Puntledge River Water Use Plan

Egg Incubation Success in Reach C

Reference: PUN-220.4D

Puntledge River Egg Incubation Assessment 2006-2007 Results

Study Period: Sept 2006 – March 2007

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Puntledge River Egg Incubation Assessment, 2006-2007 Results

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EXECUTIVE SUMMARY

In 1997, minimum flows in Reach C of the Puntledge River were increased from 2.7 m^3/s to 5.7 m^3/s. This higher minimum flow was retained as a recommendation by the Puntledge Water Use Plan Consultative Committee (PUN WUP CC). The CC also recommended implementation of a 2 year study to monitor incubation success of salmonids in Reach C to determine whether this minimum flow constraint allowed for successful incubation of salmonids in Reach C. The specific objectives of the study were to: 1) assess incubation success of salmonid eggs in response to the 5.7 m^3/s minimum flow recommended for Reach C, and 2) develop a predictive model that links flow and associated physical parameters with incubation success.

This report presents the results of year 1 of this study (fall 2006 to spring 2007). As per the project terms of reference, the year 1 report focussed on compilation of a background information review, and presentation of methods and results for year 1. Development of the model linking incubation success, physical incubation parameters, and flow is to be performed after year 2 results are complete. Nevertheless, some preliminary relationships were examined using the year 1 data.

The literature review component covered the topics of chinook and steelhead life history, their habitat requirements, the effects of flow regulations on incubation success, status of spawning habitat in Reach C, and previous incubation monitoring projects on the Puntledge River. This information is presented at the beginning of the report (p. 2).

The field study involved monitoring at 3 sites in Reach C of the Puntledge River: pipeline crossing (Site 1) located 500 m downstream of the Browns River confluence, Bull Island Sidechannel (Site 2) located 300 m upstream of Stotan Falls, and Barbers Hole (Site 3) located 250 m downstream of the Puntledge Diversion Dam. At each site, 4 microsites were established across a transect that encompassed the best spawning gravel at the site. Three incubators were installed at each microsite. Each incubator was loaded with 200 green fall chinook eggs obtained from the Puntledge Hatchery. A control group of 6 cassettes remained at the Puntledge Hatchery incubation facility. Incubation success was assessed at the eyed, hatch, and button-up developmental stages by pulling one incubator from each microsite at each of these stages, and 2 cassettes from the control group.

Water level, water column temperature, and intergravel water temperature were monitored using continuous recording devices (15 minute intervals) installed at each site. Other environmental parameters were monitored by weekly visits to each site. These parameters include depth, velocity, intergravel and water column DO, intergravel and water column conductivity, intergravel and water column pH, and turbidity. These were either measured in triplicate at each microsite or at one representative microsite for each study site. Lastly, substrate composition at the monitoring sites was assessed 3 times during the course of the study using both visual estimation and Wolman pebble counts.

Hourly flows during the monitoring period (October 6, 2006 to March 8, 2007) ranged from 5.4 to 160 m^3/s at Site 3 (based on Gauge 6), and from 5.8 to 224 m^3/s at Site 1 (Gauge 6 + Browns). Flows captured by the weekly environmental monitoring visits were substantially less than these due to an inability to wade the river at high flows. These data were collected under flows up to 13 m^3/s at Site 1 and 28 m^3/s at Site 3. Site 2, being a sidechannel with a deflector weir at the entrance, was more manageable, and we collected environmental data at mainstem flows up to 32 m^3/s (Gauge 6).

Mean daily intergravel temperatures during the study period were similar at Sites 2 and 3, but averaged 2 °C lower at Site 1. The lower temperatures at Site 1 were attributed to cold water input from the Browns River. The range in mean daily intergravel temperature over the incubation period was 2.3 to 14.3 °C at Site 1, 4.0 to 14.8 °C at Site 2, and 4.4 to 15.2 °C at Site 3. Water column temperatures were very similar to intergravel temperatures at all sites.

Assessment of spawning substrates using pebble counts showed similar particle size distributions and particle size descriptors at Sites 1 and 3 (Pipeline and Barbers Hole), while Site 2 (Bull Island Sidechannel) substrates showed a narrow distribution spanning 16 to 90.5 mm. The particle size characteristics at Site 2 were due to placement of screened gravels into Bull Island Sidechannel in 2002 and 2003. Visual substrate assessments, though more subjective, were better at providing information on the level of fines at the monitoring sites. These assessments indicated that Sites 1 and 2 had relatively low percentages of fines (averages of 9 and 2%, respectively), while Site 3 (Barbers Hole) had a higher percentage of fines (average of 17%).

Weekly environmental monitoring resulted in 14 visits to Sites 1 and 2, and 12 visits to Site 3. Some field trips had to be cancelled due to high flows. Intergravel and water column DO tended to increase over the monitoring period, perhaps due to increased solubility of oxygen as water temperatures declined. Site 3 appeared to have lower intergravel oxygen levels than the other two sites, perhaps due to the greater percentage of fines at this site. Depths and velocities were variable among visits and were directly related to flows during the site visit.

Incubation survival from green egg to fry for Sites 1, 2, 3, and the control were 13.6, 40.0, 14.0, and 52.6%, respectively. Thus, overall survival was lowest (and similar) at Sites 1 and 3, higher at Site 2, and highest at the hatchery control. The above values are probably negatively biased due to poor fertilization success in most cassettes. Stage-specific survival rates for the fertilization to eyed egg developmental stage are probably not reliable for this same reason. Stage-specific survival rates generally suggested that the greatest mortality occurred during the hatch to fry developmental stage.

Analysis of year 1 data was confounded by two main factors. First, poor fertilization success due to the use of green eggs introduced external effects and additional variability into the incubation results. Second, the syringe used to extract intergravel oxygen samples yielded suspiciously high DO readings, and resulted in us questioning whether we were getting true “intergravel” values. Methods to improve on the study design and overcome these limitations are provided in the recommendations.

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1. INTRODUCTION

In 2003, the Puntledge River Water Use Plan (PUN WUP) Consultative Committee (CC) recommended several operational changes to BC Hydro's Puntledge River facilities to improve flow conditions for spawning, rearing, and access for the watershed's key indicator fish species (chinook salmon and steelhead trout). One recommendation was to retain the Reach C minimum flow of 5.7 m^3/s that was implemented in 1997 (prior to 1997 the minimum was 2.8 m^3/s). This decision was based on the assumption that increased flow has a positive influence on incubation success by avoiding the extreme low flows that may occur under natural hydrograph situations (BC Hydro 2005). However, no information is available to confirm that the increased minimum flow has elicited an increased in fish production. Therefore, the CC recommended a two-year study to assess incubation success of salmon and steelhead eggs in the reach between the Puntledge Diversion Dam and the powerhouse, (Reach C).

The objectives of the incubation monitoring program are as follows:

1. Assess incubation success of chinook eggs in response to the minimum flow recommended for Reach C in the Puntledge Water Use Plan (5.7 m^3/s).

2. Develop a predictive model that links flow and associated physical parameters with incubation success (to be developed after collection of year 2 data).

The results of this two-year study will assist in determining whether the implementation costs of providing base flows below Puntledge diversion dam are justified by the benefits for egg-incubation.

This report summarizes results from the first year of field evaluations on incubation success at three sites in the Puntledge River, and the environmental variables collected at each site. Fall chinook eggs were used in the assessment and it is assumed that results can be extrapolated to steelhead trout. The report includes a literature review where live history information, flow regulation impacts, status of spawning habitat, and previous incubation studies are summarized. This is followed by presentation of the 2006/2007 field program (methods, results, discussion, and recommendations).

This report does not include presentation of the predictive model (objective 2) – this component will be developed after acquisition of year 2 data, and will be presented in the final report. However, in this report we do explore some preliminary relationships between flow and collected data.

2. LITERATURE REVIEW

2.1 Life History Synopsis

Chinook

The Puntledge River supports both an early (summer) run and a late (fall) run of chinook salmon. The two runs have discrete migration timings and spawning distribution in the river. However both stocks spawn at the same time (October to early November).

Summer-run chinook salmon enter the river from May to August. Females are predominantly 4 years old (range 3-5 yrs) while males are mainly 3 and 4 years old (range 2-5 yrs). Although the two stocks are genetically distinct, it is suspected that the summer-run stock evolved from early migrants of the fall-run stock that were able to negotiate Stotan and Nib Falls as flows decreased after peak spring freshet between June and August (Marshall, 1972). Summer-run adults originally utilized spawning habitat above Stotan Falls and more predominantly, in a 4 kilometre section of river immediately below the outlet of Comox Lake. This section is presently located between BC Hydro's Diversion Dam and the Comox Lake Impoundment Dam and is referred to as the Headpond. Historically, summer chinook adults entering the system would have migrated upstream quickly, and likely held in the cooler depths of Comox Lake during the summer to escape elevated river temperatures until they were ready to spawn in the fall. They would then drop back downstream and spawn below the lake outlet while some would have spawned in the main lake tributaries, notably the Cruickshank and Upper Puntledge Rivers.

Fall-run chinook salmon are larger than the summer-run chinook and enter the river from September to October. Adults normally spawned downstream of the Browns River confluence (Bengeyfield and McLaren, 1994). However, modifications to Stotan and Nibs Falls to improve fish passage in the 1960s and 70s have now facilitated the access of fall chinook and other salmon species into the upper reaches of the river.

Annual escapement records for summer-run chinook show numbers averaging around 3,000 prior to 1955 followed by a rapid decline to an average of 460 for the 20 year interval through the 1960s and 70s. The population began to recover slightly through the 1980s with a high of 1,950 and then declined again through the 1990s to a ten-year average of 430. Fall-run chinook salmon follow a similar trend in declines from an average of 3000 prior to 1960 to an average of 560 in the subsequent 4 decades. During the 1980s, three successive years of escapement levels below 100 prompted a transplant of fall-run chinook eggs from Big Qualicum and Quinsam rivers in attempts to create a new Puntledge River stock and restore the population. Escapements in the past 5 years have been well above historical levels.

Puntledge summer and fall-run chinook are predominately "ocean-type" fish. After emerging from the gravel in March, fry begin to move downstream with peak migration occurring in April.

Lister (1968) noted that many chinook fry from natural spawning in the Upper hatchery channel in 1966-1968 migrated downstream in March/April while the remainder stayed until June/July reaching lengths of 70-80 mm before dispersing downstream. Since the commencement of sex specific counts of returning adults in 1965, the ratio of male to female spawners has varied significantly among both stocks. The ratio has ranged from 2 males for every female to as high as 9 to 1 (Trites et al. 1996).

Steelhead

The Puntledge River supports both summer and winter-run steelhead. Summer steelhead enter the river between August and September while winter steelhead enter the river from December (early component) through May (late component; C. Beggs, pers. comm.). Both runs spawn from February until April/May (Bengeyfield and McLaren, 1994). Historically, the main spawning area for summer steelhead was in the reach below the lake outlet (Headpond) and the Cruickshank and Upper Puntledge River tributaries. Winter-run steelhead spawn below Stotan Falls and in the Browns and Tsolum rivers (Bengeyfield and McLaren, 1994). Presently, both the summer and winter-run steelhead in the Puntledge River are considered to be at a high risk of extinction with returns in the last decade below 100 and recent returns below 30 (Rimmer et al., 1994; Wightman et al., 1998). The trend of declining steelhead returns is mirrored in several other east coast Vancouver Island and lower mainland streams. This has lead to the development of “A Recovery Plan for East Coast Vancouver Island steelhead trout” (Wightman et al. 1998), and more recently the “Greater Georgia Basin Steelhead Recovery Action Plan” (Lill 2002). The main objective of the recovery plan is to reverse the declining trends and return steelhead stocks to secure and self-sustaining numbers.

One “stock rebuilding” technique being implemented through the recovery plan is the Living Gene Bank (LGB) program. The objective of the LGB is to accelerate the rebuilding of 3 wild steelhead populations (Little Qualicum River, Quinsam River and Keogh River) to self-sustainable levels while maintaining the genetic diversity found within these stocks. A similar captive breeding program was initiated in 1998 for the Puntledge River steelhead stocks (both summer-run and winter-run). While the overall goal of stock rebuilding was the same, the emphasis was on sport fishery enhancement rather than preservation of wild stock genetics. The Puntledge LGB program was terminated in 2002 after a 5 year period (Anon. 2005), due to the low adult returns and limited success of the program observed in this watershed (C. Wightman, pers. comm.).

Habitat Requirements

Depth and velocity habitat suitability index (HSI) curves for spawning chinook and steelhead developed for the WUP process are illustrated in Figure 1 (chinook) and Figure 2 (steelhead). These provide generalized depth and velocity preferences for spawning by these species. Spawning and incubation requirements for various parameters suggested by other authors are provided in Table 1. Predicted embryonic development times for chinook and steelhead based on accumulated thermal units (ATU’s) are summarized in Table 2.

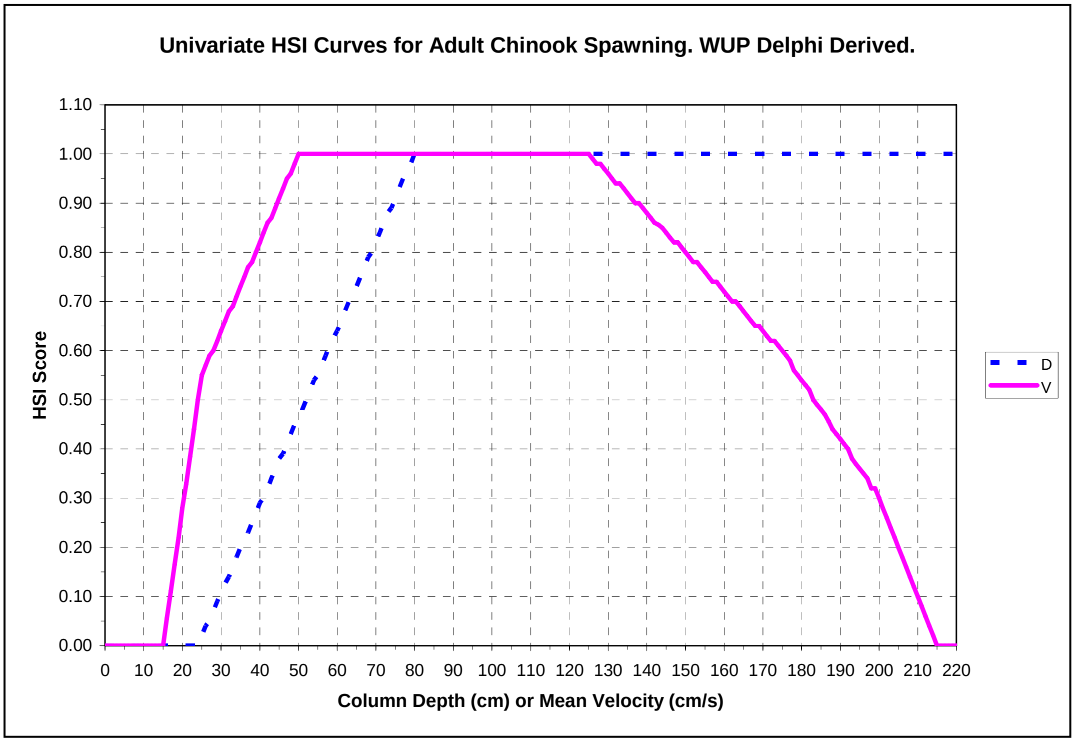

Figure 1. Depth and velocity habitat suitability index (HSI) curves for chinook spawning developed for WUP projects (developed in 2001 by Delphi process).

