Effects of Simulated Blasting on Mortality of Rainbow Trout Eggs

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Abstract.—Blasting in or near water can negatively affect fish. In Canada, there are maximum allowable limits for blasting-induced overpressure (100 kPa) and peak particle velocity (PPV; 13 mm/s) to protect fish and their incubating eggs, respectively. No studies, however, have related PPVs from blasting to egg mortality. To address this information gap, we developed a laboratory blast simulation procedure for relating egg mortality to different levels of PPV exposure. Eggs of rainbow trout *Oncorhynchus mykiss* were subjected to PPVs of up to 245.4 mm/s during six sensitive developmental stages. Eggs also were exposed to a previously described drop height method, in which the final velocity of the eggs is used to estimate PPV exposure; we tested both the original out-of-water treatment and an in-water drop height treatment. Using blast simulation, egg mortality increased at only one developmental stage and only from exposures greater than 132.3 mm/s. Mortality was greater when eggs were placed in spawning gravel versus free in containers, although mortality generally increased at the same PPV level for both treatments. In the drop height method, eggs held out of water were more sensitive to a given exposure level than were eggs held in water. The drop height method may not provide an accurate assessment of blasting-induced PPVs, especially when eggs are out of water, but should be suitable for comparing the egg sensitivity of different species or development stages. Our controlled, laboratory-based results indicate that the Canadian PPV guidelines provide ample protection for rainbow trout eggs within spawning beds.

Explosives are used in or near fish-bearing waters for many activities, including geophysical exploration, open-pit mining, construction, and other industrial development. If the resulting blasts are great enough, the pressure and seismic waves produced can negatively affect fish and incubating eggs in the vicinity. The sudden pressure deficit (measured indirectly as overpressure [kPa]) resulting from an explosion can rupture the swim bladder and other soft organs of juvenile and adult fish (Wright 1982; Keevin et al. 1999). In contrast, developing eggs may be damaged more by the shaking of the substrate (Wright 1982), which is typically measured as peak particle velocity (PPV). Appropriately, guidelines in Canada provide maximum allowable limits for both overpressure (100

kPa) and PPV (13 mm/s) to protect fish and incubating eggs, respectively (Wright and Hopky 1998).

Substrate shaking induced by the motion of seismic compressional, shear, and surface waves results in time-varying motion of the ground. The particle displacement ($u[t]$), velocity ($v[t]$), and acceleration ($a[t]$) that are produced can be quantitatively measured using different transducers. Each measure of the seismic wave is related to the others: $a(t) = d[v(t)]/dt = d^2[u(t)]/dt^2$ (Mulligan 1991). Geophones, which record the passing seismic waves in terms of ground particle velocity versus time, are the simplest and most robust seismic wave detectors and hence are commonly employed. Typically, the resulting seismogram is distilled to a single-attribute PPV, which is the maximum value of $v(t)$ observed when the wave passes.

Previous exposure studies have related overpressure to the mortality of fish (Wright 1982), but no studies have related PPV to the mortality of fish eggs. However, the sensitivity of eggs to physical distur-

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bance (of which PPV is one type) has been documented for many species of cultured fish. This work has primarily focused on identifying sensitive developmental stages. Although concentrated on Pacific salmon *Oncorhynchus* spp. (Smirnov 1954, 1955; Jensen and Alderdice 1983, 1989; Johnson et al. 1983, 1989; Dwyer et al. 1993; Jensen and Collins 2003), investigations have been performed for other cultured species (Holmefjord and Bolla 1988; Crisp 1990; Fitzsimons 1994; Hilomen-Garcia 1998; Gwo et al. 1995; Krise 2001; see also Battle 1944). Typically, the period of egg sensitivity starts soon after fertilization and ends when eggs have reached the eyed stage. For most species, peak sensitivity occurs during epiboly, when the mesodermal sheath replaces the vitelline membrane around the yolk (Velsen 1987). The duration of this sensitive stage varies among species depending on developmental rate.

Because of the focus on determining sensitive developmental stages, many studies provide only qualitative assessments of physical shock exposure. Indeed, quantification of mechanical disturbance exposures have only involved measurements of the height from which eggs are dropped into water (Battle 1944; Smirnov 1954), dropped onto mesh (Gwo et al. 1995), or are dropped, free of surrounding fluid, inside an enclosure (Jensen and Alderdice 1983, 1989; Crisp 1990; Krise 2001). However, it has been suggested that the latter technique allows conversion from drop height to more explicit units of exposure, such as energy (Jensen and Alderdice 1989) or PPV (Jensen 2003), via simple physical principles. Thus, after determining the velocity of eggs at the end of a fall, Jensen (2003) suggested that the lowest PPV to cause 10% mortality in eggs of the most sensitive Pacific salmon species (Chinook salmon *O. tshawytscha*) was 140 mm/s, which is more than an order of magnitude greater than the Canadian maximum allowable PPV.

Dropping eggs inside an enclosure produces physical shock exposures that are similar, but not identical, to the particle velocities associated with the propagating seismic waves produced during blasting. In particular, dropping eggs from a height produces an abrupt, large pulse upon impact, whereas the blast-induced shaking of eggs may last a few seconds (Faulkner et al. 2006). Additional concern that the drop height method may not adequately represent blast-generated exposures stems from Jensen and Alderdice's (1983) evidence that eggs held in water had lower mortality than eggs held out of water. Therefore, the comparability of the drop height technique to blasting exposure in an aquatic environment is unknown.

Seymour and Nakatani (1967; cited in Post et al. 1974) and Post et al. (1974) recorded peak vertical accelerations experienced by eggs of Dolly Varden *Salvelinus malma* and rainbow trout *O. mykiss*, respectively, at sensitive developmental stages during exposure to actual or simulated nuclear detonations. Egg mortality did not increase despite multiple exposures to $|a(t)|$ values up to 98 m/s^2. As noted above, particle acceleration is related to particle velocity and hence PPV, but PPV cannot be determined without additional knowledge. In the only known study to quantify PPV exposure due to blasting, Faulkner et al. (2006) found no elevated mortality in eggs of lake trout *S. namaycush* in Lac de Gras, Northwest Territories, despite exposures (28.5 mm/s) from an adjacent open-pit mine that were more than double the Canadian PPV limit. For comparison, the PPV of 28.5 mm/s measured by Faulkner et al. (2006) had a peak vertical acceleration of only 2.1 m/s^2, suggesting that PPVs in the nuclear tests (Seymour and Nakatani 1967; Post et al. 1974) were well above the Canadian guidelines.

