimation had not occurred at this stage. Brown trout subjected to acute handling stress show a much more pronounced peak of cortisol (over 100 ng/ml) 1–2 h post-stress [34]. This difference may be accounted for by the fact that brown trout are a species more sensitive to stress than rainbow trout, but may also indicate that the fish in this experiment had begun to acclimate to the emersion stress to some extent. However, mature and maturing trout also show a substantially reduced response to stress compared to immature fish [35]. Since the fish in this case became gradually more mature as the experiment proceeded, this effect could also have been involved in the attenuated stress response observed. The important point, though, is that the fish subjected to emersion stress had significantly higher plasma cortisol levels than the unstressed control fish. After 20 wk of exposure to repeated, intermittent emersion stress some degree of acclimation would be expected to have taken place [36], although this would have been prevented to some degree by the random timing of the stress; this slow but steady acclimation could have continued until spawning, possibly resulting in the acute stresses becoming progressively less effective. It was important to maintain the efficacy of the stresses during the final half of the experiment since this is probably one of the most sensitive phases in the reproductive cycle, when blood concentrations of the reproductive hormones increase in both male and female rainbow trout as spawning approaches. Hence, the number of stresses to which the

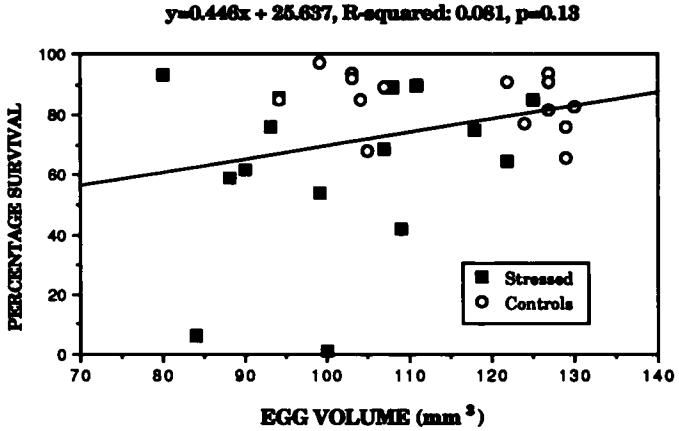

FIG. 8. Simple regression analysis of egg size (volume) and progeny survival revealed no significant correlation between these two parameters. When the data from the two treatment groups is analyzed separately or combined to generate the regression line (as above), no significant correlation between egg size and egg and fry survival is noted. Regression equation for stressed group: y = 0.487x + 13.989, r = 0.237, p = 0.395; Regression equation for controls: y = −0.226x + 110.696, r = 0.316, p = 0.251.

fish were subjected during the 9 mo prior to spawning was increased between Weeks 22 and 32 of the experiment (early September to December), at which time plasma levels of sex steroids and vitellogenin are rapidly increasing in the maturing females [37] and plasma androgen levels are increasing in maturing males [38]. The frequency of the acute stress was then reduced and eventually stopped in early January when spawning was imminent. From late December, the females were checked every few days for signs of ovulation. The frequency of the emersion stress had to be reduced at this time to prevent a possible deterioration in fish health due the additive effects of emersion and the handling stress associated with gamete collection [39]. Since both groups of fish were subjected to equal amounts of handling stress during the stripping procedure, the effects of stress on reproduction observed in this study were likely to have been caused by the earlier repeated exposure to emersion stress. The viability of the progeny from the control fish that ovulated late in the spawning season was no different from that of the first fish to ovulate; thus, although the former fish were subjected to more handling stress than the latter fish immediately prior to ovulation, this had no obvious effect on the quality of the eggs produced. Once ovulation had commenced, the female fish were checked every 1–2 days and the eggs were removed from the body cavity by manual stripping when individuals were found to have ovulated. This was important since ovulated eggs of oviparous teleosts become overripe if retained in the body cavity and these eggs show a progressive reduction in viability [40, 41]. This process of post-ovulatory aging is poorly understood, but it is known that it has a profound effect on the ability of the eggs to be fertilized and to develop further through the stages of eying, hatch, and swim-up [42–46]. The eggs from control females in this study had fertilization, eying, and hatching rates of over 90%, which compare very favorably with the maximum survival rates obtained by Springate et al. [47] and other authors.