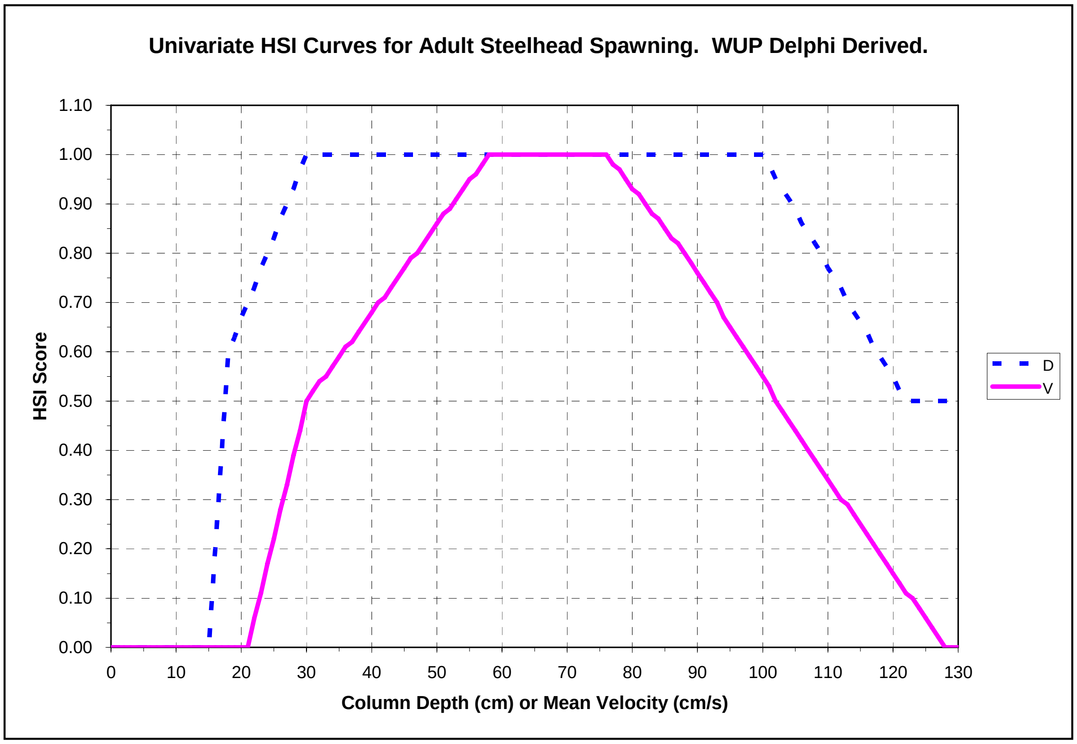

Figure 2. Depth and velocity habitat suitability index (HSI) curves for steelhead spawning develped for WUP projects (developed in 2001 by Delphi process).

Table 1. Spawning and incubation requirements of chinook and steelhead.

Parameter	Chinook	Steelhead	Source
Spawning			
Substrate	1.3–10.2 cm	0.6–10.2 cm	Bell (1986); Hunter (1973)
Depth	≥ 30 cm (summer run) ≥ 24 cm (fall run) ≥ 35 cm (HSI ≥ 0.20)	— ≥ 24 cm ≥ 16 cm (HSI ≥ 0.20)	Reiser and White (1981) Thompson (1972) WUP HSI curves
Velocity	32–109 cm/s (summer run) 30–91 cm/s (fall run) — 19-205 cm/s (HSI ≥ 0.20)	— — 40–91 cm/s 25-118 cm/s (HSI ≥ 0.20)	Reiser and White (1981) Thompson (1972) Smith (1973) WUP HSI curves
Temperature	10–17 °C (observed in BC) 5.6–13.9 °C (recommended) > 22 °C (cannot spawn) 25.1 (upper lethal temp.) 5-15 °C (spawning migration)	3.9–9.4 °C	Shepherd et al. (1986) Bell (1986) Beauchamp et al. (1983) Brett (1957) Walthers and Nener (1997)
Incubation			
Substrate Fines: Particles < 2 mm Particles < 3 mm Particles < 6.35 mm	Maximum Acceptable Levels : 10% 19% 25%	Maximum Acceptable Levels : 10% 19% 25%	BC Approved Water Quality Guidelines (MoE 1998) Canadian Water Quality Guidelines (CCME 1999)
Intergravel Oxygen	5.0 mg/L 6.0 mg/L (instantaneous min.) 8.0 mg/L (30-day mean)	— 6.0 mg/L (instantaneous min.) 8.0 mg/L (30-day mean)	Leitritz and Lewis (1980) BC guidelines (RIC 1998)
Temperature	5.0–14.4 °C	—	Bell (1986) J. Jensen's studies

Table 2. Predicted embryonic development times (in days and ATUs) for Chinook salmon and Steelhead using models listed in Table 3 in Billard and Jensen 1996.

Species	Temp	Yolk plug closure		Eyed stage		50% hatch	
	(°C)	Days	ATUs (°C-days)	Days	ATUs (°C-days)	Days	ATUs (°C-days)
Chinook	5	26.7	133.5	51.5	257.5	102.4	511.8
	7.5	17.9	134.5	34.2	256.6	70.3	527.5
	10	13.4	133.5	24.9	249.2	52.6	526.4
	12.5	10.6	132.1	19.2	240.5	42.1	525.7
Steelhead	5	17.6	88	34.3	171.4	70.7	353.4
	7.5	11.7	87.5	23.9	179.5	47.2	354
	10	8.5	84.6	17.1	171	32.9	328.6
	12.5	6.5	81.1	12.5	155.9	24.8	309.8

2.2 Effects of Flow Regulation on Incubation Success

Effects of flow regulation on incubation success in the Puntledge River have not been specifically studied. Incubation studies have been conducted on the river but these have been to assess the merit of gravel placement in the headpond reach, and to monitor incubation success within gravel installed in Bull Island Sidechannel (Wright and Guimond 2003). Given the lack of previous studies, effects can only be surmised based on changes in flow that have occurred since regulation and by habitat conditions observed in this reach today. The following are possible effects of flow regulation on incubation survival in Reach C.

Impoverished and poor quality spawning habitat

A common consequence of dam installation is that these structures intercept sediments preventing their transport to downstream reaches. This leads to depletion of streambed sediments in these reaches, the severity of which depends on the extent of sediment inputs from banks and tributaries downstream of the dam. If downstream sediment sources are inadequate, the streambed may eventually be scoured to a state where all that remains is an armoured layer of the larger particle sizes (Kondolf 2000). Depletion of sediments (spawning gravels) has been suggested for reaches downstream of the diversion dam on the Puntledge River (Reaches C and D) (Bengeyfield and McLaren 1994; Guimond 2002) though the extent of the change is uncertain due to lack of documentation of pre-regulation habitat conditions. Today natural spawning habitats in Reach C are limited to Barbers Hole, the area around Bull Island, the Gas Pipeline area, and isolated pockets along the river margin (Lough 2003). Furthermore, spawning habitat at these locations is of poor quality in that bed thicknesses are shallow, gravels are mixed with larger substrates (cobbles), and compaction is moderate.

Because of the poor quality of these spawning habitats, fish utilizing these sites may be unable to dig redds as deep as normal, which in turn may result in higher egg mortalities from scour during high flow events. Montgomery (1999) suggested that species spawning in a given reach are adapted to the high flow events of that reach whereby species spawn deeper than the average scour depth, or they spawn at times when scour is not an issue. Species that historically spawned in Reach C include summer chinook, which probably spawn relatively deep, and summer steelhead, which spawn in the spring when freshet events are less likely to be an issue. Thus, effects of diminished gravel supply in Reach C may not only include reduced spawning area, but reduced egg survival in existing habitats due shallower redds and greater susceptibility to scour during flood events.

Geologic data reviewed by Guimond (2002) indicated that the headpond reach flows through a band of post-glacial deltaic sand and gravel deposits. Thus the banks in this region may have historically been a major source of gravel recruitment to downstream reaches. Another sediment source in this reach was likely Supply Creek as evidenced by a large gravel deposit sustained off its mouth today. Thus prior to dam installation, Reach C likely had a more balanced sediment budget whereby gravel recruitment matched downstream movement. It seems plausible that spawning habitats were more abundant, with deeper, looser (less compacted), and more uniformly graded particle sizes.

Reduced flows during incubation

Incubation studies on the Cowichan River found that intergravel oxygen levels at monitoring sites were positively correlated with flow (Burt et al. 2005). The mechanism here is that stream flow directly influences hydraulic gradient across a given redd, which affects intergravel velocity, which in turn affects oxygen supply to the redd (and removal of metabolic wastes) (Wu 2000). Thus, if flow regulation on the Puntledge River has resulted in reduced flows during incubation, a corresponding reduction in intergravel oxygen is expected. Thus, surface flow has the potential to impact incubation survival if it causes oxygen levels within redds to drop below critical levels (the BC guideline for intergravel oxygen include an instantaneous minimum of 6 mg/L and a 30-day mean of 8 mg/L; RIC 1998).

Dewatering of Redds

If flow regulation or unexpected flow reductions due to operational problems result in dewatering of redds during incubation, then egg or alevin mortalities can occur. Under such circumstances, the extent of mortalities are influenced by a number of physical factors including increased or decreased temperatures, drying (desiccation), reduced dissolved oxygen, increased concentration of biotic wastes, and settling of the gravel (Neitzel and Becker 1985). The magnitude and duration of the flow reduction would also be expected to influence egg and alevin survival.

Elevated Water Temperatures during Migration and Incubation

Like many BC rivers, the Puntledge River experiences high water temperatures during the months of July through September. During low summer flows, average daily temperatures in the lower Puntledge River frequently exceed 20 °C and daily maximum temperatures often reach 25 °C (Griffith, 2000). Temperature data has been collected from the Puntledge Hatchery since 1965 and at six additional sites in the river since 1998 (Sweeten 2002). River temperatures in Reach C are for the most part regulated by the thermal surface mass of Comox Lake. This waterbody warms slowly in the Spring and cools slowly in the Fall. Furthermore, it has been noted that the river temperatures tend to increase as one moves downstream in Reach C. The intensity of this warming trend depends largely on the snowpack and air temperature. In 1998 for example, a year with below normal snowpack and warm air temperatures, significant warming by (up to 4.5 °C) occurred between the impoundment dam at Comox Lake and the Browns River ~ 8 km downstream (Griffith, 2000). A mathematical model developed by Sweeten (2002) shows the effect of mean air temperature and mean daily flow on water temperature but requires more temperature data to improve model predictions at the higher temperature ranges. The elevated temperatures in the Puntledge River are likely to have some impact on the productivity of those stocks that are present in the system during this period, namely pink salmon and summer runs of chinook salmon and steelhead.

Although the effects of exposure to elevated water temperatures on various life stages of juvenile and adult salmonids has been investigated (Berman, 1990; Servizi and Jensen, 1977), little information exists on the effects of exposure to high temperatures on the latter stages of egg maturation, gamete quality, fertilization success and egg development. Recent studies in the Puntledge River have attempted to demonstrate the influence of high water temperatures on the latter phases of maturation in pink and summer-run chinook salmon (Jensen et al. 2004; Jensen et al. 2005; Jensen et al. 2006). In the earlier study pink salmon adults were captured during their upstream migration in the river and exposed to three declining water temperature regimes prior to spawning. One group was exposed to a chilled regime (mean 15.1 °C; range 11.6 – 19.4 °C), one group to an ambient regime (mean 18.4 °C; range 15.0 – 21.8 °C) and the third group to a heated regime (mean 21.3 °C; range 16.6 – 24.0 °C). The study clearly demonstrated the negative influence of elevated temperatures on the latter stages of pink salmon maturation. Adults exposed to higher temperatures experienced increased pre-spawn mortality, delayed maturation rates and reduced gamete viability (Jensen et al. 2004). The study was repeated using summer chinook adults in 2004 and 2005. Despite several unanticipated and unexplainable technical difficulties encountered in both years, results from these studies also demonstrated a delay in maturation (2004) and a higher pre-spawn mortality (2004 and 2005) in the adults exposed to warmer temperatures (17-19 °C) compared to the “chilled” (8–9 °C) group (Jensen et al. 2005; Jensen et al. 2006).

Effects of High Flows on Incubation Success

High flows can scour spawning beds, and if the scour occurs to the depth of the deposited eggs, then the eggs can be washed away. According to Montgomery et al. (1999), salmonid species utilizing a given river reach tend to be species or of size where eggs are buried deeper than average scour conditions. For example, if typical winter floods scour spawning beds to a depth of 20 cm, species that spawn in that section will tend to plant their eggs deeper than 20 cm (e.g., chinook), or will spawn in the spring when there is less risk of scour (e.g., steelhead). However, if regulation results in regular spill events that exceed the average flood discharge with associated increased depth of scour, then the incubation survival of the species using these reaches will be compromised.