To better assess recent suggestions that the PPV guidelines provide ample or more than ample protection for incubating eggs (Jensen 2003; Faulkner et al. 2006), studies are needed that combine the control and replication of laboratory experiments with a more realistic form of physical shock and direct measurements of PPV. We developed a laboratory-based blast simulation technique to examine effects of PPV on egg survival. We sought to produce PPVs that were representative of well-described out-of-water (substrate-based) blasting events (Faulkner et al. 2006; Welz 2006) and PPVs with greater amplitudes, frequencies, and total exposure levels. Eggs held loose in containers within the water were subjected to single and repeated exposures. We compared these results to those in which eggs were placed in spawning gravel, and we also explored the accuracy of the drop height approach by exposing eggs both in and out of water to the technique used by Jensen (2003).

Methods

We focused our research on rainbow trout eggs owing to their availability and relevance to previous studies (Post et al. 1974; Jensen and Alderdice 1989). We obtained fertilized, water-hardened rainbow trout eggs during fall 2004 and 2005 from the Raven Brood Trout Station (Alberta Sustainable Resource Development, Caroline). These eggs were placed in a cooler of oxygenated water and transported by vehicle (3 h) to the Biosciences Aquatics Facility at the University of Alberta. Upon arrival, eggs were disinfected using a weak iodine solution (1:600 by volume). Eggs were then randomly apportioned to the different treatments. Eggs for the drop height exposures were loaded loosely
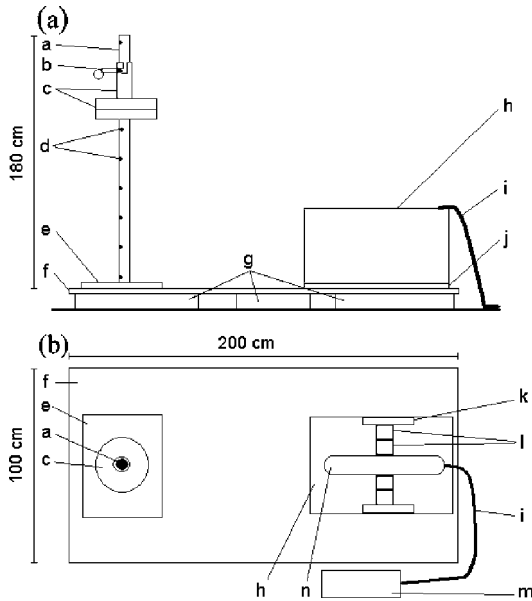
FIGURE 1.—(a) Side view and (b) top view of a weight drop apparatus used in 2004 to simulate blast-induced peak particle velocities (PPVs). Labeled structures are as follows: (a) steel pole secured at the top with straps (not shown); (b) pin used to release the weight apparatus; (c) weight set-up, including an aluminum sleeve and cast-iron weights; (d) holes drilled in the pole, spaced 15 cm apart; (e) 1-cm rubber mat; (f) 0.64-cm-thick steel base plate; (g) 5-cm foam padding; (h) fiberglass exposure tank; (i) sensor cable; (j) 2.5-cm rubber mat, (k) Plexiglas spacer; (l) containers (6 × 6 × 5 cm) used to hold rainbow trout eggs; (m) Instantel Mini Blast Mate monitor; and (n) OYO Sea Array 4 four-component underwater geophone sensor. For the 2005 experiments, the exposure tank was rotated 90°.

into a tray of a vertical stack incubator (Heath trays), whereas eggs for the blast simulation studies were loaded into plastic containers within a separate vertical stack incubator. These containers (6 × 6 × 5 cm) were open at the top and covered on the bottom and sides with 1.5-mm-mesh screening. Both vertical stack incubators were hooked up to the same recirculation tank, which supplied water at 8 ± 0.5°C in 2004 and 10 ± 0.5°C in 2005. A portion of the water was changed frequently to prevent high ammonia and low oxygen concentrations.

Blast simulation (weight drop apparatus).—We designed a weight drop apparatus to simulate blasting events. The base of the apparatus consisted of a steel plate measuring 100 × 200 × 0.64 cm that was insulated from the floor by three evenly spaced pieces of 5-cm foam padding (Figure 1). A 180-cm-long, 1.9-cm-diameter pole was mounted in the middle of the plate (25 cm from one end of the base, 175 cm from the

other end) and was secured at the top by three straps attached to nearby walls. The pole had a series of holes spaced 15 cm apart to allow for drop heights ranging from 2 to 137 cm. An 18.1-kg weight was attached to 30 cm of oversized aluminum pipe, which slid freely over the pole and ensured a smooth drop. The weight was held at a desired height by fitting a pin into an L-shaped slot cut into the top of the aluminum sleeve. To release the weight, the pin was quickly pulled out, allowing the weight to fall. A 1-cm rubber mat was placed at the base of the pole to cushion the shock of the weight and lower the frequencies transmitted into the steel plate. At the other end of the steel base, a fiberglass tank measuring 60 × 40 × 35 cm was placed on top of a 2.5-cm rubber mat. In 2004, the back of the tank was 2.5 cm from the end of the plate and the front was 112.5 cm from the base of the pole (Figure 1). In 2005, the tank was rotated 90°; the front was 112.5 cm from the base of the pole, and the back was 22.5 cm from the end of the plate.

Exposures were recorded with a Sea Array 4 four-component underwater geophone sensor (OYO Geospace Corporation) that was coupled to the bottom of the tank. This sensor contains three self-orienting 10-Hz geophones (OYO; Model 30CT) to monitor movement in the longitudinal, vertical, and transverse directions. The sensor was attached to an Instantel Mini Blast Mate monitor, which was used to record exposures (Figure 1). The maximum PPV that can be recorded in any channel is 256 mm/s. In 2004, the sensor was kept in the tank during exposures, whereas in 2005 the desired exposures were measured repeatedly before eggs were placed into the tank, making the presence of the sensor in the tank during the actual egg exposures unnecessary.

