SNAKE RIVER FALL CHINOOK SALMON LIFE HISTORY INVESTIGATIONS

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Executive Summary

Chapter One – This chapter was published in the Transactions of the American Fisheries Society in 2012. We conducted a three-year radiotelemetry study in the lower Snake River to answer the questions: do fall Chinook salmon juveniles pass dams during winter when bypass systems and structures designed to prevent mortality are not operated; does downstream movement rate vary annually, seasonally, and from reservoir to reservoir; and, what are some of the factors that contribute to annual, seasonal, and spatial variation in downstream movement rate? Fall Chinook salmon juveniles moved downstream up to 169 km and fast enough (7.5 km/d) such that large percentages (up to 93%) of the fish passed one or more dams during winter. Mean downstream movement rate varied annually (9.2-11.3 km/d), increased from winter (7.5 km/d) to spring (16.4 km/d), and increased (6.9-16.8 km/d) as fish moved downstream from reservoir to reservoir. Fish condition factor at tagging explained some of the annual variation ($P \leq 0.01$) in downstream movement rate, whereas water particle velocity ($P \leq 0.0001$) and temperature ($P \leq 0.0001$) explained portions of the seasonal variation. An increase in migrational disposition as fish moved downstream helped explain the spatial variation ($P = 0.05$-0.07). The potential cost of winter movement might be reduced survival due to turbine passage when the bypass systems and spillway passage structures are not operated. Efforts to understand and increase passage survival of winter migrants in large impoundments might help to rehabilitate some imperiled anadromous salmonid populations.

Chapter Two – Natural juvenile fall Chinook salmon in the Snake and Clearwater rivers exhibit two life history strategies. "Ocean-type" fish migrate out to the ocean in their first summer of life as subyearlings, but "reservoir-type" fish delay seaward migration during the summer, and some overwinter in reservoirs before continuing their migration the following spring as yearlings. Earlier emerging fish produced in the Snake River tend to adopt the ocean-type life history whereas many of the later emerging fish from the Clearwater River tend to adopt the reservoir-type life history. The underlying cause of the reservoir-type life history is poorly understood, but we believe there may be link to physiological development. We used traditional markers of the parr-smolt transformation (smoltification), including gill Na^+/K^+-ATPase activity and thyroid hormone levels, along with gene expression microarrays to assess the development of ocean-type juvenile fall Chinook salmon and then compared it to that of juvenile fall Chinook salmon from the Clearwater River. We showed that parr in the Snake River are physiologically distinct from actively-migrating smolts but smolts migrating early and late in the summer and fall are physiologically similar. Juvenile fall Chinook salmon collected from the Clearwater River were similar in size to early-migrating smolts in the Snake River but were most physiologically similar to Snake River parr. Genes differentially expressed between Snake River parr and smolts and between fish from the Clearwater River and smolts from the Snake River were involved in the cell cycle, steroid metabolism and other metabolic pathways, and DNA repair and packaging. Many of the genes differentially expressed in these comparisons had expression patterns that correlated with gill Na^+/K^+-ATPase activity, suggesting that they were related to smoltification and migration status.

Chapter Three – Natural subyearlings produced in the Clearwater River are exposed to cool (~10-12°C) temperatures when water is released from Dworshak Reservoir for summer flow augmentation. Total dissolved gas (TDG) levels range from 100-110% in the lower Clearwater

River. When fish move into the Snake River, they encounter temperatures up to 24°C at the surface which have the potential to incur gas bubble disease (GBD) in fish as dissolved gases in their bodies expand under warmer temperatures. This may result in both direct and indirect mortality, but this situation has been little studied. We conducted laboratory experiments to examine subyearling mortality rates and incidence and severity of GBD in fish that were moved between waters that varied in TDG and temperature. Fish experienced significant mortality only at temperatures of 25°C, which increased with exposure time. However there was no significance difference in mortality between fish acclimated to 100% TDG and 110% TDG. Fish that died did show signs of GBD. Generally, signs of GBD such as bubbles in the lateral line and unpaired fins were higher in fish acclimated at 110% TDG than in fish acclimated at 100% TDG, but there were few trends related to exposure temperature. Field measurements of TDG showed that TDG ranged from about 100% to 122.5% at some locations. Generally, TDG fluctuated daily, up to 8% during August and early September, and was highest late in the afternoon and lowest in the early morning. Laboratory results and field monitoring demonstrated that emigrating juvenile salmon can potentially be at risk from elevated temperatures, TDG, and GBD albeit to an unknown extent, which may increase their vulnerability to predation.