2.3 Spawning Habitat in Reach C of the Puntledge River

Reach C of the Puntledge River extends from the BC Hydro Diversion Dam to the Powerhouse 6.3 km downstream and is further divided into upper and lower sections at the confluence of the Browns River (BC Hydro 2003). This is typically a bedrock/boulder reach dominated by two sets of waterfalls (Stotan and Nib Falls). Historically, these falls were barriers to most anadromous fish except summer runs of chinook and steelhead salmon, but now are capable of providing access for a variety of salmonids since remedial work completed between 1923 and 1977 facilitated fish passage.

In a survey of spawning gravel in Reach C (MJL Environmental Consultants 2003) it was found that 90% of the functioning gravel was located in 3 discrete areas along the river (Barbers Hole, Bull Island and the Gas Pipeline crossing) while the remaining 10% was found in scattered patches along the wetted edge of the channel for a total of approximately 1,955 m^2 of functional gravel. Since that survey was conducted, additional spawning habitat has been made available in Reach C through various gravel placement projects by Fisheries and Oceans Canada and the BCCF (Table 3.).

Table 3. Summary of spawning habitat enhancement projects in the Puntledge River Reach C from 2003-2006.

Location in Reach C	Year Completed	Spawning Habitat Added (m^2)	Source
Bull Island Sidechannel	2003	1308	Guimond and Norgan 2003
Mainstem at Bull Island	2005	538	S. Sylvestri (BCCF) pers. comm.
Below Diversion Dam	2005	454	S. Sylvestri (BCCF) pers. comm.
Barbers Hole, Mainstem at Bull Island on RB	2006	~ 800	S. Sylvestri (BCCF) pers. comm.
Sub-total		3100	
Grand Total		5055	

2.4 Previous Incubation Monitoring on the Puntledge River

In the fall of 2001, surplus summer-run chinook broodstock from the Puntledge River Hatchery were allowed above the diversion dam for the first time since the diversion dam fishway was closed to adult fish passage in 1965. The significant declines in summer chinook escapement in the previous decade as a result of spawning habitat loss, combined with a high incidence in turbine mortality on juvenile salmonids lead to Fisheries and Oceans (DFO) taking the majority of returning adults for hatchery broodstock and preventing access to the historic habitat above the diversion dam.

A study was undertaken in 2001 to monitor the behaviour of adult summer chinook in the headpond and assess the survival of chinook eggs at the confluence of one of the main tributaries in the headpond reach (Supply Creek). This site was one of two remaining areas in the backflooded “headpond” that contained small amounts of functioning spawning gravel. Results from the 2001 incubation study were encouraging (survival to swim-up ranged from 11% to 81%; DFO 2001 Unpublished data). This lead to a more detailed incubation study in 2002/03 at the same location to determine whether spawning habitat in the headpond reach of the Puntledge River could support a spawning population of summer run chinook (Wright and Guimond 2003). In addition, a study was conducted concurrently at the Bull Island sidechannel to monitor incubation success in this recently rehabilitated spawning habitat.

In the headpond, mean incubation survival (egg to fry) of chinook eggs in Jordan-Scotty incubation cassettes was 55 % (range: 16% – 93%; n = 21). Cassettes were buried in water depths ranging from 0.38 to 2.01 metres. However the variability in survival among the cassettes was not correlated to water depth or substrate composition, nor could they be conclusively linked to environmental variables measured during the study (intergravel oxygen and water velocity). Intergravel dissolved oxygen measurements were only conducted in the shallow sites due to the limitations of the sampling equipment. Dissolved oxygen and velocity data were insufficient (not enough data or replicates) to explain the high variability in egg to fry survival at the 21 incubation sites across the channel. Recent incubation studies on the Cowichan River suggest that 30 – 40 dissolved oxygen samples per site are needed to reduce the variance around site means. It has also been speculated that the process of obtaining intergravel DO samples using the metal syringe may cause a positive bias in DO readings due to entrainment of surface water in the shaft hole (Burt *et al.* 2005).

In the Bull Island sidechannel, mean incubation survival of chinook eggs in two constructed spawning platforms was 98% in 2002/03 (range: 96.5% – 100%; n = 17). There was no difference in survival between the two platforms. The high egg-to-fry survival at Bull Island was not unexpected given that the substrate was comprised of screened and washed gravel placed in the channel in the summer of 2002. A follow-up monitoring program was implemented in 2005 to evaluate both the physical and biological performance of the sidechannel three years post construction. Incubation studies were conducted at the two sites used in 2002 following methods in Wright and Guimond (2003), as well as in a third spawning platform that was constructed in 2003. Incubation survival was high at all 3 sites (mean = 98.6%; range: 97% – 100%; n = 21) with no difference among the sites.

3. METHODS

3.1 Site Selection

Incubation sites in Reach C of the Puntledge River that were used for this study were identified in the Puntledge River Water Use Plan: Monitoring Program Terms of Reference (TOR). Site 1 (Gas Pipeline crossing) and Site 3 (Barbers Hole) are located in the Puntledge mainstem and were previously assessed in 2003 (Lough 2003) and associated with transect data collected by Burt (2003). Site 2 (Bull Island Sidechannel) was the focus of two prior incubation assessments (Wright and Guimond 2003; Guimond 2006). These 3 sites were revisited prior to commencement of the study to ensure that they were suitable for chinook spawning, and to identify access points and potential safety issues. A fourth ‘control’ site proposed in the Browns River was eliminated due to the lack of a suitable and/or accessible chinook spawning site identified during a reconnaissance of this system. Locations of these sites are shown in Figure 3.

At each of the selected sites a permanent transect was established across the study area to facilitate placement of incubator microsites and to ensure that weekly spot measurements could be taken at the same location during each monitoring visit.

3.2 Environmental Monitoring

Water Level and Temperature

Solinst water level recorders were installed at each site to record water levels throughout the study. The loggers measure pressure changes produced from changes in water depth and atmospheric pressure. The instruments were placed underwater and suspended from cables in 2 inch perforated metal standpipes that were embedded in the streambed at the transect site. A third recorder (Solinst barologger) was located nearby and used to measure local barometric pressure. This data was used to compensate the water level recorder data.

A Tidbit temperature logger was buried in the gravel beside a Jordan incubator at one microsite in each incubation site to collect intergravel temperature. The logger was left in place until the final incubator check at the “button-up” stage. Water column temperatures were monitored by the temperature sensor of the water level logger. The water level loggers were periodically downloaded in order to calculate the Accumulated Thermal Units (ATU - daily mean temperature multiplied by the number of days of incubation) which was used to estimate the rate of development of eggs and schedule the incubator checks during the study.

Substrate Composition

The particle size characteristics of surface sediments at each incubation site were assessed using a) Wolman pebble counts (Wolman 1954) and b) visual estimation. The Woman pebble counts involved an observer traversing a transect line across each incubation site with heel-to-toe steps. With each step the observer, while looking away, picked up the first particle touched by a pointed index finger at the tip of the boot. The intermediate diameter of the selected particle was measured with a ruler to the nearest millimetre. This was performed until 100 particles were measured. The crew member completing this task differed for some sample dates, however, on any given date the same observer was used at all 3 sites.

The visual substrate assessment involved an observer scanning across the transect line and visually estimating the percentage of each substrate size class. The observer also scooped up a handful of bed material from under the top layer so that subsurface sediments could be incorporated into the visual estimate. Percentages were assigned according to the Wentworth scale (RIC 2000). Table 4 shows these size classes and the visual cues used as an aid in this assessment.

The Wolman pebble counts and visual estimates were conducted 3 times during the study: shortly following incubator installation (late October), at the hatch stage (December), and at the "button-up" stage (Feb-Mar).

The Wolman pebble counts have the benefit of repeatability among different observers, however, they tend to be biased toward larger particle sizes. This bias is because operators tend not to select particles smaller than the tip of the index finger or particles that are too large to pick up. The visual estimates are better able to incorporate smaller particle sizes (e.g. fines) but suffer from poor repeatability (variance) among different observers. Despite the drawbacks of each method, they provided quick and simple means for gathering some quantitative and qualitative data for assessing substrate differences among sites and possible changes in substrate composition over the incubation period.

Table 4. Particle size categories used and visual cues used in the visual substrate assessments.

	Fines	Small Gravel	Large Gravel	Small Cobble	Large Cobble	Boulder	Bedrock
Abbreviation	FI	SG	LG	SC	LC	BO	BR
Size Range (mm)	≤2	>2 – 16	>16 – 64	>64 – 128	>128 – 256	>256 – 4096	>4096
Visual Cue	< lady bug	grape	Tennis ball	Soft Ball	Soccer Ball	> Soccer Ball	> car

Figure 3. Puntledge River Reach C showing locations of egg incubation sites and other features.

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Water Column and Intergravel Parameters

Water column and intergravel parameters were assessed during weekly visits to the 3 incubation sites. Intergravel data were obtained using a 30 cm long metal syringe apparatus to extract a water sample from 20 – 28 cm below the substrate surface (see Photo 12, Appendix B). The water sample was then extruded into a 500 ml nalgene container for measurement of water quality variables. To avoid any equipment bias, the metal syringe was also used to collect water column samples. The following physical parameters were measured during each visit:

Dissolved oxygen — water column and intergravel dissolved oxygen was measured in triplicate (in mg/L and percent saturation) at each cassette microsite using an OxyGuard Handy Mk II oxygen meter.

Depth and Velocity — Water column depth and velocity was measured at the same location at each microsite on each visit. A measuring tape stretched across the site transect line ensured that depths and velocities were recorded at the same spot on each visit. Velocity was measured with a Swoffer Model 2100 propeller type flow meter mounted to a 1.5 m top-setting rod. Readings were taken at 0.6 of the depth with the meter set to display a 40-second average. Depth was measured using the graduations on the top-setting rod. Both depth and velocity were measured in triplicate at each microsite on each visit.

Conductivity — conductivity was measured in triplicate on intergravel and water column samples collected at one representative location at each incubation site using a Horiba Twin B-173 conductivity meter.

pH — pH was measured in triplicate on intergravel and water column samples collected at one representative location at each incubation site using an Oakton waterproof pHTestr.

Turbidity — water clarity was measured directly in the water column in triplicate at each incubation site using a McVann Analite NEP160 turbidity meter for the first five visits. A HACH 2100P Turbidity meter was used for the remainder of the visits. For the latter method, water column samples were collected at each site in 250 ml sample bottles (in triplicate) and taken to Puntledge Hatchery for measurement.

The number of intergravel and water column measurements for each of the above parameters collected at each site per visit are summarized in Table 5. Sites 1 and 2 were visited 14 times while site 3 was visited 12 times. Fewer site visits were made than proposed due to the extended periods of high flows during the study period. The ability to safely complete water sampling activities at Site 1 was hindered at flows exceeding 25 cms (combined Gauge 6 and Browns River discharge), while flows in the range of 14 – 16 cms (Gauge 6 discharge) were the upper limit for monitoring activities at Site 3.

Table 5. Physical parameters measured at each microsite (1, 2, and 3) or at a representative location at each site (4, 5, and 6) and number of measurements during each spot visit.

WQ Variable	Intergravel (# Measurements per Site per Visit)	Water Column (# Measurements per Site per Visit)	Total # Measurements per Site per Visit
1) Dissolved Oxygen (mg/L & % saturation)	12	12	24
2) Average Depth (m)	-	12	12
3) Velocity (m/s, 40 sec average)	-	12	12
4) Conductivity (μS/cm)	3	3	6
5) pH	3	3	6
6) Turbidity (NTU)	-	3	3

3.3 Installation and Monitoring of Incubators

On the day before the incubators were scheduled to be installed in the river, holes were pre-dug in the streambed at Sites 1 and 3 to accommodate Jordan-Scotty Incubation cassettes in an effort to reduce the site preparation time on the day of installation and hence the holding time of the gametes. Holes for the cassettes at Site 2 were prepared on the morning of the incubator installation. The Jordan-Scotty Incubators have blocked escape holes to allow for assessment to the “button-up” stage.

Eggs and sperm were collected from Fall-run chinook salmon at Puntledge Hatchery on 6 October 2006. Eggs from 2 females were collected in separate containers and then pooled and loaded into 43 Jordan incubation cassettes (36 for the study group and 7 for the control group). Milt from 13 males was collected in individual Whirlpak bags. Each milt sample was visually inspected for quality (absence of water or blood) and then all samples were combined and then divided into three 50 ml containers. Loaded cassettes and milt were stored in coolers and transported to the three incubation sites in 2 batches. The first batch of cassettes was transported to Sites 2 and 3. The crew then returned to the hatchery to load the final cassettes for Site 1 and the hatchery control group. Once at the site, cassettes were fertilized on shore with a syringe containing 3 – 4 ml of milt. The syringe ingredients were discharged onto the cassette and the cassette immediately immersed in a basin of water and gently agitated to allow the sperm to thoroughly mix with the eggs in the cassette (approximately 1 minute). The fertilized cassette was then passed to a technician who buried it in the streambed at a minimum depth of 40 cm (i.e. approximately 25 cm of gravel over the cassette). The process was repeated for all cassettes. Each cassette was secured by a 50 cm length of ¼ inch polypropylene rope to a 40 cm length of rebar, painted fluorescent orange, and situated beside the cassette. At each incubation site, 3 cassettes were buried in one of 4 microsites along the transect, for a total of 12 cassettes per site.

A control group of eggs remained at Puntledge Hatchery to compare survival in optimum "hatchery" conditions to natural river conditions. This group was treated similar to the study group wherever possible. Eggs were loaded into 6 Jordan cassettes and fertilized with remaining pooled milt from the 13 males. Cassettes were then placed in Heath incubation trays.

Assessment of incubation success was checked during three stages: 3 – 4 weeks after installation at the eyed stage; 8 – 9 weeks after installation at the hatching stage; and 16 – 18 weeks after installation at the button-up/swim-up stage (Table 6). The incubators used in this study (Jordan-Scotty Incubators) have blocked escape holes which permits assessment to the "button-up" stage.

For each stage, one cassette from each microsite was removed and assessed. The contents of the incubator were emptied into a shallow basin and the number of dead and live eggs/alevins and fry were enumerated. Cassettes were not replaced in the gravel after assessment due to the amount of site disturbance that would be necessary to excavate the incubator hole. This disturbance could alter the intergravel conditions of flow, permeability and dissolved oxygen, thereby altering the incubation environment of the cassettes remaining in the gravel.