The weight drop apparatus was used to generate PPVs in a controlled, repeatable setting and to emulate the duration, frequency, acceleration, and energy content of actual blasting events recorded during our earlier field study (Faulkner et al. 2006). The PPV levels were reported as the peak vector sum of the movement in the three geophone channels. Duration was defined as the period (s) of significant shaking during which the amplitude was at least 2% greater than the maximum amplitude of background noise levels. Frequency (Hz) was reported as the zero crossing frequency for each of the three geophone channels, which is the frequency of the largest peak. Peak acceleration (m/s^2) was reported for all three channels. To estimate energy, we used the formula for kinetic energy (KE), namely,

$$KE = \left(\frac{1}{2}\right) m \sum_{t_0}^{t_n} v(t)^2,$$

TABLE 1.—Characterization of weight drop blast simulations conducted with rainbow trout eggs during 2004 and 2005 and actual blasting events recorded at the Diavik Diamond Mine, Inc. (DDMI) A154 pit at Lac de Gras, Northwest Territories (see Faulkner et al. 2006). Variables include peak particle velocity (PPV), reported as the peak vector sum of movement in the transverse (tran), vertical (vert), and longitudinal (long) directions; duration of the shaking event; peak acceleration measured in each direction; zero-crossing frequency for each direction; power; and relative power of the waveforms. All values are means (SEs in parentheses).

Year	N	PPV (mm/s)	Duration (s)	Acceleration (m/s²)			Zero-crossing frequency (Hz)			Power	Relative power
				Tran	Vert	Long	Tran	Vert	Long		
2004	3	12.4 (1.0)	1.2 (0.4)	5.9 (0.6)	2.9 (0.6)	5.4 (0.2)	67.7 (7.8)	63 (20)	107 (61)	4.6 (0.3)	0.06
	3	31.0 (2.1)	0.84 (0.03)	12.3 (0.5)	20.3 (3.4)	22.2 (2.0)	41.5 (2.5)	59 (11)	133 (38)	46.3 (1.2)	0.63
	3	107.3 (7.4)	0.68 (0.04)	22.7 (2.0)	52.9 (5.6)	32.9 (2.8)	10.6 (1.0)	39.5 (7.0)	70.0 (3.1)	319 (27)	4.37
	3	117.7 (3.3)	0.71 (0.06)	25.1 (3.8)	62.1 (2.2)	39.4 (4.1)	9.8 (0.6)	38.5 (6.5)	100 (14)	435 (21)	5.96
	3	141 (12)	0.80 (0.03)	25.7 (2.6)	66.2 (6.0)	40.6 (5.0)	21.3 (6.7)	35.9 (5.4)	78 (32)	618 (97)	8.47
	3	219.3 (1.2)	0.89 (0.05)	27.7 (1.8)	137 (12)	39.7 (3.2)	18.4 (1.9)	41.4 (7.1)	70 (24)	1,313 (56)	18.0
2005	15	36.3 (0.4)	0.53 (0.01)	3.3 (0.1)	12.4 (0.1)	10.5 (0.3)	44.2 (3.0)	48.0 (0.5)	91.4 (2.6)	43.7 (0.5)	0.60
	15	132.3 (2.4)	0.90 (0.34)	11.6 (0.5)	51.3 (1.8)	34.9 (0.7)	19.0 (1.2)	35.3 (0.6)	71.8 (0.3)	385.1 (7.0)	5.28
	15	199.1 (2.6)	0.82 (0.01)	27.3 (3.8)	184.5 (4.5)	41.7 (0.7)	11.9 (1.7)	38.0 (1.5)	77.1 (6.3)	1,466 (21)	20.1
	15	245.4 (3.0)	0.88 (0.02)	25.9 (2.0)	243.5 (3.1)	48.0 (0.5)	12.9 (2.3)	26.1 (2.6)	109.8 (1.3)	1,846 (16)	25.3
DDMI	10	15.9 (1.9)	3.4 (0.22)	1.2 (0.1)	1.2 (0.1)	1.4 (0.1)	9.2 (0.8)	12.7 (1.9)	9.2 (0.4)	73 (14)	1

where m is mass, $v(t)$ is particle velocity of the substrate during shaking (not to be confused with the wave propagation velocity), and t_0 and t_n are the initial and final times, respectively. Because mass can be considered constant in this application, the kinetic energy is directly proportional to $v(t)^2$. Thus, an estimate of the power of the entire waveform, including its duration and all particle velocities, can be calculated as follows:

$$\text{Power} = \sum_{t_0}^{t_n} v(t)^2.$$

By normalizing this value, we can directly compare among all blasting events, whether simulated or real. The power of a typical Lac de Gras operational blast was calculated, and those of all simulated events were divided by this value to give a relative power for each event.

Although more than one delay is common during blasting events (i.e., more than one detonation is set off with short intervals between detonations), the current Canadian PPV guidelines do not address delays in blasting. Therefore, in 2004, our simulations focused on single exposures with no delays. In 2005, however, we used repeated exposures to simulate multiple-delay blasting events that produce vibrations with total durations in excess of 3 s.

2004: single-exposure trials.—In 2004, exposures were carried out during egg development at three times (51, 59, and 67 degree-days) corresponding to early epiboly stages (Vernier developmental stages 9 and 10; see Table A.1 in the appendix). At each developmental stage, there were six PPV treatments (12.4–219.3 mm/s; Table 1) and a handled control in which eggs were loaded into the tank but received no PPV

exposure. Other parameters of the blast simulations are given in Table 1. Replicates for each exposure consisted of four containers, each holding an average of 132 eggs (SE = 2.3; N = 84 containers). Dead eggs, characterized by a white and opaque appearance, were not loaded into the containers. There were also six nonhandled control containers, which were loaded with eggs but received no further manipulation. During the exposures, 65 L of water were transferred from the recirculation tank into the exposure tank. Water was changed between trials to ensure that temperature did not change by more than 2°C. At each developmental stage and PPV level, four containers were placed in the tank, two on each side of the sensor, parallel to the exposure source. These containers were held tightly on both sides by Plexiglas spacers, and all containers were coupled to the bottom of the tank with weights. After exposure, containers were loaded back into incubation trays to allow for further egg development.