Chapter Four – We conducted monthly beam trawling in Lower Granite and Little Goose reservoirs to describe the seasonal abundance of benthic epifauna that are potentially important as prey to juvenile fall Chinook salmon. The predominant taxa collected were Siberian prawns, the opossum shrimp *Neomysis mercedis*, and the amphipod *Corophium* sp. Prawns were relatively abundant at shallow sites in both reservoirs in June, but were more abundant at deep sites in lower and middle reservoir reaches in autumn. Prawn densities were commonly $<0.2/m^2$. Prawn length-frequency data indicated that there were at least two size classes. Juvenile prawns present in shallow water more often than adult prawns, which were generally only found in deep water by autumn. Ovigerous prawns had an average of 171 eggs, which represented about 11.5% of their body weight. Limited diet analyses suggested that prawns consumed *Corophium*, *Neomysis*, and aquatic insects. *Neomysis* dominated all catches both in terms of abundance and biomass, and they were more abundant in Lower Granite compared to Little Goose reservoir. *Neomysis* were more abundant at shallow sites than at deep sites. *Corophium* were present in our collections but were never abundant, probably because our trawl was not effective at capturing them. The caloric content of prawns (4,782 Kcal), *Neomysis* (4,962 Kcal), and *Corophium* (4,926 Kcal) indicates that these prey would be energetically profitable for juvenile salmon. Subyearling fall Chinook salmon prey heavily on *Neomysis* and *Corophium* at times, but the importance of prawns as prey is uncertain.

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CHAPTER ONE

Downstream Movement of Fall Chinook Salmon Juveniles in the Lower Snake River Reservoirs during Winter and Early Spring

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INTRODUCTION

Winter is a critical season that can greatly influence the survival and behavior of juvenile anadromous salmonids. It is generally accepted that natural fluvial processes in small streams create diverse habitat conditions that allow anadromous salmonid parr to limit their winter movement and energy expenditure, and thereby maximize overwinter survival by avoiding predation and starvation (review by Brown et al. 2011). Fish avoid predation and conserve energy when metabolic activity is reduced by seeking cover and holding (Bustard and Narver 1975; Cunjak and Power 1986; Hillman et al. 1987; Cunjak 1988a). These behaviors tend to limit winter movement, particularly for fish that conceal themselves within the substrate (Valdimarsson et al. 1997; Metcalfe et al. 1998). Parr that do move during winter usually do so while feeding over spatially restricted areas (Hillman et al. 1987; Cunjak 1988b; Bradford et al. 1995; Contor and Griffith 1995; Roussel et al. 2004; Linnansaari et al. 2005). Winter movement may also be a response to sedimentation, flooding, ice buildup, or dam operations (Hillman et al. 1987; Tschaplinski and Hartman 1983; Roberston et al. 2004; Brown et al. 2011).

To our knowledge, winter movement of juvenile anadromous salmonids in reservoirs has rarely been studied. Juveniles of the Snake River basin fall Chinook salmon population complete their freshwater life cycle in large main-stem habitats that are affected by hydroelectric dams (Figure 1). Historically, many fall Chinook salmon in the Snake River basin migrated seaward as subyearlings during spring and early summer (Mains and Smith 1964; Krcma and Raleigh 1970) to spend their first winter in the Pacific Ocean. Following the construction of Brownlee Dam in 1958 (Figure 1), Durkin et al. (1970) published the first known peer-reviewed account of overwintering in reservoirs by fall Chinook salmon. We refer to these fish as "reservoir-type" juveniles after Connor et al. (2005). Dams completed by 1975 limited spawning of Snake River fall Chinook salmon to the lower Snake River and portions of its larger tributaries, and completely impounded the migration corridor between Lower Granite and Bonneville dams (Figure 1). Most contemporary juveniles still adopt the subyearling migration tactic, but some adopt the reservoir-type tactic in reservoirs upstream of Bonneville Dam (Connor et al. 2002). After initiating migration in early summer, these fish later delay seaward migration during late summer and autumn (e.g., Tiffan et al. 2009a) while retaining morphological characteristics associated with smolts rather than parr (e.g., complete silvering and lack of parr marks, well developed teeth on the maxilla, mandible, gums, and blackening of the gums; Gorbman et al. 2003; Connor et al. 2005). The reservoir-type tactic is successful when measured in terms of adult returns. Connor et al. (2005) estimated that nearly half of the natural fall Chinook salmon adults that returned to the Snake River basin during 1998–2003 had overwintered in a reservoir or the Columbia River estuary as juveniles.