Table 6. Calculated ATUs for each developmental stage assessed during the study at each incubation site.

Date	Developmental Stage	Site 1 (Pipeline)	Site 2 (Bull Island)	Site 3 (Barbers Hole)
2-Nov-06	Eyed	338.00	355.31	367.61
8-Dec-06	Hatch	-	629.34	657.39
29-Dec-06	Hatch	602.33	-	-
9-Feb-07	Button-up	-	941.61	976.61
8-Mar-07	Button-up	826.14	-	-

4. RESULTS

4.1 General Habitat Characteristics

Site 1 – Gas Pipeline Crossing

Site 1 is located approximately 500 m downstream of the Browns River confluence. At this location, the channel is wide (~ 65 m) and has a mid-channel bar containing willow and a few shrub alder. At base flow (5.7 cms), the bar splits the river into 2 channels. The right channel is predominantly bedrock while the left channel contains a mix of gravels, cobbles, and some boulders. The incubation site was located in the left channel and situated on the best gravel at this location (see Photo 5). Substrate data collected at this site pertained specifically to the transect established across the incubator installation area. Visual estimates of this substrate indicated a dominance of small and large gravel, and small cobble within Site 1 (Table 7). At base flow (5.7 cms), the average depth along the transect was 0.32 m. The site was heavily utilized by fall chinook spawners and to a lesser degree by coho during the study period. For the 3 weeks following cassette installation, field staff found several cassettes with their surfaces exposed by spawning fish. This site was also influenced by the Browns River which resulted in cooler temperatures (water column and intergravel), a greater fluctuation in flows, and slightly higher turbidity compared to Sites 2 and 3 (details provided in the Environmental Monitoring Results section).

Table 7. Substrate characteristics of the 3 incubation study sites on the Puntledge River based on visual estimates of the percentage of Wentworth scale size categories.

	Percent of Each Size Category						
	Fines (≤2 mm)	Small Gravel (>2-16 mm)	Large Gravel (>16-64 mm)	Small Cobble (>64-128 mm)	Large Cobble (>128-256 mm)	Boulder (>256-4096 mm)	Bedrock (>4096 mm)
Site 1 (Pipeline)	9	24	25	25	13	4	0
Site 2 (Bull Island SC)	2	15	74	8	0	1	0
Site 3 (Barbers Hole)	17	23	26	22	11	1	0

Notes: Values are average percentages based on 3 separate visual estimates (October 2006, December 2007, and February or March 2007).

Site 2 – Bull Island Sidechannel

The Bull Island Sidechannel is a natural secondary channel feature located 300 m upstream of Stotan Falls. Historically it was known to be used by summer-run chinook salmon and steelhead trout. In 2002 and 2003, over 3,700 m^3 of spawning gravel was added to the sidechannel creating 2,165 m^2 of spawning habitat for chinook, coho, and steelhead. The gravel additions consisted of a mixture of 15% 6–25 mm diameter (¼ – 1 in.), 50% 25–50 mm diameter (1 – 2 in.), and 35% 50–76 mm (2 – 3 in.). The gravel was placed upstream of Newbury weirs constructed in the channel, forming 3 separate spawning platforms (Guimond and Norgan 2003). The incubation site was located at the uppermost spawning platform, upstream of a large log jam (see Photo 6). Visual estimates of substrate composition by our study indicated a dominance of large gravel (16–64 mm) with lesser amounts of small gravel and small cobble (Table 7). This size distribution is comparable to the gravel placement specifications described above. A large deflector groin was constructed at the upstream entrance of the sidechannel and this structure significantly reduces scouring flows into the channel during floods (Guimond 2006). Chinook salmon were observed spawning immediately upstream and downstream of the transect line and one redd was situated in close proximity to microsite 4. One cassette was found completely displaced on one occasion (upstream cassette at microsite 1; discovered October 20, 2007) and was reburied.

Site 3 – Barbers Hole

Site 3 is located on the river left about 250 m downstream of the Puntledge Diversion Dam. There are two islands in this area which split the river into three channels. The right and middle channels (facing downstream) are the largest and carry the bulk of flows while the left channel is smaller and carries proportionally less volume. Site 3 is located in a section of natural spawning habitat in the leftmost channel (see Photo 7). About 60 m downstream of this site is a large deep pool called Barbers Pool and all three channels empty into this pool. The substrate at the incubation site appears to be derived mainly of sandstone and is flat and more angular than the gravels found at the other sites. Nevertheless, fall chinook salmon were observed spawning in this substrate beginning about 5 m downstream of the incubation transect line. Our visual estimates of substrate at this site indicated a composition dominated by small and large gravel, small cobble, and fines (Table 7). This site had a notably higher percentage of fines than the other incubation sites, particularly on the left half of the transect line.

4.2 Environmental Monitoring Results

Stream Discharge

Flows (hourly averages) during the study period (October 6, 2006 – March 8, 2007) were obtained from BC Hydro Gauge 6 below the diversion dam (WSC Gauge No. 08HB084) and from the Browns River. Discharges at Site 3 were determined from Gauge 6 data, while Site 1 flows were determined by taking the sum of Gauge 6 and Browns River data (Figure 4). For Site 2 in the Bull Island Sidechannel, discharge was calculated using the water level data collected during the study and applying the stage-discharge relationship developed during the 2005/06 Bull Island post-construction assessment (Guimond 2006). This is illustrated in Figure 5 and compared with discharge from Gauge 6 on the Puntledge River.

Flows associated with the environmental monitoring and incubation assessment events are denoted by the symbols in Figure 4. The chart shows that there were three extended periods of high flows during the study and that these often precluded completion of spot monitoring events. Daily average flows in Reach C above and below the Browns River confluence were greater than the base flow (5.7 m^3/s) 100 % of the time and greater than 25 m^3/s 21– 27% of the time (Table 8). Our field crew found that operation of the intergravel sampler (syringe) was difficult at flows greater than 25 m^3/s at Site 1, and greater than 16 m^3/s at Site 3 (the highest hourly flow recorded for a sampling event was at Site 1 and was 27.7 m^3/s). The exception was Site 2 which was protected from high flow events by the deflector weir located at the sidechannel intake (Figure 5).

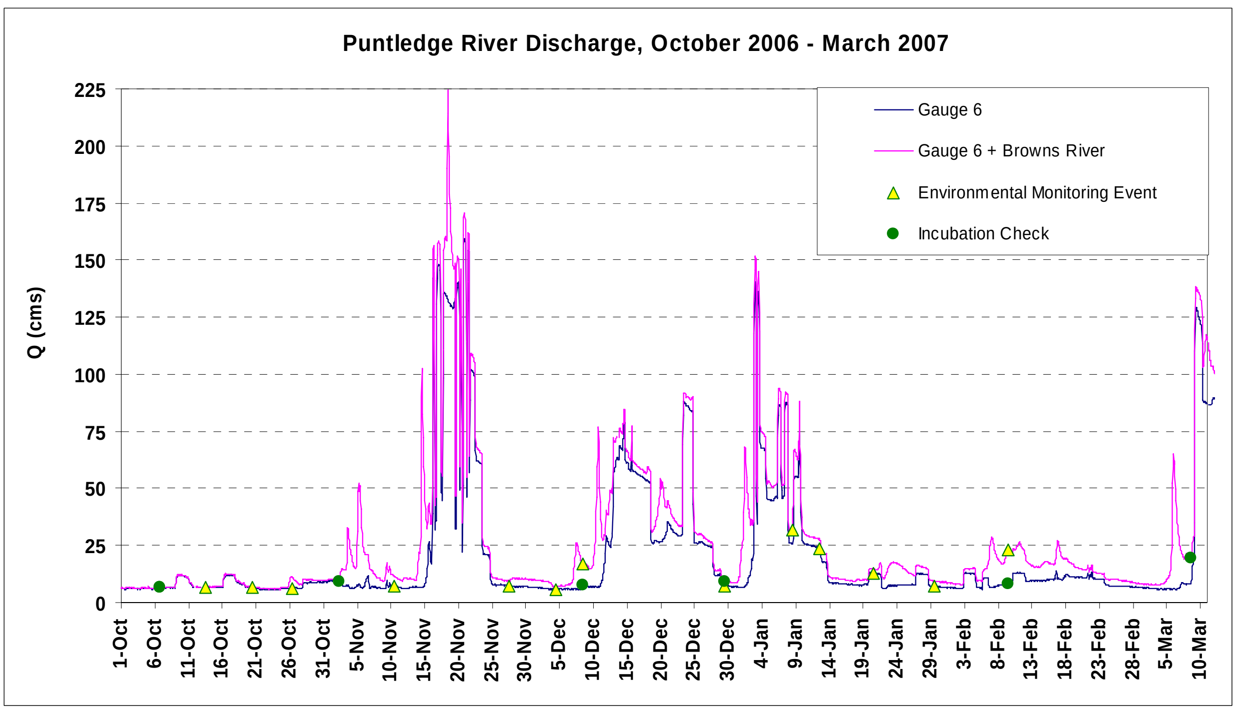

Figure 4. Hourly discharge for the Puntledge River at Gauge 6 below the diversion dam (WSC Gauge No. 08HB084) and below the Browns River confluence.

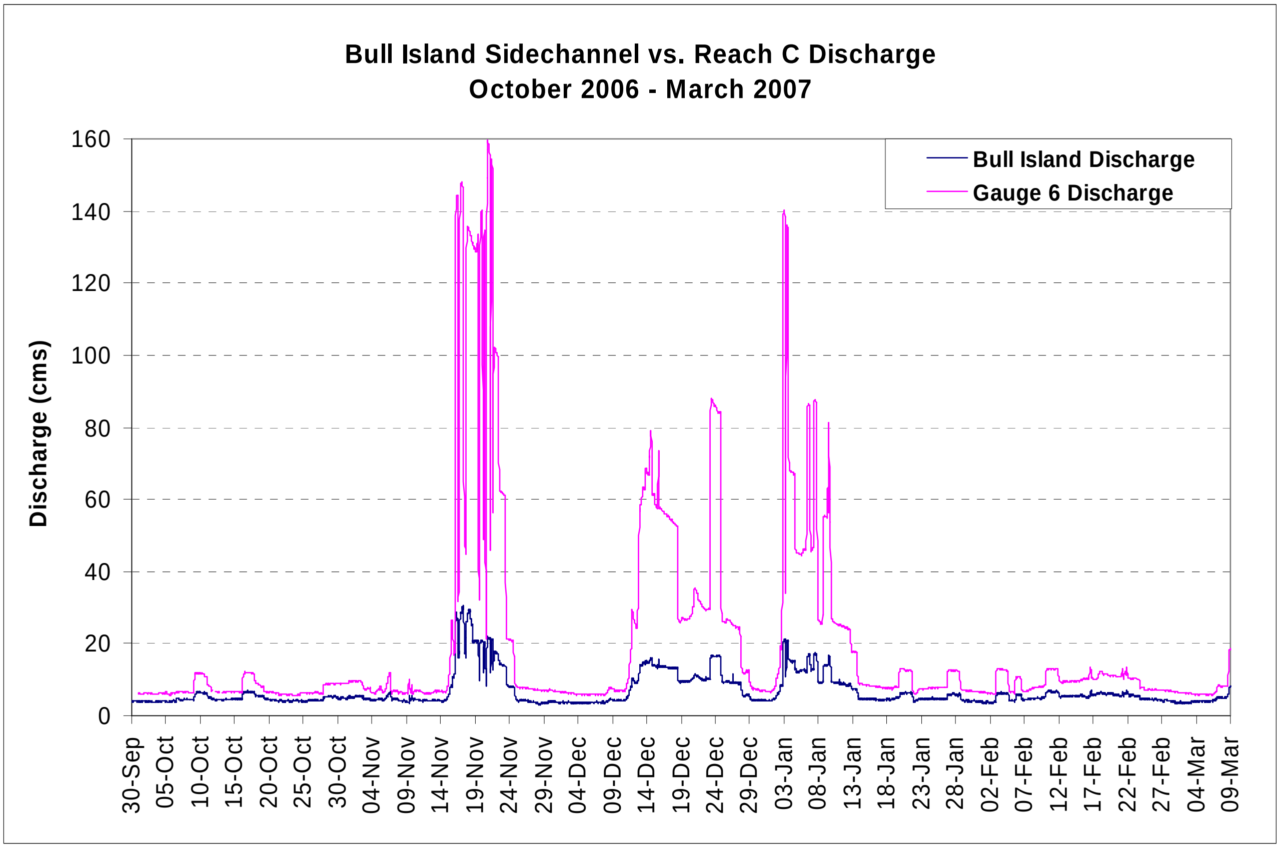

Figure 5. Comparison of hourly discharge in Bull Island Sidechannel and in the mainstem based on Gauge 6. Bull Island flows were derived from the Site 2 water level recorder and the stage-discharge rating curve developed in 2005-2006.

Table 8. Frequency of days during the study period when mean daily flows were greater than 5.7 m^3/s and greater than 25 m^3/s.

	# of Days Greater Than 5.7 cms	% of Study Days	# of Days Greater Than 25 cms	% of Study Days
Gauge 6	154	100%	32	21%
Gauge 6 + Browns River	154	100%	41	27%

Intergravel Temperatures

Mean intergravel water temperatures during the study period are shown for each site in Figure 6. Temperatures were similar at Sites 2 and 3, and thus rates of egg development were comparable for these two sites. However, intergravel temperatures at Site 1 were much lower due to the influence of cold water input from the Browns River. This resulted in a slower rate of egg development at Site 1. Average intergravel temperatures calculated for the entire incubation period from Oct 6 – Feb 9 for Sites 2 and 3 were 7.39 °C and 7.67 °C, respectively. The average at Site 1 for the period Oct 6 – Mar 8 was 5.33 °C. Incubation temperature for the control group at Puntledge Hatchery was 8.09 °C for the period Oct. 6 to Feb. 9. For the most part, the daily average intergravel and surface water temperatures were equal at all sites except during the high flow period throughout November at Site 1 when surface water temperatures were slightly warmer by as much as 2 °C.