2005: repeat-exposure trials.—In 2005, exposures were carried out at 75 and 95 degree-days, which correspond to middle and late epiboly (stages 11 and 14, respectively; Table A.1). For both stages, there were four PPV treatments (36.3–245.4 mm/s; Table 1). Figure 2 shows examples of each level of exposure. For each PPV treatment, eggs experienced zero (handled control), one, two, three, five, or seven "blasts" (weight drops) at 30-s intervals. Replicates for each exposure consisted of three plastic containers each containing an average of 99 live eggs (SE = 2.2; N = 144 containers). There were also six nonhandled controls. Water was transferred to the exposure tank as described above. During exposure trials, three rows of six containers each were situated parallel to the exposure source and were coupled to the bottom. An
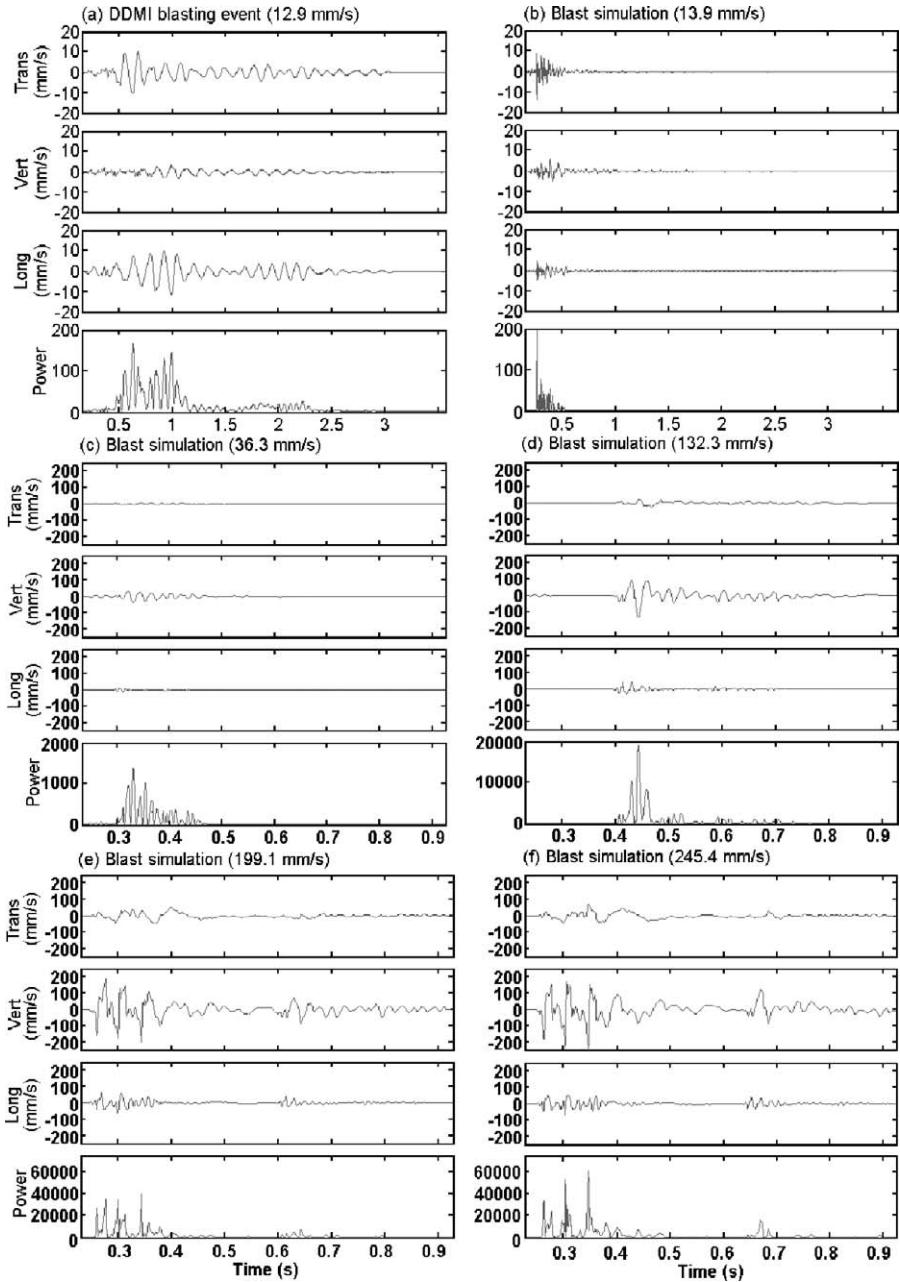
FIGURE 2.—Peak particle velocities (PPVs; mm/s) in the transverse (trans), vertical (vert), and longitudinal (long) directions and power, an estimate of the magnitude of combined movement (see text for additional information), for real and simulated blasting events. Panel **(a)** shows values measured in the substrate of Lac de Gras during an actual blasting event at Diavik Diamond Mine, Inc.'s (DDMI) open-pit mining operation. Values from laboratory blast simulations with varying PPVs are shown in the remaining panels: **(b)** 13.9 mm/s, a PPV similar to that of the actual DDMI event, **(c)** 36.3 mm/s, **(d)** 132.3 mm/s, **(e)** 199.1 mm/s, and **(f)** 245.4 mm/s. Note the differences in the time and power scales.

exposure of appropriate magnitude was then generated. At the end of the repetitions, the containers were returned to the vertical stack incubator to allow for further egg development.

Spawning gravel effects.—In 2005, a separate set of trials examined the effects of spawning gravel on the survival of eggs exposed to simulated blasting. The spawning gravel consisted of smooth river rock with a diameter of about 3 cm in the longest axis, similar to that used by Post et al. (1974). For the intergravel treatment, slightly larger (8 × 6 × 5 cm) containers were used. These containers were half-filled with gravel, and eggs were then loaded into the container and allowed to fall into the interstitial spaces between the gravel. Additional gravel was carefully added to fill the container and cover the eggs. The intergravel containers had an average of 112 eggs (SE = 2.4; N = 30 containers), while the standard nongravel containers had an average of 123 eggs (SE = 4.4; N = 41 containers). Exposures were conducted at about 90 degree-days (stage 12; Table A.1). During exposures, four containers of each treatment were placed into the exposure tank in two rows situated parallel to the exposure source and were coupled to the bottom of the tank. Five repeated exposures were carried out at the same PPV levels used in the other 2005 experiments (Table 1), including handled controls. After exposures, all containers were returned to the incubators for continued incubation; gravel was not removed during this continued incubation. There were also eight nonhandled control containers per treatment.

Mortality.—After all exposures, eggs were allowed to incubate until at least the eyed stage (stages 18–20; Table A.1), since most of the mortality due to physical shock should have occurred before this stage (Smirnov 1954, 1955; Johnson et al. 1989; Jensen and Collins 2003). Eggs were classified as dead (white or partially white in appearance or without noticeable development) or alive. Eggs were subsequently preserved in Davidson's solution (300 mL of distilled water, 200 mL of formalin, 100 mL of glacial acetic acid, and 300 mL of 95% ethanol) for later verification of developmental stage. In some cases, unfertilized eggs were difficult to distinguish from eggs that died during exposures; therefore, they were included as dead eggs. Mortality was calculated on a per-container basis as the proportion of eggs that were classified as dead (i.e., no visible pigmented eyes) when examined at the eyed stage.