Winter movement by reservoir-type fall Chinook salmon in the Snake and Columbia rivers would have implications for management. Migration timing has been studied by

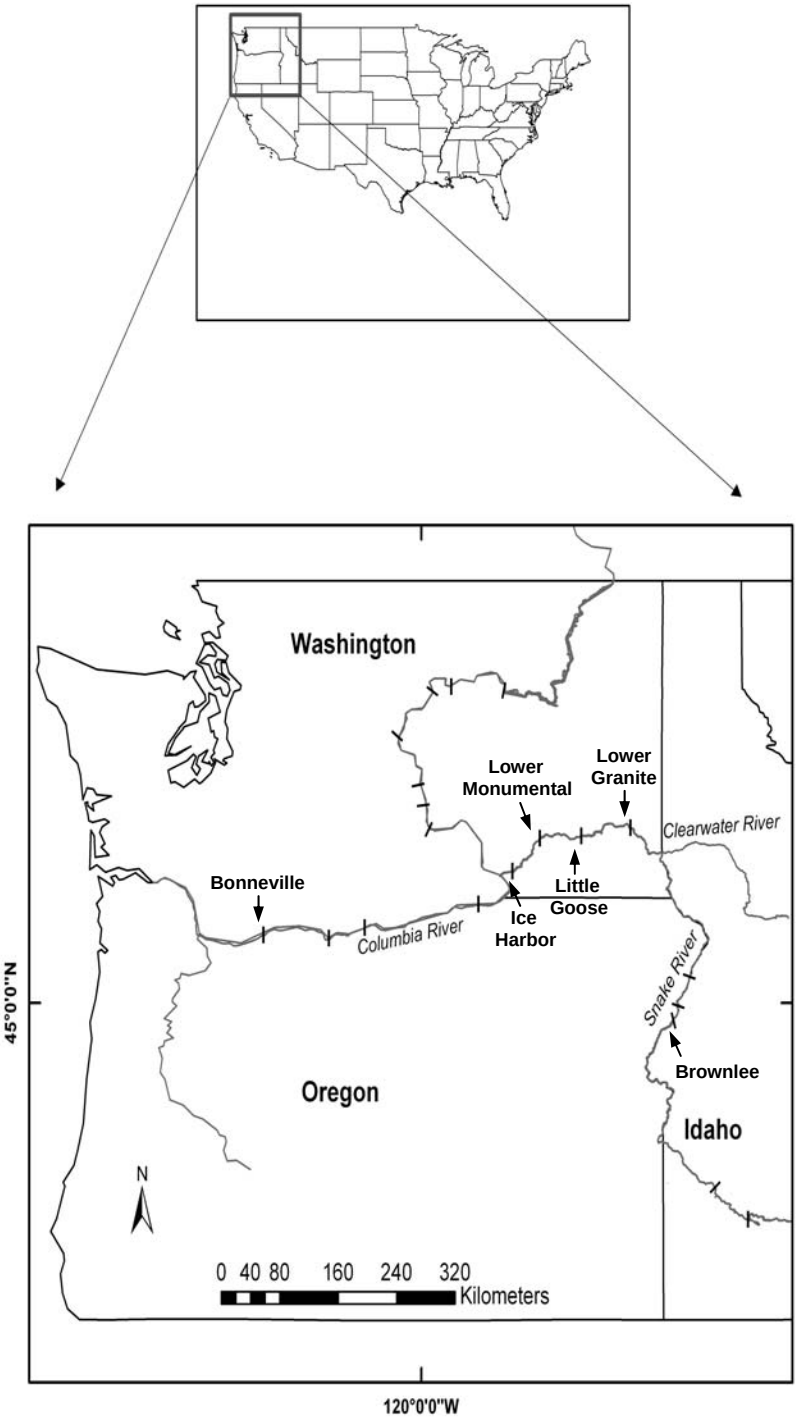

Figure 1. The study area including the major dams and reservoirs in the Snake and Columbia River basins that pertain to this study.

implanting subyearling Chinook salmon with passive integrated transponder (PIT) tags (Prentice et al. 1990a) during summer and then detecting the tagged fish as they pass the seven downstream dams equipped with PIT-tag monitoring systems (Prentice et al. 1990b; Figure 1). The fish bypass systems at main-stem dams that house the PIT-tag monitoring systems and route juveniles away from the turbines are typically not operated during winter (www.ptoccentral.org/Ptoc_OM). Detections made at the dams after fish bypass is resumed the following spring confirm the reservoir-type tactic, but do not provide information on downstream movement in the reservoirs or dam passage during winter. Water is rarely passed over the dam spillways in winter, thus it is important to understand that the only route of passage during winter when the juvenile fish bypasses are not operated is through the turbines. Extensive winter movement might increase turbine passage and result in mortality that reduces the contribution of the reservoir-type tactic to production. In the absence of data on winter movement and dam passage, there was little impetus to expend the resources to operate the juvenile fish bypass systems at the dams during winter.