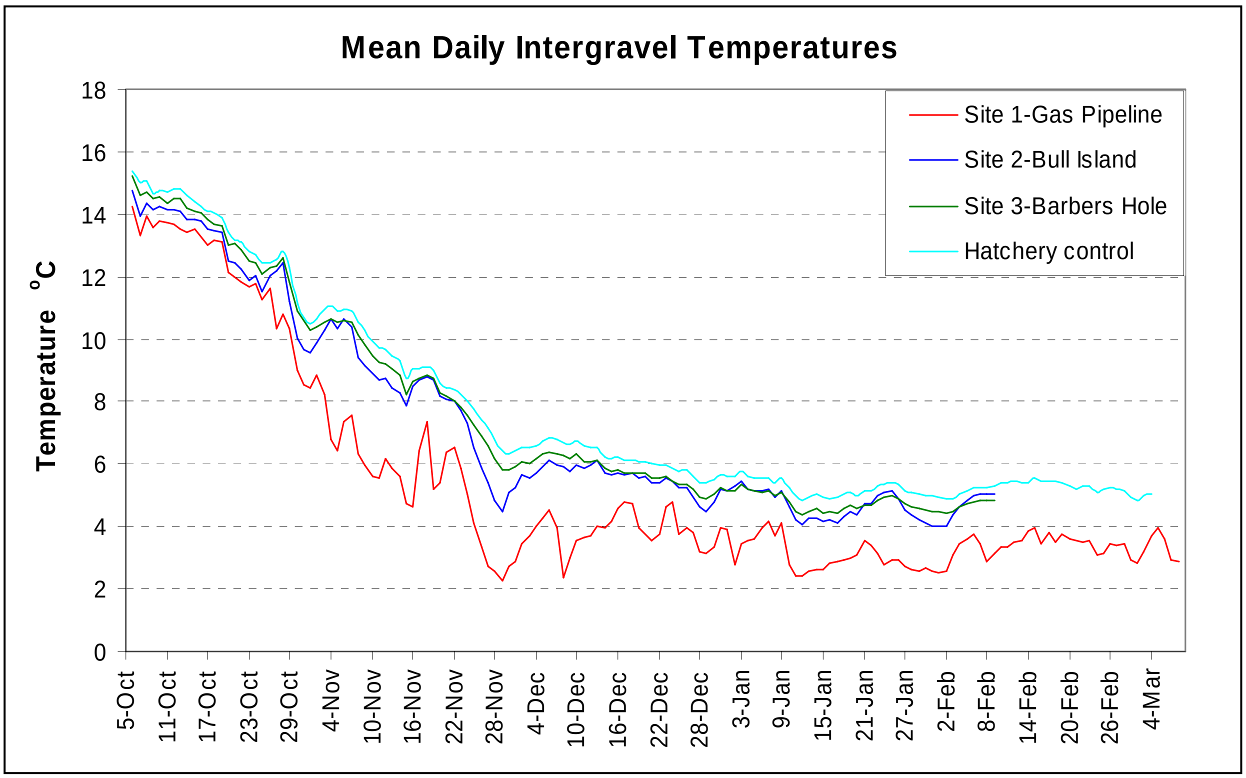

Figure 6. Mean daily intergravel water temperatures from Tidbit loggers buried at each incubation site and for the Puntledge Hatchery control incubators, October 2006 to March 2007.

Substrate Assessments

Wolman pebble counts and visual estimates of substrates were conducted at each incubation site at the beginning (October), middle (December), and end (February/March) of the incubation monitoring period. The distribution of particle sizes from the pebble counts are shown for each site and sample date in Figure 7 (sites are arranged horizontally and sample dates vertically). The data show a wide range of particle sizes for Sites 1 and 3 and a narrow range (16 – 90.5 mm) for Site 2. The distribution at Site 2 (Bull Island Sidechannel) is owing to manmade introduction of spawning gravels to this area in 2002 and 2003, whereas the other sites reflect natural grading by the river. Figure 7 also shows that there was a shift at all sites toward smaller sizes for the December sampling and then back to larger sizes for the February/March sampling.

The seasonal changes in substrate composition are also illustrated in the cumulative percentages charts in Figure 8. The vertical series on the left shows cumulative percentages from the pebble counts while the series on the right shows cumulative percentages from the visual estimates. The pebble count charts show a clear shift to the left (smaller sizes) in December and then to the right (larger sizes) for the Feb/Mar sampling. In the visual estimation charts, this pattern is only apparent for Site 1. The lack of seasonal changes in the visual estimates for Sites 2 and 3 may be due the subjectivity of this assessment method. The visual estimates however, do provide some information on the level of fines (particles $\leq$ 2 mm) not shown by pebble counts. Sites 1 and 3 showed the highest percentage of fines exceeding 20% at Site 3 on the February 9, 2007 sampling.

Possible explanations for the seasonal shifts in substrate described above include a major flood during November 15-25 (see Figure 4) and/or redistribution of substrates by salmon spawning at the incubation sites. Some of the variation in size distribution from one sampling period to the next may also be due to a change in the observer conducting the pebble counts.

Various descriptors of streambed composition at each incubation site are provided in Table 9. Median diameter (D_{50}) and geometric means (D_g) showed the same seasonal shifts previously described. The geometric sorting index (S_g) is the “degree to which fluvial processes have collected similar sized particles” (Kondolf 2000) with lower values indicating a higher degree of sorting. The sorting index values shown in Table 9 suggest well sorted substrates at Sites 1 and 3 and highly sorted substrates at Site 2. The skewness coefficient, a measure of the asymmetry of particle size distributions, was relatively low at all sites and ranged from slightly negatively skewed (-0.2) to slightly positively skewed (+0.3). Natural salmon spawning beds are typically negatively skewed (i.e. the distribution tail extends into the smaller particle sizes; Kondolf 1988). The absence of a stronger negative skewness in our data may be due to the inability of pebble counts to sample these smaller grain sizes.

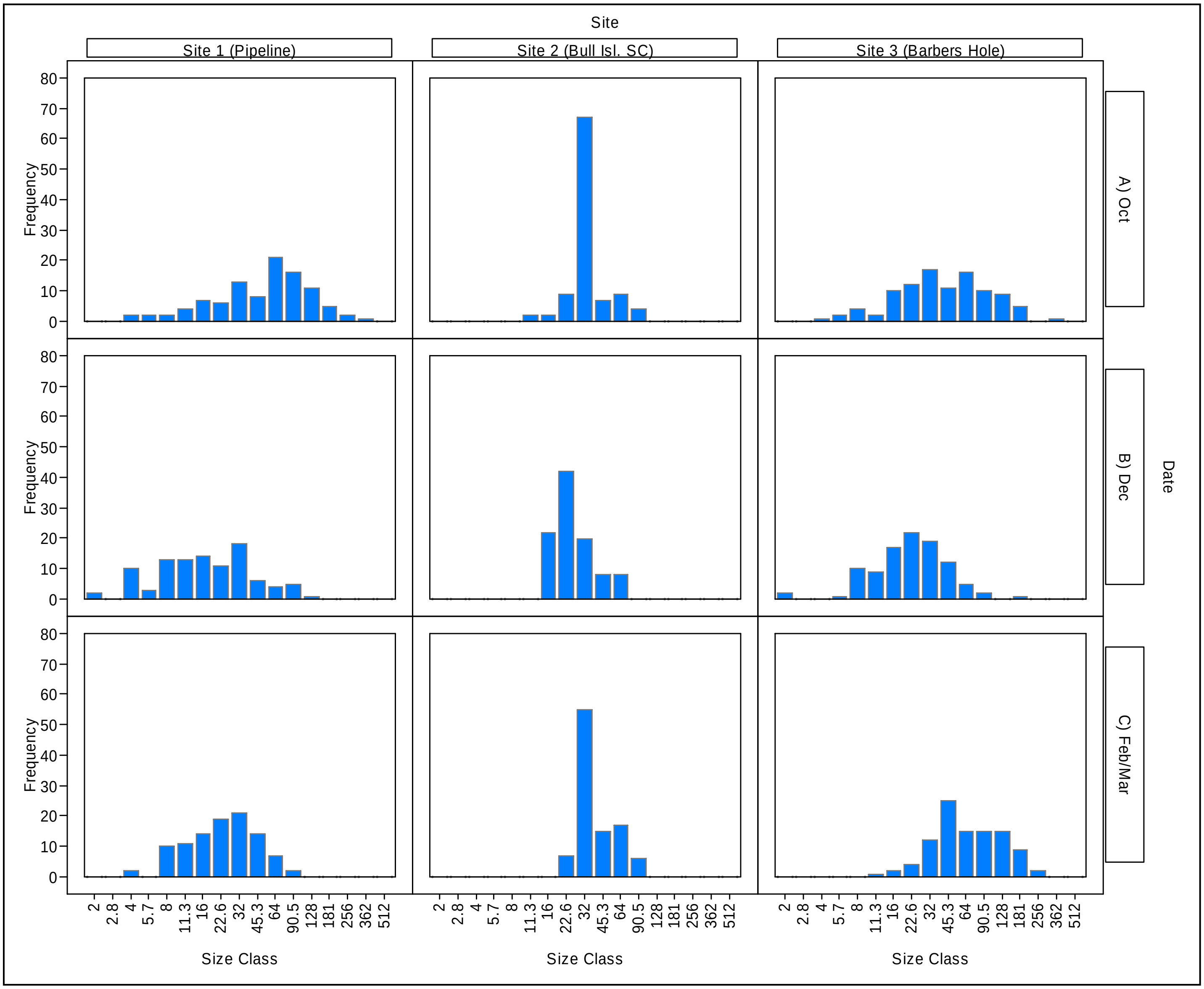

Figure 7. Particle size distributions from Wolman pebble counts. Charts are arranged horizontally by site and vertically by sample month (October, December, and February or March). Size classes (x-axis) are $\log_2$ transformation of ½ phi steps.

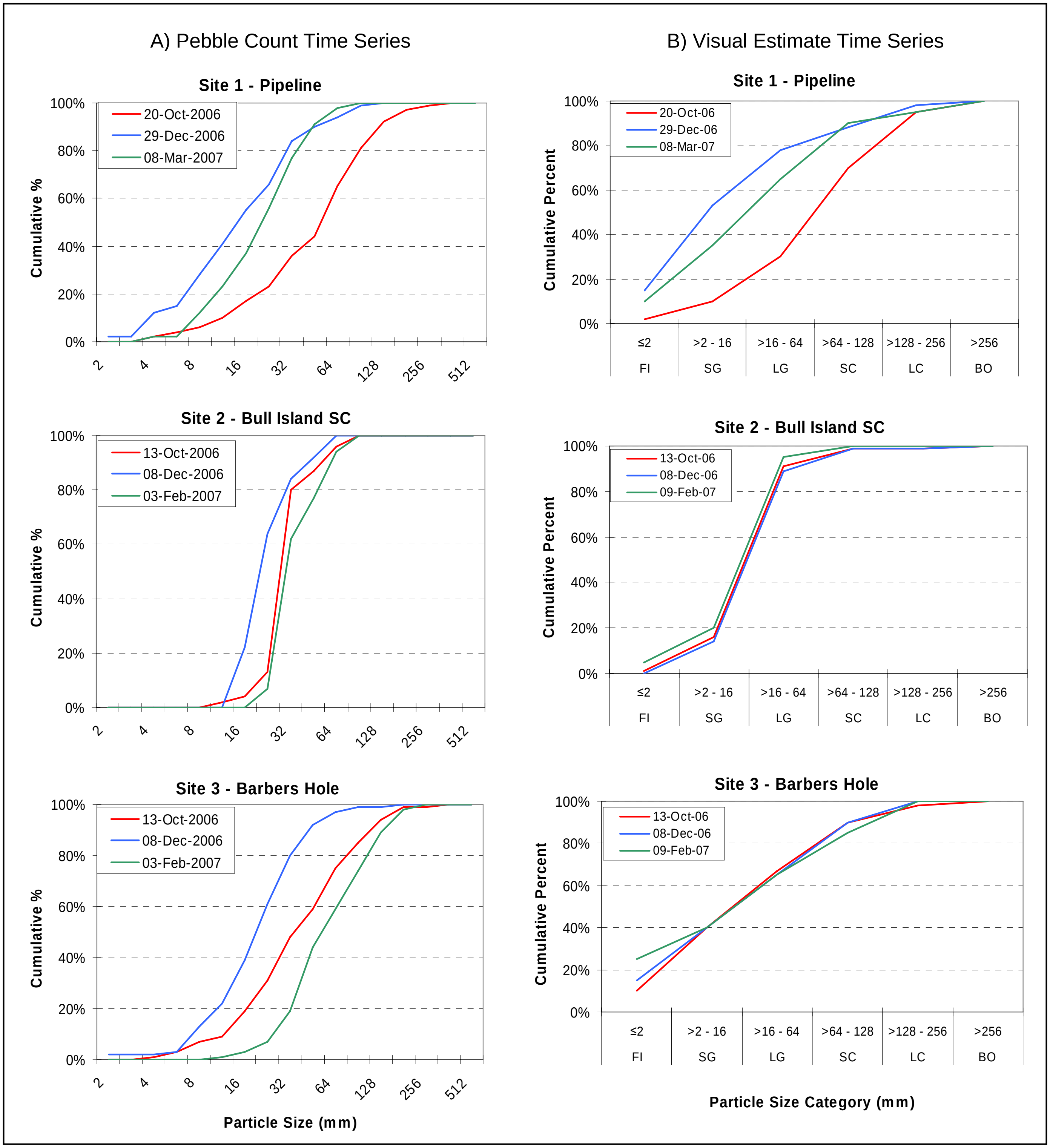

Figure 8. Changes in cumulative size distribution of particles at each study site based on pebble counts (time series A) and visual estimation (time series B).

Table 9. Summary of stream bed particle size descriptors (mm) based on pebble count data.