The effects of stress on oogenesis and spermatogenesis have been investigated in studies using fish exposed to sublethally low pH (acid stress) or pollutants. A few of these studies have examined the consequences of exposure in terms of fertility, gamete quality, and larval growth and development. For example, female brook trout exposed to acid stress have been shown to ovulate significantly later than control fish held at neutral pH and also to show reduced fecundity [48, 49]. Fish (white sucker, *Catostomus commersoni*) exposed to environmental stress in the form of bleach kraft mill effluent (BKME) also show increased age to maturity, lower fecundity, and reduced egg size [50]. Stress has also been shown to delay or completely inhibit ovulation in mammals [3, 4] including humans [1, 2].

Rainbow trout exposed to acid stress have lower plasma levels of vitellogenin, the precursor of the major yolk constituent [51], and sublethal exposure to cyanide inhibits yolk deposition by suppressing uptake at the ovarian level [52]. The reproductive disruptions in fish exposed to acid stress have been attributed to low pH suppressing gonadotropin secretion [53], resulting in delayed ovulation, and on adverse affects on carbohydrate metabolism and liver physiology reducing the liver's ability to metabolize carbohydrates and synthesize vitellogenin [54]. The synthesis of vitellogenin by the liver is stimulated by increasing levels of estradiol released from the ovary. However, the reduced plasma levels of this precursor of the major yolk constituent in acid-stressed fish cannot simply be attributed to lower circulating levels of estradiol, since Tam et al. [48] and Weiner et al. [55] reported no significant change in estradiol levels in brook and rainbow trout, respectively, following acid exposure. Reduced survival of progeny from fish exposed to acid stress has been demonstrated in a number of studies [49, 55, 56]. A study by Weiner et al. [55] showed that progeny of acid-exposed rainbow trout females and control males had reduced survival, indicating oogenesis is adversely affected by acid stress. A similar, but less pronounced, reduction in the survival of progeny from acid-exposed males and control females was also noted, suggesting that oogenesis is more sensitive to exposure to acid stress than spermatogenesis. Fathead minnows and brook trout exposed to low pH exhibited ovarian histological changes, particularly an increase in oocyte atresia and depression of reproductive success, which were directly associated with the level of environmental stress experienced [53, 57].

Although these studies suggest that it is already established that stress adversely affects gamete quality in fish, in fact interpretation of the results of the studies employing acid stress is complicated by the fact that the fish chronically exposed to sublethally low pH had reduced calorific intake and hence did not grow as well as the control fish [53]. In addition, it might be speculated that the energetic costs of maintaining homeostasis under acid conditions would divert resources from gonadal growth. Since the number of eggs produced by a female is proportional to body weight, it is not surprising that the smaller, acid-stressed fish had reduced fecundity. A similar argument applies to egg size, which is related to body size [40]. Thus the deleterious effects of acid stress on oogenesis and spermatogenesis observed in many of these studies cannot be distinguished from the effects of poor nutrition. It is well known that reduced food intake in mammals and humans is associated with hypothalamic dysfunction and reversible sterility. Similarly, in fish decreased food availability has been reported to suppress gametogenesis [58] and reduce fecundity [15]. This problem of distinguishing between the direct consequences of stress on reproduction and the indirect consequences due to nutritional factors is discussed comprehensively by Tyler et al. [26] in relation to oocyte atresia (and hence reduced fecundity) in rainbow trout. In the study reported here there were no significant differences in either somatic weight or length between the fish exposed to repeated acute stress and the unstressed control

fish. The regimen of random, repeated, acute stresses was chosen not only because in life stress usually occurs as multiple events in a series, separated by varying intervals, but also because exposure of fish to acute stress has little effect on overall food intake. For example, Pickering et al. [34] using brown trout, a species more sensitive to stress than rainbow trout [17], showed a temporary cessation of feeding following an acute handling stress; however, this was not sufficient to impair growth during the experiment. Rainbow trout subjected to acute stress by Wedemeyer [59] resumed normal feeding behavior the following day. Similarly, in this study the rainbow trout recovered very rapidly and resumed normal feeding behavior immediately following an emersion stress.