In November 2004, we initiated a three-year radiotelemetry study to increase the understanding of winter movement by reservoir-type fall Chinook salmon in three of the four lower Snake River reservoirs (Figure 1). The objectives of this paper are to: (1) determine if reservoir-type fall Chinook salmon pass dams during winter when bypass systems and structures designed to prevent mortality are not operated; (2) determine if downstream movement rate varied annually, seasonally, and from reservoir to reservoir; and (3) identify some of the factors that contributed to annual, seasonal, and spatial variation in downstream movement rate.

METHODS

*Study area***.—**We conducted our study on the lower Snake River in southeastern Washington (Figure 1). The lower Snake River is impounded by four run-of-river hydroelectric dams that are each about 30 m high and close to 1 km long with a six-turbine powerhouse, eight-bay spillway, navigation lock, and earthen wall. Ice Harbor Dam is located at river kilometer (rkm) 16 (as measured from the mouth of the Snake River), Lower Monumental Dam is located at rkm 67, Little Goose Dam is located at rkm 113, and Lower Granite Dam is located at rkm 173. Reservoir retention times range from less than 2 weeks in winter to just a few days during the spring run-off. Temperatures during the winter in the lower Snake River are likely warmer and more stable than they were historically and ice formation is rare.

Data collection.—We collected reservoir-type fall Chinook salmon for radio tagging by angling in Lower Granite Reservoir during three field seasons. During the winter of 2004-2005, we collected fish during 12-18 November and 13-17 December (field season 1). During the winter of 2005-2006, we collected fish during 26-28 October and from 29 November to 1 December (field season 2). During the winter of 2006-2007, we collected fish during 26-30

October and during 3-7 November (field season 3). We used three fishing rods each equipped with a leaded line (71g), silver "Dodger", and "wedding ring" spinner (size 8) baited with corn or maggots. Terminal gear was trolled at slow to moderate speeds approximately 27 m behind a boat while searching for fish with an electronic fish finder. We fished during daylight hours within 2.4 km of Lower Granite Dam. We landed fish with a water-filled sanctuary dip net and transferred them directly into a livewell supplied with re-circulated reservoir water. At the end of each day, fish were placed in a floating net pen (1.8 m long, 1 m deep, and 0.6 m wide) located in the forebay of Lower Granite Dam.

We removed the fish from the net pens a minimum of 24 h and a maximum of 192 h after they had been captured, and then anesthetized them in a 66 mg/L solution of MS-222. We inspected each fish for de-scaling and mouth injury. We then measured fork length to the nearest 1 mm and weight to the nearest 0.1 g, and scanned each fish for a PIT tag with a Model 2001F Portable Transceiver System (Destron-Fearing Corporation). Scanning was necessary to avoid implanting radio tags into juvenile fall Chinook salmon that had been PIT tagged the previous summer as part of other research. Injured fish, previously PIT-tagged fish, and fish smaller than 170-mm fork length were released back to the reservoir without further processing after recovery in aerated water. We used the 170-mm size criterion to distinguish between fall and spring Chinook salmon juveniles (91% accurate; Connor et al. 2005).

We had 100 coded radio tags (model NTC-4-2L; Lotek Wireless, Inc.) for each field season. We activated the tags and then surgically implanted them into fish using the methods of Adams et al. (1998). The radio tags were cylindrical and measured 18.4 mm long by 8.2 mm in diameter, weighed 2.1 g in air, and had 30-cm antennas. The tag to body weight ratio was typically less than 3%. In field season 1, tags were programmed to emit a signal every 10 s for an expected operating life of 171 d. In field season 2, tags emitted a signal every 12 s for an expected operating life of 177 d. In field season 3, new coding technology enabled tags to emit a signal every 8 s for an expected life of 221 d. Following surgery, the fish were allowed to recover for 24 h in 125-L containers supplied continuously with fresh reservoir water. After recovery, the 125-L containers of fish were transported by boat to a mid-channel release point 10 km upstream of Lower Granite Dam. We released the fish upstream of their point of initial capture to provide them with time to re-acclimate to the reservoir environment. Following each release, we continuously collected detection data in the forebays and tailraces of Lower Granite, Little Goose, Lower Monumental, and Ice Harbor dams at fixed radiotelemetry detection stations. Each fixed station comprised one to two 3- and 6-element Yagi antennas, an SRX 400 receiver (Lotek Wireless, Inc.), solar panel, and 12-V battery. We downloaded radiotelemetry detection data weekly.