Site	Sample Date	Median Diameter (D_{50})	1 SD Upper (D_{84})	1 SD Lower (D_{16})	Geometric Mean (D_g)	Geometric Sorting Index (S_g)	Skewness (sk)
Site 1 (Pipeline)	20-Oct-06	50	97	14	42	2.6	-0.2
	29-Dec-07	15	32	6	14	2.3	0.0
	08-Mar-07	21	40	9	20	2.1	-0.1
Site 2 (Bull Island SC)	13-Oct-06	28	35	24	29	1.2	0.2
	08-Dec-07	21	32	16	22	1.4	0.2
	09-Feb-07	30	55	25	34	1.5	0.3
Site 3 (Barbers Hole)	13-Oct-06	34	87	15	34	2.4	0.0
	08-Dec-07	20	36	10	18	1.9	-0.1
	09-Feb-07	55	110	30	55	1.9	0.0

Notes:

$Sg = (D_{84} / D_{16})^{0.5}$; 1 = perfectly sorted sediment, < 2.5 indicates well sorted, ~ 3 is considered normal, > 4.5 is poorly sorted.

$sk = \log(D_g/D_{50}) / \log(S_g)$

Water Column and Intergravel Monitoring Results

Physical monitoring was conducted from 13 October 2006 to 8 March 2007. During this period Sites 1 and 2 were visited 14 times while Site 3 was visited 12 times. During each visit, dissolved oxygen, depth, and velocity were collected from each microsite (in triplicate) while pH, conductivity, and turbidity were collected from one representative location per site (in triplicate). Figure 9 presents site averages for all parameters except turbidity, and illustrates differences among sites and how these parameters varied over the course of the monitoring period. Figure 10 presents site averages for turbidity over the same period.

Intergravel and water column DO tended to increase over the monitoring period, perhaps due to increased solubility as water temperatures declined. Depth and velocity were variable from one visit to the next and would be directly associated with discharge on the day of the spot check. pH and conductivity (intergravel and water column) tended to decrease over the monitoring period. In terms of site differences, Site 3 appeared to have lower intergravel oxygen levels than Sites 1 and 2. This is probably related to the greater proportion of substrate fines at this site. Velocities at Site 1 were highly variable among visits and much higher than at the other sites. This would be related to freshet events from the Browns River whereas flows at the other two sites are largely dictated by releases at the diversion dam.

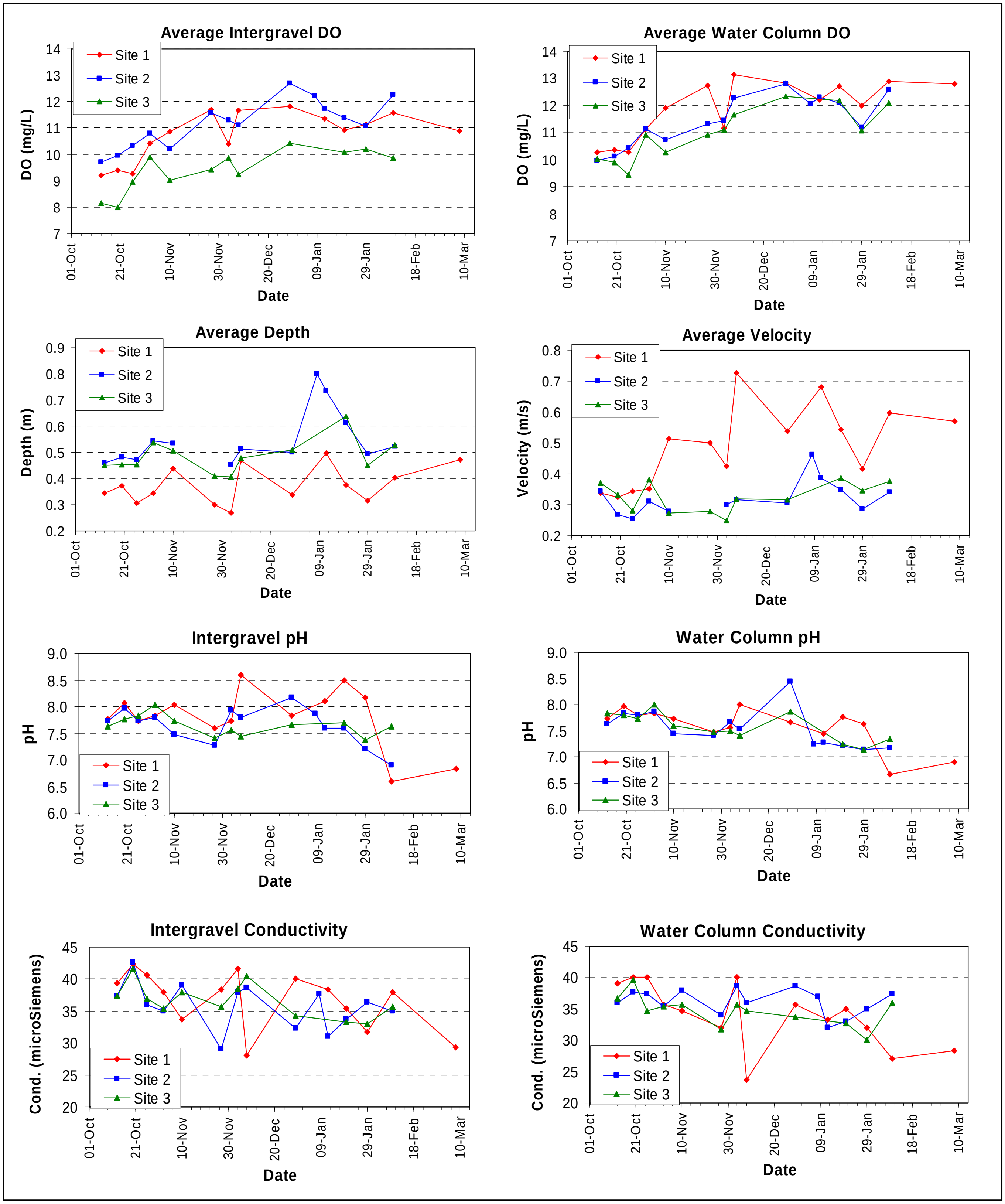

Figure 9. Time series for physical parameters measured at each site. Values are the average of all replicates pooled per site by date (12 readings per average for DO, depth and velocity; 3 readings per average for pH and conductivity).

Turbidity readings (Figure 10) were relatively low for all sites over the monitoring period (< 8 NTU). Values were highest and most variable at Site 1 (Pipeline), again due to the influence of freshet events from the Browns River.

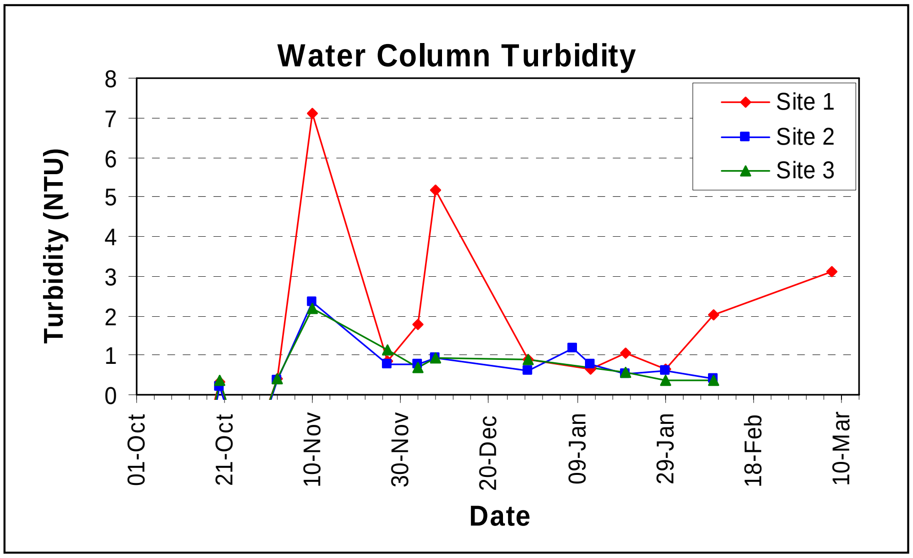

Figure 10. Mean water column turbidity (NTU) by site and date (each average is based on 3 replicate readings).

4.3 Incubation Success

Incubation survival results for the various developmental stages for the 3 incubation sites and the hatchery control group are summarized in Table 10 and in Figure 11. We experienced some difficulty analyzing the data for the fertilization to eyed egg stage at Site 2. The 4 incubators selected at this site for determination of survival to eyed stage yielded an average survival of 35%. This turned out to be lower than the survival to hatch produced from the remaining 8 incubators at this site (which yielded a survival to hatch of 51.9%). Thus, these incubators underestimated the survival to eyed stage relative to the remaining incubators at Site 2. In order to improve the interpretation of the results at this site for the remaining stages, we assumed that survival to the eyed stage was at least as good as survival to hatch, hence a value of 52% was used in Table 10 to calculate cumulative and stage specific survival rates for this site.

The cumulative mean survival rates for fertilization to fry ranged from 13.6% to 40 % for the study groups versus 52.6% for the control group. Using the revised fertilized-to-eyed survival rate for Site 2, this site had the best survival of the three sites in Reach C. Similarly, stage-specific survival rates for eyed-to-hatch and hatch-to-fry were greatest at Site 2 (99.8% and 77.1% respectively). These results are consistent with expectations that recently placed screened spawning gravel would provide high quality incubation conditions as demonstrated in previous incubation studies at this site. Sites 1 and 3 had poor survival overall (cumulative mean survival = 13.6% and 14% respectively). Survival from eyed-to-hatch was high at Site 1 but decreased significantly in the latter stage of development likely due to the increase in oxygen demand by the developing embryo. Stage-specific survival was more consistent from eyed-to-hatch and hatch-to-fry at Site 3. At the microsite level, variability in survival was greatest at Sites 1 and 3 compared to Site 2, with some cassettes having 100 percent mortality in the final incubation check (Appendix A)

Table 10. Cumulative survival rates (A) and stage-specific survival rates (B) from the 3 Puntledge River study sites and the hatchery control. Values are averages across all microsites per site with associated 95% confidence limits.

Site	A) Cumulative Mean Survival and 95% CL (%)					
	To Eyed		To Hatch		To Fry	
Site 1 (Pipeline)	62.0	±18.2	60.8	±15.7	13.6	±17.0
Site 2 (Bull Island SC)	52.0[1] (35.0)	±15.7	51.9	±15.7	40.0	±17.0
Site 3 (Barbers Hole)	75.8	±15.8	31.5	±15.7	14.0	±17.0
Control (Hatchery)	71.5	±22.3	63.9	±18.1	52.6	±17.0
Overall Means (Sites 1-3)	63.3		48.1		22.5	

Site	B) Stage-Specific Mean Survival (%)[2]		
	Fert.-to-Eyed	Eyed-to-Hatch	Hatch-to-Fry
Site 1 (Pipeline)	62.0	98.1	22.4
Site 2 (Bull Island SC)	52.0	99.8	77.1
Site 3 (Barbers Hole)	75.8	41.6	44.4
Control (Hatchery)	71.5	89.4	82.3
Overall Means (Sites 1-3)	63.3	79.8	48.0

Notes:

1. Site 2 incubators selected for determination of survival to eyed stage had a lower survival than those selected from this site for the survival to hatch stage (35% vs. 51.9% for the incubators selected for survival to hatch). Thus, these incubators underestimated the survival to eyed stage relative to the remaining incubators at Site 2. For this reason, it was assumed that survival to the eyed stage was at least as good as survival to hatch, hence the value of 52% used in the above table. However, this also results in a stage-specific survival rate near 100% for the eyed to hatch survival rate.
2. Calculation of stage-specific survival rates involves dividing the survival from fertilization to that stage by the survival from fertilization of the former stage and multiplying by 100. For example, the stage-specific mean survival for eyed-to-hatch for site 1 = 60.8 ÷ 62.0 × 100 = 98.1.

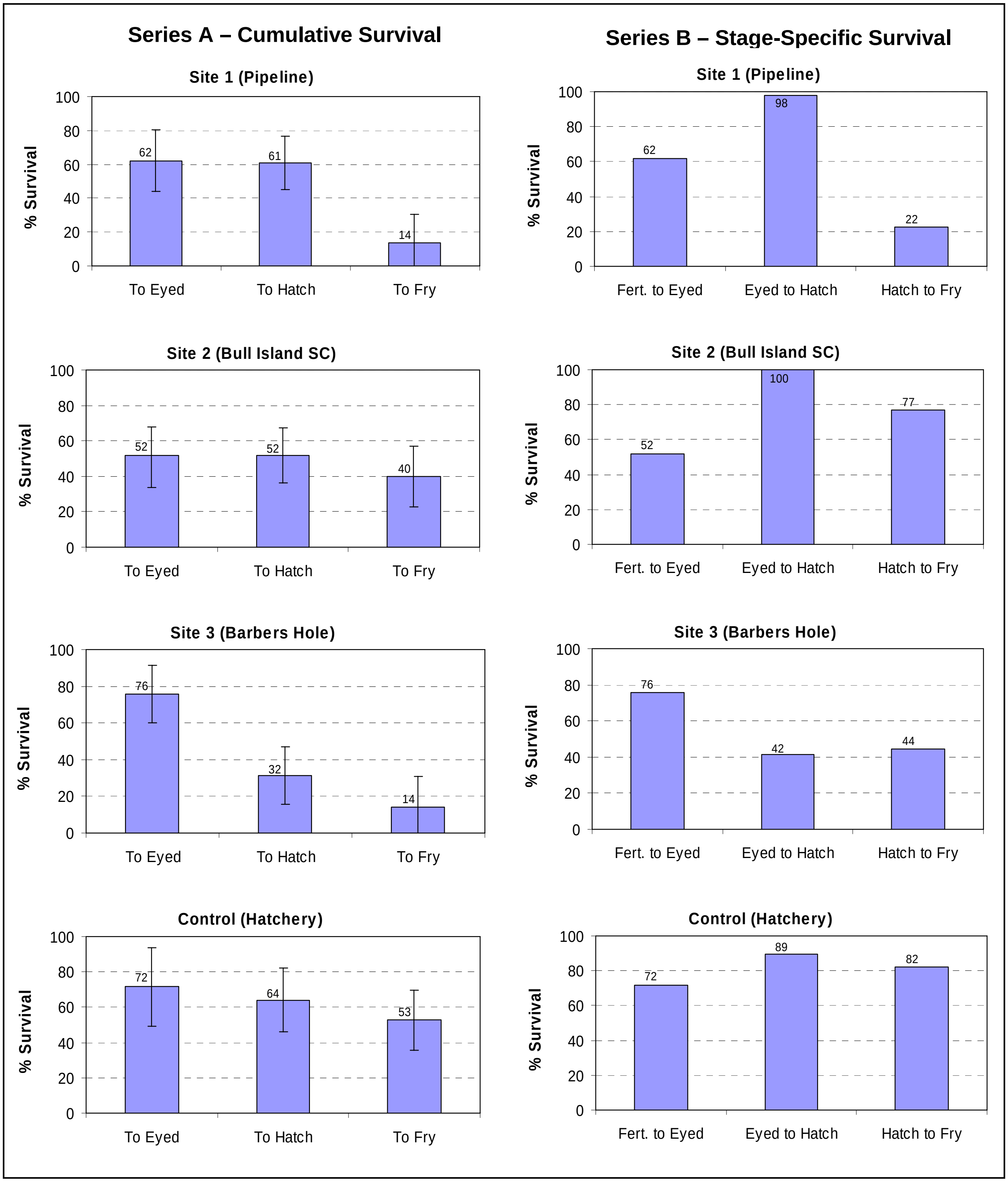

Figure 11. Cumulative survival (series A) and stage-specific survival (series B) from incubators at the 3 Puntledge River study sites and from the hatchery control. The data are averages across all microsites per site; error bars are 95% confidence limits. Note: Cumulative survival to hatch presented for Site 2 is the adjusted value (see footnote 1 in **Table 10** for explanation).