In this study, stressed female trout produced significantly smaller eggs than unstressed control fish. However, there was no significant correlation between egg size and egg and fry survival rates ($p = 0.13$; Fig. 8). These results indicate that egg size has no direct implication as far as overall egg quality and fry survival are concerned. The importance of egg size has been difficult to ascertain because of conflicting results from various studies and because of problems in separating the effects of egg size on egg/fry survival rates from the effects of other factors such as age, strain, and nutritional status of the parent fish. In some studies, smaller eggs have been shown to experience increased mortality [60, 61]. However, Springate and Bromage [62], using rainbow trout, found that egg size had no direct effect on egg quality or fry survival, a finding supported by this study. Similarly, in studies using brook trout and orangethroat darters, egg volume was positively correlated with juvenile size at hatching and size at yolk sac resorption, but had no significant effect on embryonic survival, developmental time, or subsequent growth rate of hatchlings [63, 64]. The present uncertainty surrounding the effects of egg size on progeny survival has occurred because of differences in age and size of parental fish, varying culture conditions, and, most importantly, uncontrolled variation in the ripeness of eggs [41]. At the present time the balance of evidence indicates that egg size has no direct implication as far as egg quality is concerned. The female trout exposed to repeated acute stress in this experiment did not show any change in fecundity (Fig. 5). In female rainbow trout, the reproductive cycle starts approximately 12 mo prior to spawning, and the number of eggs recruited for development that year is decided at a very early stage. The fish in the present study were not exposed to stress until 9 mo prior to spawning, probably too late in the cycle to affect fecundity.

At present, there are no truly dependable criteria for estimating sperm quality. In mammals, humans, and fish, the length of time and intensity of spermatozoon motility [66–70], the percentage of motile spermatozoa [71], sperm density [68, 72], and the chemical composition of the seminal plasma [73–75] are all parameters that have been measured in an attempt to assess sperm quality. However, there is little hard evidence directly linking any of these parameters with fertility. In salmonids, length of time and intensity of spermatozoon motility are not invariably positively correlated with fertilizing ability [76]. Since fertility and motility do not reside in the same portion of the sperm, it is possible to obtain highly motile sperm incapable of fertilizing eggs [77]. Using low sperm concentrations, near the critical sperm:egg ratio, Moccia and Munkittrick [68], using rainbow trout, showed a significant positive correlation between fertilization rate and subjective motility assessments early in the reproductive season. Later in the season, however, this correlation was lost; instead, a positive correlation between initial sperm density and fertilization rate was evident. They concluded that the critical factor for fertilization was the number of motile sperm, not the motility per se [68]. In the present study, no seasonal change in sperm density was evident, although our study covered only a portion of the period when the males were ripe.