Distance moved and dams passed in winter.—We used dam tailrace detections at Lower Granite, Little Goose, Lower Monumental, and Ice Harbor dams to indicate dam passage. We

assigned a season (winter, 1 November to 19 March; spring, 20 March to 31 May) to the tailrace detection for each detected fish. We identified the downstream-most tailrace detection for each fish detected in winter and then calculated the distance (km) between that tailrace and the release point in Lower Granite Reservoir. We then calculated the number of days (rounded to the nearest whole day) that elapsed between release and the detection. This calculation provided measures of net downstream distance moved and movement duration based on a single unique detection for each fish detected in winter. To describe passage during winter at each dam, we calculated percent winter passage (number of winter tailrace detections divided by the total number of tailrace detections [winter and spring combined]) by dam and field season. The sample sizes used in this analysis of winter passage and all remaining analyses were based on repeated measurements on individual fish.

Downstream movement rate.—To determine whether downstream movement rate depended on field season, season, and reservoir, we calculated downstream movement rates (nearest 0.1 km/d) for individual fish through Little Goose, Lower Monumental, and Ice Harbor reservoirs. Downstream movement rate was equal to the number of days that elapsed between detection in the tailrace of the dam that bounded the upper end of the reservoir and detection in the forebay of the dam that formed the reservoir, divided by distance between the fixed stations in the tailrace and forebay. We assigned each downstream movement rate a season as described previously. Downstream movement rates were not calculated and analyzed for Lower Granite Reservoir because all of the fish were released there in the winter.

As mentioned earlier, the analyses on downstream movement rate were conducted on repeated measurements on individual fish. Thus, we used a mixed model to determine whether mean downstream movement rate (± 95% C.I.) depended ($\propto = 0.05$) on season (winter, spring) and reservoir (Little Goose, Lower Monumental, Ice Harbor). Season, reservoir, and the season × reservoir interaction were the fixed class variables. Field season (1, 2, 3) and the field season × reservoir interaction were random terms. We conducted a square-root transformation of downstream movement rate to stabilize the variance and then fitted the full mixed model described above, a mixed model without interaction terms, and a mixed model without the interaction terms or the random term for field season. The effects of repeated measurements were modeled using an autoregressive error structure with heterogenous variances (Littell et al. 1996). Of the three models, we selected the model with the lowest Akaike's information criterion (AIC) value for subsequent analyses and refer to it as mixed model 1 of downstream movement rate. We used Fisher's protected test for least-significant differences to make pairwise comparisons of least-square transformed means between seasons and reservoirs ($\propto = 0.05$). We report untransformed arithmetic means.

Explaining variation in downstream movement rate.—Condition factor (K; weight divided by length^3 multiplied by 10^5) was selected *a priori* to explain annual variation in

downstream movement rate. We only measured K before release in Lower Granite Reservoir during winter, thus it did not vary seasonally or spatially. To explain seasonal and spatial variation in downstream movement rate, we selected water particle velocity (0.1 km/d) and temperature (0.1°C) as variables *a priori*. We calculated daily water particle velocity from 1 November to 31 May for each field season and reservoir by dividing daily reservoir volume by daily reservoir outflow (data from www.cbr.washington.edu/dart and www.fpc.org) and reservoir length. We calculated mean water particle velocity by averaging the daily water particle velocities for a given fish and reservoir between the detection dates used to calculate downstream movement rate for that fish in that reservoir. Daily mean temperature data were only available for the upper end of Little Goose Reservoir and the tailrace of Ice Harbor Dam (www.cbr.washington.edu/dart). We regressed daily mean temperature measured at these two locations from 1 November to 1 June of each field season against each other (range of r^2 values, 0.92–0.95). The slope coefficient (approximately 1.1 for all three regression models) was divided by the distance between the upper end of Little Goose Reservoir and the tailrace of Ice Harbor Dam to calculate the rate of downstream warming (0.007°C/km). We then used observed daily mean temperatures in the upper end of Little Goose Reservoir, the rate of downstream warming, and downstream distances between reservoirs to estimate daily mean temperature for Lower Monumental and Ice Harbor reservoirs. We then calculated a mean temperature for individual fish in a given reservoir as described for mean water particle velocity.