Data analysis.—All mortality rates were arcsine–square-root transformed before statistical analysis to meet test assumptions (Zar 1999). Control mortality was analyzed both within and among treatments (developmental stage, handled versus nonhandled) to assess mortality due to handling. The three experiments were subjected to separate analyses of variance (ANOVAs) owing to differences in the source batch of eggs and handling. Differences in mortality were assessed, as appropriate, among exposure levels, development stages, number of exposure repetitions, and gravel treatments. For all treatments, Fisher's least-significant-difference multiple comparison test was used to identify exposure levels that differed from those of the handled control (Zar 1999).

For all statistical tests, a significance level of 0.05 was used. Statistical analyses were performed using SYSTAT version 10 (SPSS 2000) unless otherwise noted. If the exposure treatments generated sufficient mortality relative to that of the handled controls, the exposure levels that caused 5% and 10% mortality (LD5 and LD10, respectively) were calculated using Probit analysis (PriProbit version 1.63; Sakuma 1998). Correction for natural and handling mortality was performed using Abbot's correction (Finney 1971).

Drop height apparatus.—A drop height apparatus was built according to specifications provided by Jensen and Alderdice (1989; their Figure 1). The apparatus has an aluminum carrier with a compartment to carry eggs in a small petri dish (60-mm diameter ×15-mm thickness). This carrier is attached to a release platform with a quick-release mechanism, which can be moved to heights between 0 and 1,000 mm. Upon release, the carrier is guided by two wires to ensure a smooth, straight drop. Friction is minimized by using oversized sleeves in the carrier guides and using lubricant on the wires. The carrier is weighted with lead shot so that it comes to an abrupt stop upon landing.

Exposures were conducted at 51, 59, 67, 75, and 95 degree-days of egg development, which ranges from early to late epiboly (stages 9–14; Table A.1). The first three developmental stages were exposed during trials in 2004, and the last two stages were exposed in 2005. Eggs were dropped from heights of 0 (control), 10, 20, 50, 100, 400, and 800 mm. Assuming no effect from friction, these drop heights can be translated into final velocities (mm/s) using the following equation:

$$V_t = \sqrt{V_0^2 + 2gh},$$

where V_t is final velocity (mm/s), V_0 is initial velocity (0 mm/s), g is acceleration due to gravity (9,806 mm/s^2), and h is drop height (mm). Using this equation, the final velocities (PPVs) of the exposures were calculated as 0, 443, 626, 990, 1,400, 2,801 and 3,961 mm/s. Drop heights less than 10 mm were unreliable owing to apparatus limitations.

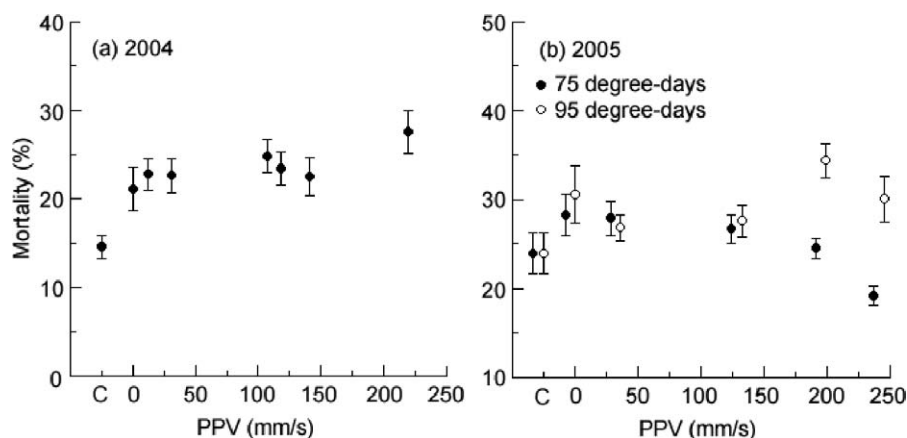The average weight of exposed eggs was calculated in 2005 from six samples of 30 eggs. From this, the

FIGURE 3.—Mortality (mean ± SE) of rainbow trout embryos in weight drop blast simulations **(a)** 2004 (51, 59, and 67 degree-days combined) and **(b)** 2005 (75 and 95 degree-days; offset to facilitate comparison); PPV = peak particle velocity. The mortality of handled control embryos is plotted at 0 mm/s; that of nonhandled control embryos is plotted at C. No exposure groups had mortality greater than that of the handled control group (ANOVA: $P > 0.05$).

energy imparted onto the eggs could be estimated as follows:

$$E = M \times g \times h,$$

where E is energy (J) and M is the mass of the eggs (kg) (after Jensen and Alderdice 1989).

Exposures were conducted as in Jensen and Alderdice (1983, 1989). For each exposure, we placed an average of 39 healthy-looking eggs (SE = 1.3; $N =$ 72 groups) into a petri dish free of surrounding fluid and fit the dish into the slot at the top of the carrier. The carrier was released from the appropriate height to achieve the desired exposure (out-of-water treatment). Exposures were also performed with the eggs submersed in water (in-water treatment). All exposures were done in triplicate. After exposures, eggs were loaded into separate incubation compartments and allowed to incubate until at least the eyed stage was reached. Mortality was calculated as described above.

Owing to differences in stock source, each year of exposure was analyzed separately. Arcsine–square-root-transformed mortality rates were analyzed with ANOVA to assess differences among exposure levels, treatments (out-of-water and in-water), and developmental stages (Zar 1999). The LD5 and LD10 values were calculated as above. These LD5 and LD10 PPV values were also converted to joules for comparison with previous studies. Statistical tests were performed as described above.

Results

Blast Simulation (Weight Drop Apparatus)

Although broadly similar, our simulated blasts differed from actual blasts at an open-pit mine in some characteristics (Table 1). At similar PPVs, frequencies and accelerations of simulated blasts were higher than those of actual blasts, but durations were shorter (Figure 2). The latter difference was addressed in 2005 with the seven repeated exposures. Simulated blasts also had lower relative power than actual blasting events at similar PPVs; however, the repeated exposures in 2005 more than compensated for this (Table 1).