5. DISCUSSION

Survival to the eyed egg stage at the three incubation sites as well as the control group at Puntledge Hatchery was less than anticipated. In addition, the lowest survival rate for this developmental stage was observed at Site 2 (35%), which was completely unexpected given recent restoration activities and the quality of gravel at this site. This left us wondering if something in our methodology may have compromised fertilization success. Further evidence to this possibility was that fall chinook spawned by the hatchery on October 5, 2006 (the day prior to the incubator installation) produced a fertilization-to-eyed egg survival of 85.4% (Laurent Frisson, Puntledge Hatchery, pers. comm.), which was greater than our hatchery control (72%). The following is a list of factors related to egg collection and fertilization procedures that may have influenced survival to the eyed egg stage.

- On the day scheduled for the installation, hatchery staff sorted through several hundred broodstock and found only two females ready for spawning. Although the quantity of eggs was sufficient, this resulted in a sample group of eggs for the study originating from a smaller pooled group. Also, a small percentage of 'over-ripe' eggs was observed from one of these females ('over-ripe' eggs cannot be fertilized), though this did not seem to be a concern by hatchery staff.
- While loading the green eggs into the cassettes, care was taken to avoid breaking eggs, as broken eggs in the basin or cassette can hinder the process of fertilization of other eggs that have come in contact with the albumen. Despite best efforts some eggs were broken during the loading process. The influence of broken eggs can have a significant effect on egg fertilization success. Wilcox et al. (1984) found that 1% concentration of broken eggs reduced egg fertilization in coho salmon from 94% to 40%.
- There may have been some loss in viability of the eggs and milt after being taken from the fish before fertilization took place. The time from taking eggs and milt at the hatchery to fertilization on site was between 1 and 1.5 hrs for the first group and 3.5 to 4 hours for the second group. The first group of cassettes were transported and installed at Incubation Sites 2 and 3, while the remaining group of cassettes were installed at Site 1 and at the hatchery (control). However, Jensen and Alderdice (1984) found that chum salmon gametes could withstand short-term storage at 15 ^{o}C for up to 12 hours without any significant loss in viability.
- In our study the chinook eggs were loaded into the incubators, transported to the site, and then fertilized in a water bath on site. The approach of fertilization after being loaded into the incubators and transported to site was recommended due to the increased sensitivity to shock of fertilized eggs. This method was performed successfully by DFO Resource Restoration Division at previous incubation studies. However, using this method essentially requires a "wet fertilization" approach in contrast to the "dry fertilization" method conducted at many salmon hatcheries. In a few words, the dry fertilization method involves adding the milt to the eggs and

allowing the sperm to mix with the ovarian fluid before adding water. Advocates of this method claim it results in greater and more consistent fertilization success because sperm motility is prolonged in ovarian fluid and the micropyle (the small opening in the egg through which the sperm enters) remains open longer. On the other hand, with the wet fertilization approach the sperm lose their motility rather quickly and fertilization success tends to be more variable (John Jensen, Pacific Biological Station, pers. comm.).

- Water temperatures at all three sites during fertilization and for the first 10 days of incubation were on the threshold of potentially causing increased risk of mortality. Studies conducted on fall chinook salmon at the Puntledge Hatchery showed a 5% mortality to the eyed stage when incubated at a constant temperature of 13 °C (John Jensen, unpublished data). For our study, average temperatures for the first 10 days of incubation were 13.7 °C, 14.1 °C, 14.5 °C and 14.8 °C for Sites 1, 2, 3 and the hatchery control respectively. The average incubation temperatures for the period to the eyed-egg stage were 12.1 °C, 12.7 °C, 13.1 °C and 13.3 °C for Sites 1, 2, 3 and the hatchery control respectively.

While the use of unfertilized gametes for experimental incubation studies more closely mimics natural spawning at the study sites and provides an evaluation of embryo survival during the earliest developmental period, it also creates many challenges for the researchers. Previous incubation studies conducted at the Bull Island Sidechannel (Wright and Guimond 2003; Guimond 2006) and Supply Creek (Guimond 2007) used eyed chinook eggs as a starting point. Although this may have resulted in an overestimate of survival since egg mortality during the earlier stages were not included, it eliminated many of the difficulties associated with using unfertilized gametes that can influence early developmental survival as discussed above. This allows incubation results to be more easily compared from one year to the next and correlates incubation survival to physical parameters.

Though we questioned the reliability of our intergravel oxygen results, there nevertheless appeared to be potential correlations between these data and various stages of incubation survival. Figure 12 shows scatter plots of eyed-to-hatch survival and hatch-to-fry survival versus mean intergravel oxygen during these same periods. These plots suggest decreasing survival with decreased intergravel oxygen. The values of dissolved oxygen at which survival is low or nil however are much higher than the intergravel DO limits for incubation reported in the literature. This may be due to the problems associated with the intergravel sampling apparatus (syringe). It is conceivable that actual intergravel oxygen levels were systematically lower than what the instrument was indicating (see Section 6).

Ignoring the survival to the eyed stage at all sites/control and focusing on the later developmental stage-specific survivals, the results are closer to what we would expect for Sites 2 and the control group (eyed-to-hatch = 99.8 % and 89.4 % respectively and hatch-to-fry = 77.1 % and 82.3 % respectively). Site 3 had poor survival for the above stages which we would expect, based on the higher percentage of fines and lower intergravel DO measurements recorded at this site. The oxygen demand of the hatching embryo and post-hatch phase is much greater than the pre-hatch phases (Becker et al. 1983). Consequently, early eggs that may have tolerated low dissolved oxygen conditions in pre-hatch stages may show a significant increase in mortality during the hatching stage and afterwards in similar low DO conditions.

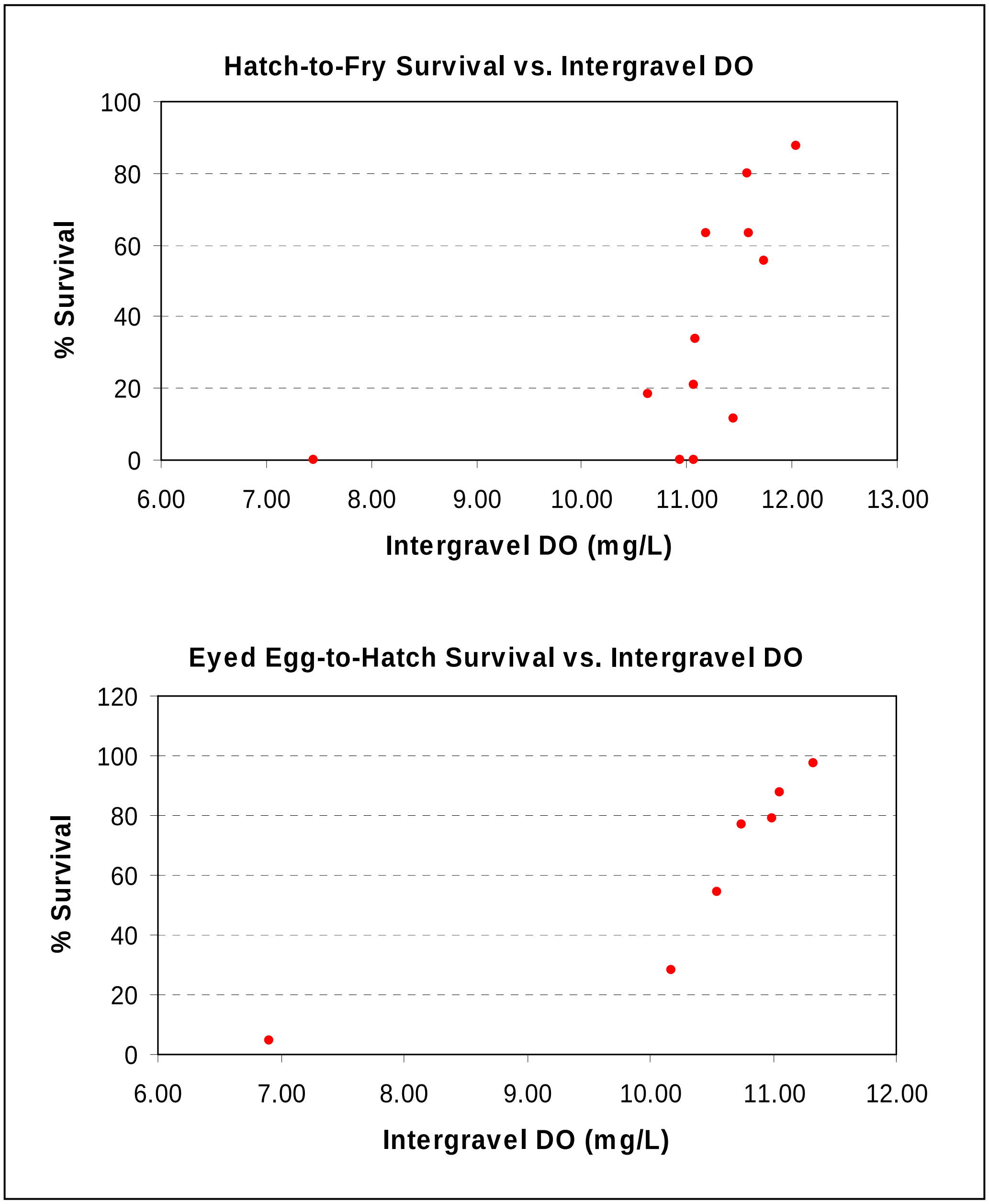

Figure 12. Scatter plots of eyed egg-to-hatch survival and hatch-to-fry survival versus mean intergravel dissolved oxygen during the respective life stage.

6. RECOMMENDATIONS

The main conclusions from Year 1 of the Puntledge Egg Incubation Assessment are as follows:

1. The study should use fall chinook eyed eggs from Puntledge Hatchery rather than green eggs. This would eliminate the risk of confounding results from egg mortality due to embryo quality, egg collection and fertilization procedures, with egg mortality from water/substrate quality characteristics. The challenge however will be to obtain eyed eggs as early as possible in the fall (i.e. use eyed eggs from the earliest spawned chinook) and install them in the river before flows increase. Preparing holes in the streambed and burying cassettes at flows greater than the minimum base flow of 5.7 m^3/s will be extremely difficult, if not impossible except at Site 2 in the Bull Island sidechannel. However, acquiring eyed-eggs from the hatchery is much easier than green eggs, and delaying installation due to flows would not result in a lost opportunity with eyed-eggs. A flow outage request for the day from BC Hydro may also be an option.

   Burying eyed-eggs rather than green eggs reduces the risk of cassettes becoming exposed or getting dug out of the gravel by spawning fish, as occurred at Site 1 and 2. However, there would be more chance that recently spawned eggs would be inadvertently disturbed while preparing the holes in the gravel for the cassettes.

2. The collection of replicate measurements of intergravel and water column conductivity and pH are not likely to provide any valuable information regarding egg survival as it relates to flow, and should be removed from the regular environmental monitoring program. The time and effort (and associated costs) saved from monitoring these parameters can be used towards collecting better quality and quantity data for intergravel dissolved oxygen.

3. The methodology for extracting intergravel water samples for dissolved oxygen measurements (i.e. syringe) should be investigated further. Although this method has provided more reliable results in past studies, it frequently yielded questionable results in our study. For instance, collecting a water column sample with the syringe often yielded lower dissolved oxygen measurements than by taking a grab sample from the water column. It was also observed that measurements of intergravel dissolved oxygen were sometimes higher than water column measurements. It is suspected that the intergravel dissolved oxygen measurements are being overestimated due to entrainment of surface water down the insertion point of the syringe in the gravel. Also, the process of expelling the sample into the container, even when done slowly, may still be adding oxygen to the sample.

Possible options that should be explored to improve the collection of intergravel dissolved oxygen include:

- Having the syringe apparatus repaired and calibrated with a more robust method such as the standpipe method prior to the commencement of the 2007/08 field investigation.

- Replace the use of the syringe apparatus method with a standpipe method at all incubation sites or use of buried probes that could be monitored at a greater range of flows.
- Having the syringe apparatus repaired and calibrated for use in the 2007/08 field season, and establish one incubation site with standpipes for more intensive monitoring at a base flow, a high flow (that can safely and logistically be monitored), and one or two flows in between. As an alternative, the DO data loggers from BC Hydro could be used at one site (if the equipment is operational or can be easily serviced).

The method used will depend on the cost of equipment repair and acquisition of new equipment, the logistics of using new methodologies such as the standpipe method at the incubation sites (i.e. transporting equipment to the site, installing and leaving standpipes in the river over winter, etc.). This may also influence the total number of spot measurements that can collected during the incubation period. A trial will be conducted this summer at one site to determine the best option before data collection commences in the fall.

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Personal Communications

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Craig Wightman	Ministry of Environment

Appendix A. Cumulative survival rates by microsite for each incubation site.