We substituted mean K for field season as a random term in mixed model 1 (described previously) to produce mixed model 2 of downstream movement rate and then compared the AIC values of models 1 and 2. The AIC values would be the same if mean K fully accounted for the variability associated with field season. We added mean water particle velocity and mean temperature as covariates to form mixed model 3 of downstream movement rate. We then formed competing mixed models by removing fixed terms and covariates. The final mixed model had a low AIC value relative to other models and only included fixed terms and covariates with slope coefficients that varied significantly ($P \leq 0.05$) from zero. If mean water particle velocity and temperature were seasonal and spatial factors for downstream migration rate, we expected these two covariates to replace the fixed terms for season and reservoir in the final mixed model of downstream movement rate. We used the final mixed model to make partial regression plots between downstream movement rate and mean K, mean water particle velocity, and mean temperature. These plots were made as shown in the following example for variable X in the simple model $Y = \beta_o + \beta_1 X + \beta_2 Z$. Predicting Y from Z alone (i.e., $Y_{pred} = \beta_o + \beta_1 Z$) provides residuals ($Y - Y_{pred}$). Each residual is referred to as an "adjusted" Y (Y_{adj}) because the variability in Y attributable to Z has been removed. Next, predicting X from Z (i.e., $X_{pred} = \beta_o + \beta_1 Z$) provides a set of residuals ($X - X_{pred}$) each of which is referred to as an adjusted X (X_{adj}). The partial residual plot is constructed by plotting Y_{adj} against X_{adj} and fitting a predicted regression line to the data ($Y_{pred\ adj} = \beta_o + \beta_1 X_{adj}$).

For reasons described in the results, mean water particle velocity and temperature failed to explain the spatial variation in downstream movement rate. Thus, we examined migratory disposition as a factor for the spatial variation. We calculated the ratio between the downstream movement rate of an individual fish through a given reservoir and the mean water particle velocity for that fish and reservoir. We classified all fish with ratios less than or equal to 1.0 as "slow movers" and those with ratios greater than 1.0 as "fast movers" (after Tiffan et al. 2009b). To determine if we could pool the data across field season, we used a 3 × 2 chi-square analysis ($\propto = 0.05$). We then used a probit model with a random term for individual fish to account for correlations among repeated observations on the same fish (Littell et al. 1996) to determine if the percentage of fast movers depended on season and reservoir. The probability of a fish being a fast mover was modeled as p = probit (season, reservoir, fish) in the full probit model describing migratory disposition. Season and reservoir were fixed terms and fish was a random term. We fitted the full probit model, a probit model without a term for season, and a probit model without a term for reservoir. We used AIC values, *P*-values, and parsimony to select the final probit model for determining whether the percentage of fast movers depended on season and reservoir.

RESULTS

Distance Moved and Dams Passed in Winter

We captured 114 (field season 1), 74 (field season 2), and 238 (field season 3) reservoir-type juveniles. Of these 426 fish: 181 were not implanted with radio tags because (1) they had been previously PIT tagged, (2) the number of fish captured exceeded the number of available tags, (3) the tags failed to activate, or (4) the fish were injured during angling. Only 23 fish were smaller than our 170-mm fork length criterion and were not tagged. We observed no mortality during tagging in any field season or the 24-h post-tagging holding period during field season 1. Totals of 1 and 3 tagged fish died during holding in field seasons 2 and 3, respectively. Two new fish were tagged to replace two of the mortalities in field season 3. Mean fork lengths ranged from 188 to 201 mm and mean weights ranged from 75.6 to 84.1 g. The total number of tagged fish released was 90, 48, and 99 during field seasons 1–3, respectively. Of these, totals of 89 (98.9% of 90), 48 (100% of 48), and 83 (83.8% of 99) fish were detected during winter and spring combined.

None of the 89 fish detected during field season 1 moved the 169 km required to pass all four Lower Snake River dams during winter compared to 9 of 48 fish (18.8%) in field season 2 and 15 of 83 fish (16.9%) in field season 3. Totals of 43, 36, and 70 of the detected fish were detected in the winters of field seasons 1–3, respectively. These were the fish for which we identified the downstream-most tailrace detection and calculated mean distance moved and movement duration during winter. The mean distance moved and duration of movement during

winter was 26.6 ± 32.0 km and 39 ± 27 d in field season 1, 76.3 ± 65.3 km and 60 ± 34 d in field season 2, and 60.5 ± 65.9 km and 37 ± 33 d in field season 3.