Control mortality.—The mortality of handled controls was similar among the three stages of development in 2004 ($F_{2,9} = 1.58$; $P = 0.26$) and between the two stages in 2005 ($t = 0.18$, df = 22, $P = 0.86$). There was a small (6.6%) increase in mortality between the handled and nonhandled controls in 2004 ($t = 1.95$, df = 16, $P = 0.035$) but not in 2005 ($t = 1.43$, df = 28, $P = 0.08$; Figure 3). During the spawning gravel study, mortality was similar between the handled and non-handled controls in both gravel treatments (intergravel: $t = 0.50$, df = 23, $P = 0.31$; nongravel: $t = 1.77$, df = 12, $P = 0.051$; Figure 4).

2004: single-exposure trials.—In 2004, mortality did not differ among the three stages of egg development ($F_{2,75} = 1.84$; $P = 0.17$) or the seven PPV exposures ($F_{6,75} = 1.17$; $P = 0.33$; Figure 3a). With the stages combined, mortality ranged from 21.1% (SE = 2.3%) in the handled control to 27.5% (SE = 2.2%) at a PPV of 219.3 mm/s.

2005: repeat-exposure trials.—In 2005, mortality differed among developmental stages ($F_{1,134} = 15.92$; $P < 0.001$), and eggs exposed at 95 degree-days had higher mortality than other groups; therefore, each stage was analyzed separately. At 75 and 95 degree-
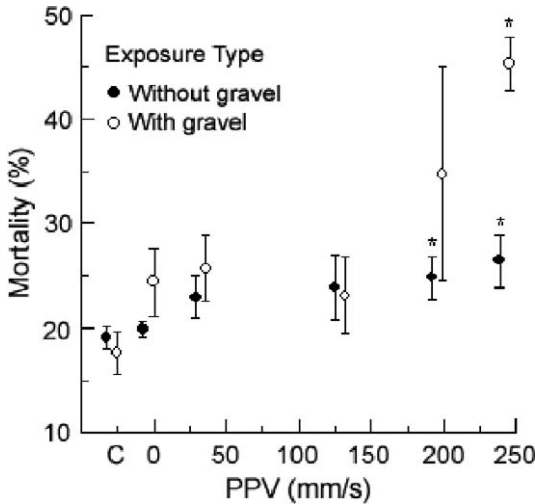
FIGURE 4.—Mortality (mean ± SE) of rainbow trout embryos at 90 degree-days of development exposed to simulated blasts by the weight drop method while being held in containers with and without spawning gravel. Asterisks indicate exposure groups with mortality significantly greater than that of the handled control (Fisher's least-significant-difference test: $P < 0.05$). See Figure 3 for additional details.

days, mortality was similar among the different levels of repeat exposures (75 degree-days: $F_{4,52} = 2.45$; $P = 0.06$; 95 degree-days: $F_{4,52} = 1.72$; $P = 0.16$). At 75 degree-days, mortality differed among exposure levels ($F_{4,67} = 5.33$; $P = 0.001$), although no exposures had mortality higher than the handled control (Figure 3b). At 95 degree-days, mortality did not differ among exposure levels ($F_{4,67} = 2.26$; $P = 0.07$) and was actually slightly less than the handled control in all but the 199.1-mm/s exposures. Overall, there was no increase in mortality due to exposure size or number of repeat exposures over the ranges we tested.

Spawning gravel effects.—Mortality was higher when eggs were held in spawning gravel than when they were held loose within the test containers ($F_{1,39} = 9.50$; $P = 0.004$; Figure 4). Egg mortality also differed among exposure levels for loose eggs ($F_{4,18} = 2.93$; $P = 0.050$); mortality was greater than that of the handled control at 199.1 and 245.4 mm/s (least-significant-difference test: $P < 0.05$). Mortality also differed among exposure levels in the intergravel treatment ($F_{4,17} = 4.95$; $P = 0.008$); however, mortality was only greater than the handled control at 245.4 mm/s (least-significant-difference test: $P < 0.05$).

Probit analysis identified an LD5 of 145 mm/s (95% confidence interval [CI] = 106–167 mm/s) and an LD10 of 168 mm/s (95% CI = 134–185 mm/s) for the

intergravel treatment. Mortality in the nongravel treatment was not high enough to generate LD values.

Drop Height Apparatus

The mortality of handled controls did not differ among stages of development within years or between in- and out-of-water treatments (ANOVA, *t*-tests: $P > 0.05$). However, mortality differed between years ($t = 3.34$, df = 28, $P = 0.002$), probably because of a difference between egg batches. Consequently, each year was analyzed separately.

In 2004 (51, 59, and 67 degree-days), egg mortality differed between treatments (in- and out-of-water: $F_{1,115} = 47.3$; $P < 0.001$) and among exposure levels ($F_{6,115} = 31.3$; $P < 0.001$) but not among developmental stages ($F_{2,115} = 0.86$; $P = 0.43$). Out-of-water mortality differed among exposure levels ($F_{6,55} = 75.2$; $P < 0.001$), and all exposures of 1,400 mm/s or more had mortality higher than that of the handled control (least-significant-difference test: $P < 0.05$; Figure 5a). For the in-water treatment, mortality also differed among exposure levels ($F_{6,56} = 10.3$; $P < 0.001$), but only the 2,801- and 3,961-mm/s exposures had mortalities higher than that of the handled control (least-significant-difference test: $P < 0.05$; Figure 5b).

In 2005 (75 and 95 degree-days), egg mortality also differed between treatments ($F_{1,75} = 28.05$; $P < 0.001$) and among exposure levels ($F_{6,75} = 112.0$; $P < 0.001$) but not among developmental stages ($F_{1,75} = 2.26$; $P = 0.14$). Mortality in the out-of-water treatment differed among exposure levels ($F_{6,35} = 296.1$; $P < 0.001$) and was higher than that of the handled control for 990-mm/s and greater exposures (least-significant-difference test: $P < 0.05$; Figure 5a). For the in-water treatment, mortality also differed among exposure levels ($F_{6,35} = 60.7$; $P < 0.001$); eggs exposed to 2,801 mm/s or more had mortality higher than handled control eggs (least-significant-difference test: $P < 0.05$; Figure 5b).