	Microsite	Site 1	Site 2	Site 3	Control
Percent Survival to Eyed Stage	1	59.0	18.0	67.5	82.0
	2	missing	32.5	84.0	61.0
	3	80.0	31.5	75.0	
	4	47.0	58.0	76.5	
	Mean	62.0	35.0	75.8	71.5
	95% CL	18.2	15.8	15.8	22.3
Percent Survival to Hatch Stage	1	63.5	93.0	6.0	61.5
	2	61.5	27.0	18.5	51.5
	3	49.0	32.5	40.5	
	4	48.5	32.0	34.5	
	1	84.5	56.0	0.0	66.5
	2	21.5	30.0	29.0	61.0
	3	77.0	63.5	74.5	79.0
	4	81.0	81.0	49.0	64.0
	Mean	60.8	51.9	31.5	63.9
	95% CL	15.7	15.7	15.7	18.1
Percent Survival to Fry Stage	1	28.5	35.5	0.0	60.5
	2	0.0	24.0	0.0	41.5
	3	9.0	55.5	47.0	65.0
	4	17.0	45.0	9.0	43.5
	Mean	13.6	40.0	14.0	52.6
	95% CL	17.0	17.0	17.0	17.0

Appendix B. Selected Photos

Photo 1. Loading unfertilized (green) chinook eggs into a Jordan cassette loader at Puntledge hatchery, Oct. 6, 2006.

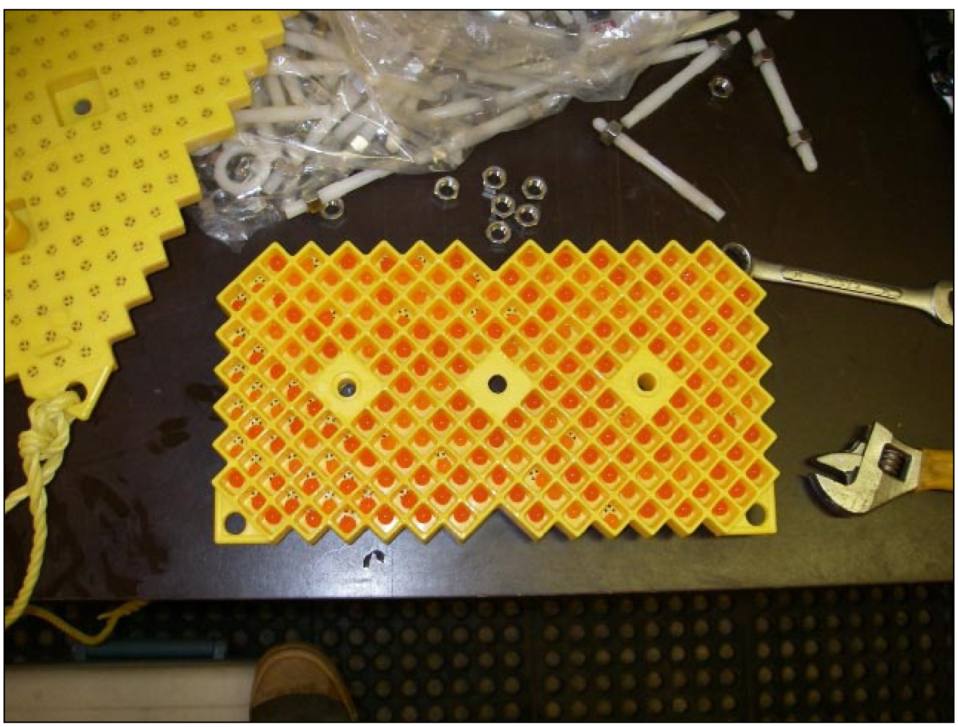

Photo 2. Jordan-Scotty incubation cassette loaded with chinook eggs prior to closing.

Photo 3. Measuring pooled milt into three separate containers for transport to the incubation sites, Oct. 6, 2006.

Photo 4. Fertilizing a cassette on shore at an Incubation site, Oct. 6, 2006.

Photo 5. Puntledge River Reach C Incubation Site 1 – Gas Pipeline crossing, below the Browns River confluence, with transect indicating location of microsites.

Photo 6. Puntledge River Reach C Incubation Site 2 – Bull Island Sidechannel, upstream of log jam, with transect indicating location of microsites.

Photo 7. Puntledge River Reach C Incubation Site 3 – Barbers Hole, with transect indicating location of microsites.

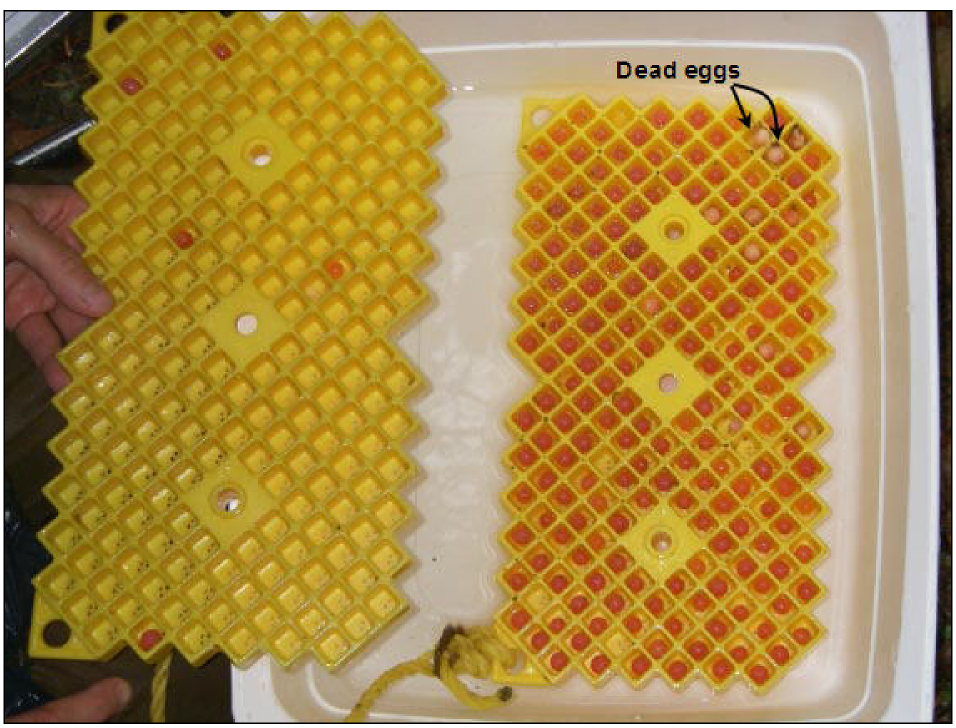

Photo 8. One of 4 cassettes removed from Site 3, Nov. 2, 2006 to assess survival to the eyed stage. White/pale pink eggs are dead.

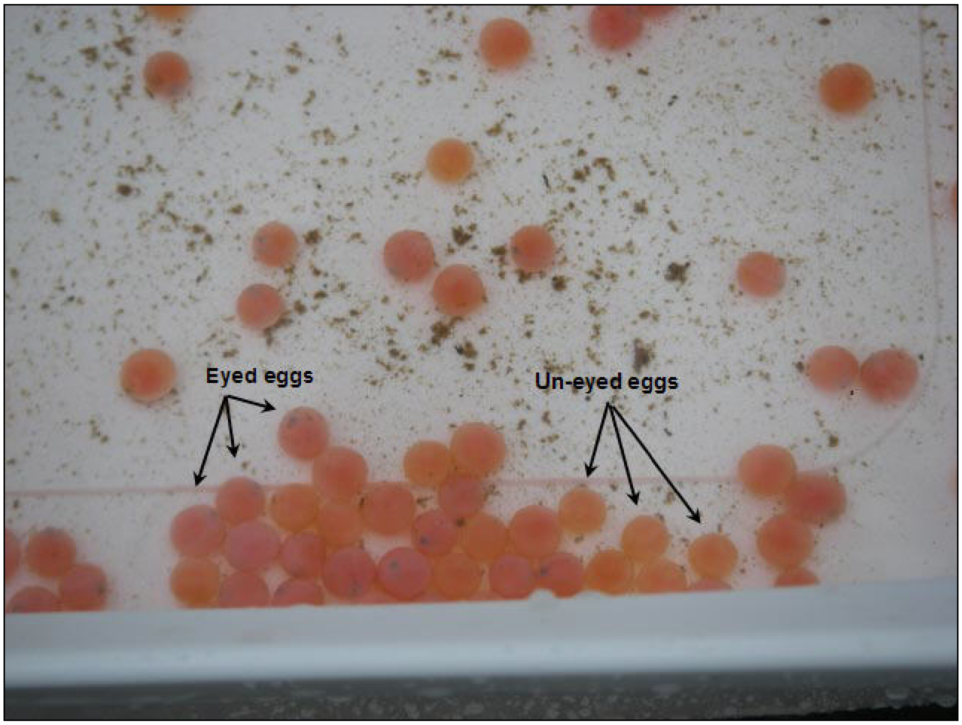

Photo 9. Close-up of eyed-eggs from a cassette showing obvious eyed eggs versus un-eyed eggs that are either unfertilized or dead but have not yet turned white.

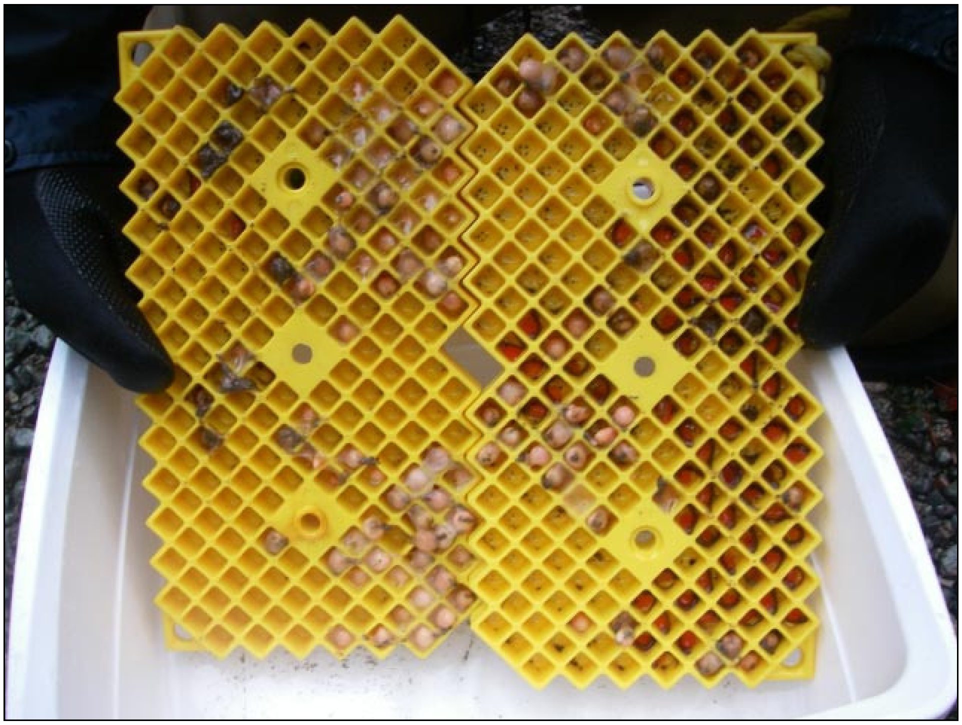

Photo 10. One of 4 cassettes removed from Site 3, Dec. 8, 2006 to assess survival to the hatch stage. White/pale pink ones are dead, dark ones are fungus and dirt covered, and red ones are live alevins with yolk sacs.

Photo 11. One of 4 cassettes removed from Site 3, Feb. 9, 2007 to assess survival to the "button-up" stage. Remaining eggs/alevins in cassette are all dead (dark ones are fungus and dirt covered). Live fry are in basin.

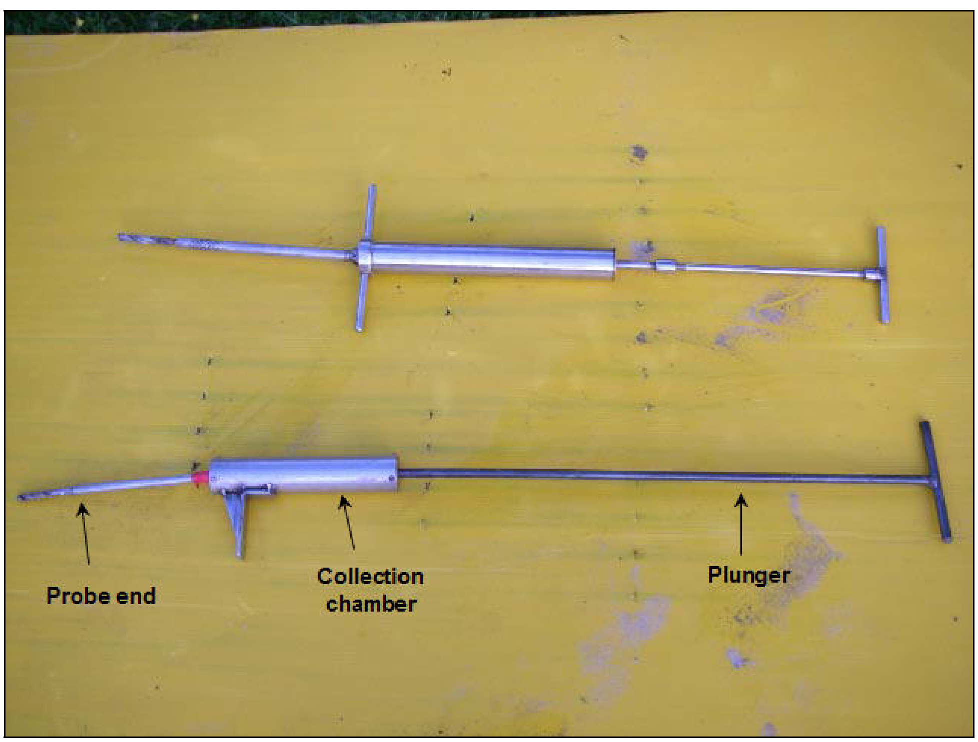

Photo 12. Syringe apparatus used to extract intergravel water samples. The narrow probe end is inserted into the gravel with the help of the foot pedal, to its full length. The plunger is pulled, drawing the water sample into the collection chamber.