The 89, 48, and 83 fish detected in winter and spring combined provided 120, 108, and 189 repeated-measures observations for calculating percent winter passage (i.e., *N* in Figure 2 panel). Spatially, percent winter passage generally decreased from Lower Granite Dam to Ice Harbor Dam within each field season as fish moved downstream and time progressed (Figure 2). Percent winter passage ranged from a high of 93.2% at Lower Granite Dam in field season 2 to a low of 0.0% at Ice Harbor Dam in field season 1 (Figure 2).

Downstream Movement Rate

A total of 117 reservoir-type juveniles provided 222 repeated-measures observations of downstream movement rate. The mixed model fitted without interaction terms was selected as mixed model 1. Field season was retained in model 1 because mean downstream movement rate varied by field season (Table 1). Downstream movement rate was dependent on season because fish moved downstream significantly ($P \leq 0.0001$) slower on average during winter than during spring (Table 1). Downstream movement rate was also dependent on reservoir because mean downstream movement rate was significantly different among all reservoirs (range of *P*-values, ≤ 0.0001–0.03) and it increased from reservoir to reservoir (Table 1).

Explaining Variation in Downstream Movement Rate

Mean K (± SD) was 0.92 ± 0.13 in field season 1, 1.11 ± 0.6 in field season 2, and 1.17 ± 0.07 in field season 3. Mean water particle velocity (± SE) associated with the 222 observations of downstream movement rate was 8.3 ± 4 km/d in field season 1, 14.1 ± 1.1 km/d in field season 2, and 9.1 ± 0.3 km/d in field season 3. Reservoir-type juveniles experienced lower mean water particle velocities during winter (7.9 ± 0.3 km/d) than during spring (15.0 ± 0.9 km/d). Though mean water particle velocity generally increased in both winter (6.1-10.1 km/d) and spring (10.2-19.2 km/d) as fish moved downstream from Little Goose to Ice Harbor reservoirs, the mean water particle velocities experienced by fish during winter in Lower Monumental (10.0 km/d) and Ice Harbor reservoirs (10.1 km/d) were similar. Mean temperature (± SE) associated with the 222 observations of downstream movement rate was 7.2 ± 3°C in field season 1, 5.6 ± 0.3°C in field season 2, and 7.0 ± 0.3°C in field season 3. Reservoir-type juveniles experienced lower mean temperatures during winter (4.9 ± 0.1°C) than during spring (9.1 ± 0.9°C). Fish experienced relatively similar mean temperatures (4.9-5.0°C) as they moved downstream from Little Goose to Ice Harbor reservoirs during winter, whereas the mean temperatures experienced in spring increased slightly (8.6-9.7°C) as the fish moved downstream.