The estimated LD5s for the out-of-water treatment at 51–67 and 75–95 degree-days (794 and 821 mm/s, respectively) were 66% and 77% of those for the in-water treatment (Table 2). Similarly, the estimated LD10s for the out-of-water treatment during these same periods (970 and 925 mm/s, respectively) were 55% and 74% of those for eggs in water (Table 2). The differences between out-of-water and in-water LD5 and LD10 values were smaller during the later-stage exposures.

Discussion

Blast Simulation (Weight Drop Apparatus)

The weight drop apparatus attempted to simulate the effects of an out-of-water blast based on field
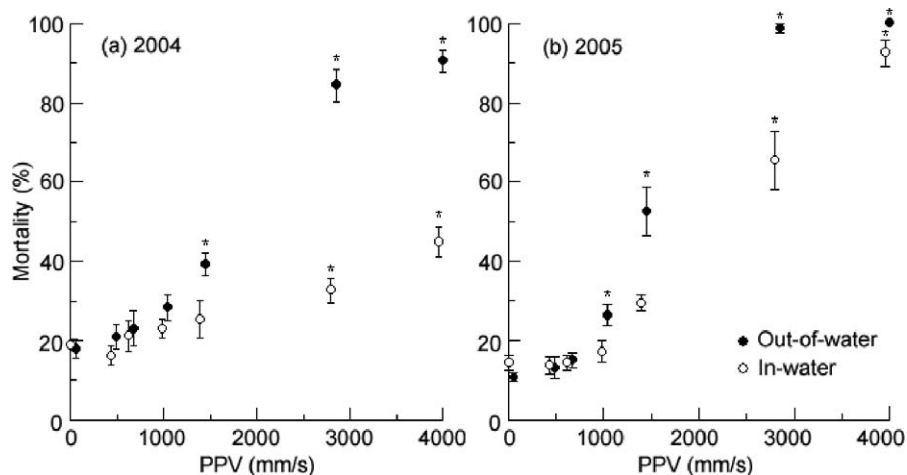
FIGURE 5.—Mortality (mean ± SE) of rainbow trout embryos exposed to simulated blasts by the drop height method while being held in or out of water in **(a)** 2004 (51, 59, and 67 degree-days combined) and **(b)** 2005 (75 and 95 degree-days combined). Asterisks indicate exposure groups with mortality significantly greater than that of the handled controls (Fisher's least-significant-difference test: $P < 0.05$). See Figure 3 for additional details.

measurements in Lac de Gras (Welz 2006). The Canadian guidelines use PPV to establish maximum allowable blasting exposure, and therefore it is the variable measured in monitoring around blasting operations (Wright and Hopky 1998). Although PPV is believed to be the most important component affecting fish eggs, other factors of blasting may also be important, such as the duration of the exposure, the peak acceleration of the substrate, and the energy content of the seismic waves.

The weight drop procedure initially produced higher peak accelerations than those measured in Lac de Gras. In response, we added padding and made other improvements to the apparatus; however, frequencies remained higher, largely because of the small scale of the apparatus. Higher frequencies probably result in greater exposure at a given PPV due to the increased acceleration and the small mass of each egg.

TABLE 2.—Drop height exposures of rainbow trout eggs (peak partical velocity [mm/s]; mean and 95% confidence interval) estimated by probit analysis to cause 5% and 10% mortality (LD5 and LD10, respectively) for out-of-water and in-water treatments at 51, 58, and 67 degree-days (stages combined) and 75 and 95 degree-days (stages combined) of development.

Treatment	Stage (degree-days)	LD5	LD10
Out of water	51–67	794 (464–1,059)	970 (620–1,238)
In water	51–67	1,195 (459–1,663)	1,756 (983–2,182)
Out of water	75–95	821 (747–884)	925 (856–985)
In water	75–95	1,060 (902–1,200)	1,254 (1,095–1,393)

The relative power of single simulated blasting events was lower than that of real blasting events of comparable PPVs because the simulated events had shorter durations and higher frequencies. By using repeated exposures in 2005, the laboratory simulations achieved durations and energy levels similar to those of the actual blasts. The mortality of eggs did not increase from these repeated exposures; therefore, it is likely that the peak energy content of the wave (estimated by PPV) is indeed the most important factor in predicting egg mortality due to blasting.

Because mortality was calculated at the eyed stage (stage 18 [about 150 degree-days]; Table A.1), some additional mortality prior to hatching was possible. When some eggs were left to incubate, however, increases in mortality were relatively small for both the weight drop and drop height treatments; the maximum increase was only 3.6% (S.G.F., unpublished data). More importantly, the increased mortality was similar among all exposure sizes within all treatments. Therefore, using mortality at the eyed stage probably did not bias our results.

It is also important to note that calculations of mortality at the eyed stage included damaged eggs, characterized as being partially white, marbled in appearance, or containing a small white streak. Although most mortality occurs within 2 d of exposure (e.g., Smirnov 1954, 1955; Johnson et al. 1989; Jensen and Collins 2003), delayed mortality has not yet been reported. In our study, many damaged eggs developed embryos with pigmented eyes, indicating that development had continued for many days after exposure.

TABLE 3.—Comparison of drop height, peak particle velocity (PPV), and energy estimated by probit analysis to cause 10% mortality in eggs of Pacific salmon. Data are from (1) the current study and (2) Jensen and Alderdice (1989). Most exposures involved eggs free of surrounding water. The results for our in-water treatment are included for comparison.

Species	Source study	Exposure in water?	Height (cm)	PPV (mm/s)	Energy (J; $\times 10^{-5}$)
Steelhead[a]	2	No	0.94	429	1.40
Chinook salmon	2	No	0.39	276	1.40
Coho salmon *O. kisutch*	2	No	0.65	357	1.80
Rainbow trout	1	No	4.37	925	2.64
Chum salmon *O. keta*	2	No	1.8	594	4.40
Pink salmon *O. gorbuscha*	2	No	2.4	686	4.80
Sockeye salmon *O. nerka*	2	No	3.8	863	5.00
Rainbow trout	1	Yes	8.02	1,254	4.84

[a] Anadromous rainbow trout.

Many of these eggs continued to develop to the hatching stage; however, all died at or just before hatching. Thus, including damaged eggs as dead eggs is imperative for an accurate assessment of mortality from exposures during earlier developmental stages. Although our study focused on exposures during the sensitive epiboly and convergence stages of development (Velsen 1987), shock and other physical disturbance in later stages may induce premature hatching, which may increase subsequent mortality of larvae (D. Wright, Fisheries and Oceans Canada, personal communication).