The need for assessment of sperm function for many different assisted-reproductive techniques has resulted in the development of increasingly sophisticated techniques to evaluate sperm quality, including the analysis of movement characteristics of human sperm, which assess not only the number of motile sperm but also the quality of that movement [66, 78]. It is now widely recognized that functional tests of sperm quality probably provide a more valuable means of assessing likely fertility and quality of sperm [79]. By reproducing in the laboratory the natural events leading up to fertilization, some aspects of human and mammalian sperm quality can be evaluated, for example, by monitoring progress through cervical mucus or gels and assessing the ability of the sperm to penetrate the membrane of hamster eggs [78]. Despite the value of these functional tests in assessing sperm quality, it is still true that the ability of sperm to successfully fertilize eggs and produce viable offspring remains the only truly dependable test of sperm quality. Not enough is known about the specific importance of individual attributes of sperm function, and further studies are needed to identify the most valuable diagnostic components of semen quality. Evidence from human, mammal, and fish studies is contradictory regarding the importance that should be placed on various parameters, although sperm density and motility remain the most widely used and reasonably useful estimates of sperm quality at the present time. In humans [66] and fish [68], sperm density has been shown to be a useful assessment of sperm quality in certain circumstances; for example, it is generally accepted that if sperm density in the human ejaculate falls below 10–20 million/ml then men tend to be infertile [80]. In this study, sperm counts—and hence sperm densities—were significantly reduced in male fish exposed to the regimen of repeated acute stress compared to unstressed control fish. The differences in sperm density between the stressed and control groups had no effect on the fertilization rate, possibly indicating

no effect of stress on sperm quality. However, despite the relatively high dilution of sperm used, there may still have been a sufficient excess of sperm to mask any reduction in quality. Ideally, one would need to titre out the sperm, but this would necessarily involve very large numbers of crosses, too many to be manageable. The lower progeny survival of the stressed crosses does indicate a stress-induced reduction in gamete quality that could be attributed to deleterious effects on sperm, or eggs, or both. This reduction in gamete quality is evident only after the fertilization stage. Little work has been carried out on the effects of stress on sperm, although it has been shown that frequent stripping (each stripping involving handling stress) results in a decrease in sperm density, total sperm number, and reduced sperm motility [33, 81].

In this study, the reduced survival of the progeny from the stressed fish compared to the controls attests to the fact that exposure to repeated acute stress reduces the quality of the gametes produced. These results are of interest, not only from a fundamental point of view, but also because at a very practical level they have direct implications in the field of aquaculture. These results show that the conditions to which fish farm broodstock are subjected during sexual maturation will be an important factor in determining the quality of gametes produced, and this will be reflected in the subsequent progeny survival rate. The regimen of repeated acute stress used in this experiment may mimic conditions on some fish farms, where trout are irregularly, but fairly frequently, disturbed by the various farming procedures. Future research should expand this experimental design by fertilizing eggs from stressed females with milt from control males, and vice versa, in an attempt to determine in which the gametes are most susceptible to the deleterious affects of stress.

The increased mortality observed in the progeny from the stressed fish occurred primarily between eying and hatch. Progeny of brook trout exposed to acid stress also show reduced survival to hatch and lower hatching success than progeny from unstressed control groups [49, 55]. The mechanisms causing these mortalities are not known. One possibility is chromosomal errors, which would lead to problems during mitosis. In fact, a number of studies do implicate chromosomal factors in determining egg quality and subsequent embryo survival. Genetic studies in cod revealed that eggs of poor quality have a high percentage of abnormal mitoses [82]. In humans, the importance of chromosomal factors in the fertilization rate during in vitro fertilization has also been investigated and the reasons for fertilization failure in in vitro fertilization have been attributed to immature oocytes (30%), poor quality spermatozoa (20%), and chromosomal aberrations (10%). However, in 40% of all unfertilized oocytes, the reasons are unknown [83]. Postovulatory depletion of high energy phosphate adenine nucleotides has been suggested as a relevant index of carp egg and embryo viability [84]. The decrease in energy charge in aged oocytes and the hormonal stimulation of follicle growth and ovulation used during in vitro fertilization procedures can lead to general disorganization of the spindle, the apparatus responsible for chromosomal segregation [85, 86]. Such changes could induce defects in chromosome alignment, which predispose to aneuploidy and reduction in embryo development. Whether stress affects gamete quality through a mechanism involving chromosomal aberrations remains to be investigated.

In conclusion, we have shown that stress during reproductive development results in delayed ovulation and reduced egg size in females, significantly lower sperm counts in males, and significantly lower survival rates for progeny from stressed fish compared to progeny from unstressed control fish.

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