All six stages of egg development exposed to our weight drop simulations were within the sensitive period (Johnson et al. 1983, 1989; Jensen 2003; Jensen and Collins 2003) and corresponded to all stages of epiboly. Of course, care must be taken when handling eggs at this time owing to the potential to cause mortality (e.g., Johnson et al. 1983, 1989). Comparison of handled and nonhandled controls indicated that handling did cause some mortality (up to 6.6%); however, our experiments minimized this potential bias by using exposure- and stage-specific handled controls.

When eggs were held loosely in the test containers (i.e., nongravel treatment), there was an increase in egg mortality only when exposures were conducted at 90 degree-days. However, the statistical significance of increased mortality (by 4.9%) after repeated exposures of 199.1 mm/s or more resulted primarily from exceptionally low variability in the handled controls at this stage, which produced an exceptionally sensitive analysis. Indeed, exposures of 219.3 mm/s did not increase mortality relative to handled controls.

Spawning gravel increased egg mortality and variability in mortality. As a result, only at 245.4 mm/s was mortality significantly above that of handled control eggs. Although the mean mortality of intergravel eggs at 199.1 mm/s was also above that of the handled control eggs (by 10.3%), exposures of at least 132.3 mm/s showed no sign of increased mortality. The

reason for the increased mortality in intergravel eggs is not known, but the movement of gravel substrate in some of the containers might have been a factor.

Our spawning gravel results were generally consistent with those of Post et al. (1974), who used similarly sized gravel in their blast simulation. They found no increase in mortality despite five repeated vertical acceleration exposures up to approximately 98.0 m/s^2, which is comparable to our findings that vertical accelerations exceeding 51.3 m/s^2 (and probably closer to 184 m/s^2) were required to increase mortality during the most sensitive period of egg development.

To best define a PPV response curve via probit analysis, much higher mortality (~50%) and thus higher PPV levels should be used. The limitations of our sensors (maximum detection level of 256 mm/s in each orthogonal direction) prevented the use of higher PPV levels in this study. Such extreme PPV levels would be much higher than values expected in blasting operations in or near spawning shoals; however, the resulting response curve could improve the comparison of mortality in the weight drop and drop height methods.

Drop Height Apparatus

In the out-of-water treatment, the average LD10 for mid- to late-epiboly stages (PPV = 925 mm/s) was somewhat higher than the average (429 mm/s) for a similar stage reported by Jensen and Alderdice (1989; Table 3), although the 95% CIs apparently overlapped (J. O. T. Jensen, Fisheries and Oceans Canada, personal communication), suggesting that the differences were not significant. Furthermore, our LD10 for energy (J) was within the range Jensen and Alderdice (1989) reported for Pacific salmon (Table 3). We made every attempt to replicate their apparatus and methodology. Still, there may have been differences in sensitivity between the source populations. Jensen and Alderdice (1989) used eggs from a wild anadromous source, whereas our source was a domestic freshwater strain (but see Krise 2001).

The LD5 and LD10 were greater for in-water than for out-of-water exposures (similar LD10 results were seen for Chinook salmon; Jensen and Alderdice 1983); the magnitude of the difference was considerably greater for early-epiboly trials (51–67 degree-days) than for later-stage trials (75 and 95 degree-days). This suggests that although water consistently provides protection, the degree of protection is reduced when eggs are at a more sensitive stage. Eggs held in water probably experience less deformity on impact because of the greater density and viscosity of water relative to air. This, in turn, suggests that Jensen's (2003) LD10 estimates are conservative for eggs in an aquatic environment, assuming that estimated final velocities achieved in the drop height approach accurately simulated the PPVs produced by blasting.

Lacking overlap in either PPV exposure levels or mortality for similar stages tested by the weight drop and drop height procedures, we cannot directly assess the latter's ability to realistically simulate a blasting event. The lowest exposure to cause significant mortality in our weight drop simulations was 199.1 mm/s, which corresponds to a drop height of only 2 mm. At such low heights, the drop height apparatus could not reliably produce PPV exposures.

Although the accuracy of the drop height method remains unproven in an absolute sense, it does provide a relatively simple method for comparing the sensitivity of eggs among species and developmental stages. It has already been used to describe the sensitivities of Atlantic salmon *Salmo salar* (Krise 2001), milkfish *Chanos chanos* (Hilomen-Garcia 1998), Chinook, chum, coho, pink, and sockeye salmon, and steelhead (anadromous rainbow trout) (Jensen and Alderdice 1983, 1989). However, egg mass must be taken into account when comparing sensitivities among species, because the energy imparted on an egg in the drop height procedure is directly related to its mass.

Although the current PPV guideline may provide more than ample protection to incubating eggs from shaking of the substrate, further research may be needed to ensure the structural integrity of spawning habitats at higher PPVs. Indeed, the 13-mm/s guideline also represents a level below which the explosives industry is confident that structures in the vicinity of the detonation will not be damaged (D. Wright, personal communication). Future work on blasting effects should also focus on determining the egg sensitivity of other species of interest using both the weight drop and drop height methods.

Acknowledgments

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Appendix: Salmonid Embryo Stages

TABLE A.1.—Salmonid embryonic developmental stages relevant to our study and the approximate corresponding degree-days for these stages in rainbow trout (from Velsen 1987 and Jensen 2003).

Vernier stage	Degree-days	Description
9	50–60	Appearance of embryonic shield, germ ring, and terminal node
10	60–70	One-third epiboly; germ ring one-third of the way toward total overgrowth of the yolk; neural groove on the embryonic shield
11	70–80	One-half epiboly; overgrowth of the yolk half completed and germ ring at the equator; formation of axial strand and neural keel; first somites; Kupffer's vesicle
12	80–90	Three-fourths epiboly; germ ring one-third overgrown; optic anlagen and three brain vesicles
13	85–95	Yolk plug less than head width; germ ring narrowing toward vegetal pole; blastoderm nearly covering entire yolk; otic and optic placodes
14	90–100	Yolk plug closed; yolk enclosed in cellular envelope (blastoderm)
18	130–150	One-fourth of yolk surface vascularized; pectoral fin buds present; posterior half of body free from yolk sac; faint eye pigmentation
20	180–210	Eyes fully pigmented; yolk sac three-fourths vascularized; head free and mouth open; cerebral hemispheres open
21	190–220	Caudal flexing of vertebral column; mesenchyme concentrations in caudal and anal fins; pectoral fins twitching