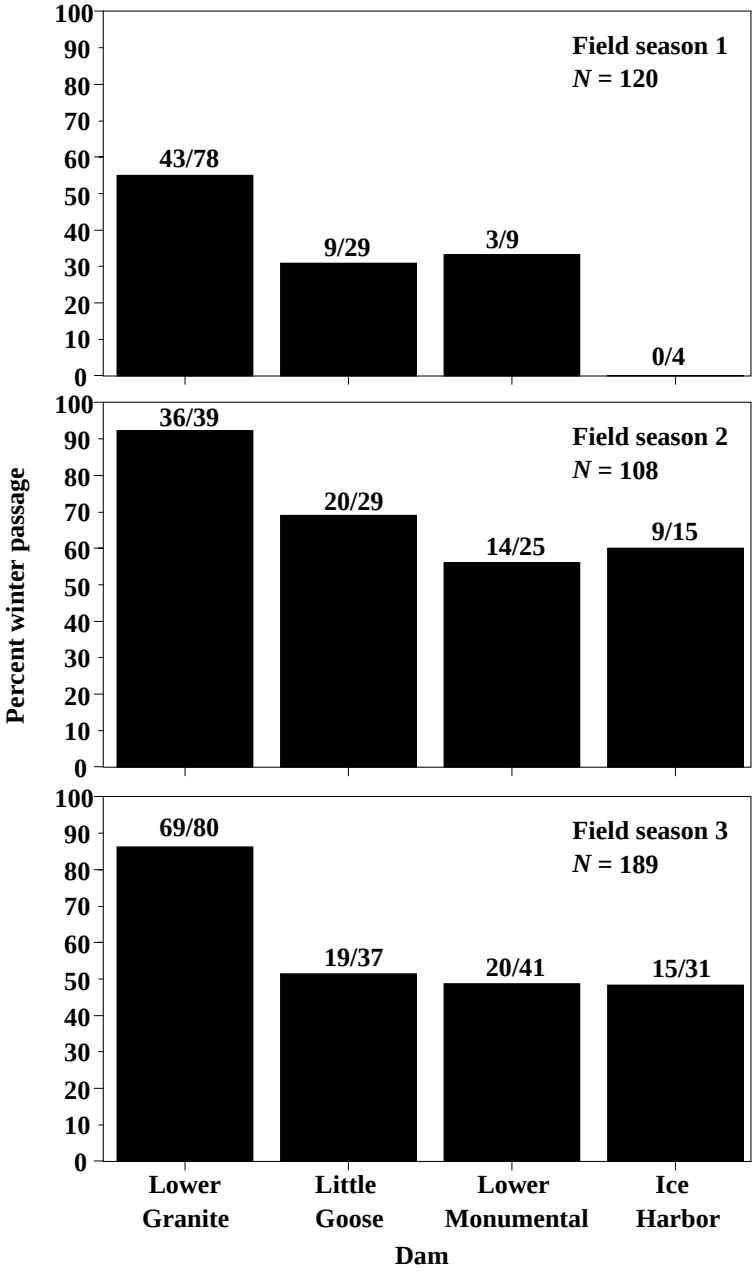

Figure 2. Percent winter (1 November to 19 March) passage at Lower Granite, Little Goose, and Ice Harbor dams for radio-tagged reservoir-type fall Chinook salmon juveniles initially released 10 km upstream of Lower Granite Dam during field seasons 1–3. The numerator above each bar is the number of winter detections at the given dam, the denominator is the total number of detections (both winter and spring) at the given dam, and *N* is the grand total number of detections.

Table 1. Downstream movement rates (km/d ± 95% C.I.) of radio-tagged reservoir-type fall Chinook salmon in the lower Snake River reservoirs by field season, season, and reservoir.

Variable	Level	*N*	Downstream movement rate
Field season	1	57	11.3 ± 2.6
	2	78	12.7 ± 2.6
	3	87	9.2 ± 1.4
Season	Winter	135	7.5 ± 1.0
	Spring	87	16.4 ± 2.4
Reservoir	Little Goose	104	6.9 ± 1.3
	Lower Monumental	69	12.9 ± 2.0
	Ice Harbor	49	16.8 ± 3.4

Substituting mean K as a random term in model 1 formed model 2 (AIC 620.5) which is equivalent to model 1 (AIC 619.4) suggesting that mean K explained most of the annual variability in downstream movement rate. After adding mean water particle velocity and mean temperature to mixed model 2 to form mixed model 3, the slope coefficient for season did not vary significantly ($P = 0.2$) from zero. We therefore dropped season from model 3 to produce our final mixed model of downstream movement rate. Our final model included a fixed term for reservoir ($P \leq 0.0001$), mean water particle velocity ($P \leq 0.0001$) and mean temperature ($P \leq 0.0001$) as covariates, and mean K as the random term. Thus, water particle velocity and temperature explained seasonal variability in downstream movement rate, but did not fully explain spatial variability in downstream movement. Adjusted downstream movement rate generally decreased as K increased ($P \leq 0.01$), increased as mean water particle velocity increased ($P \leq 0.0001$), and increased as mean temperature increased ($P \leq 0.0001$; Figure 3).

Changes in migratory disposition explained the increase in downstream movement rate as fish moved from reservoir to reservoir. The percentage of fast movers was not dependent on field season (3 × 2 chi-square analysis; $P = 0.9$), thus we pooled the data across years to fit the probit model. The effect of season was not significant ($P = 0.1$) in the probit model that included both season and reservoir (AIC = 296.4) because 52% of the 96 fish classified as fast movers moved downstream in winter and 48% moved downstream in the spring. The probit model that only included reservoir had an AIC value that was slightly higher (297.2) than the AIC value of the probit model that included both season and reservoir, but the reservoir-only model was more parsimonious and did not include a non-significant fixed-effects term. Results from the reservoir-only probit model generally showed that marginally significant ($P = 0.05$-0.07) percentages of fast movers increased as fish moved downstream from Little Goose Reservoir (31.7%; $N = 104$) to Lower Monumental Reservoir (46.4% $N = 69$) to Ice Harbor Reservoir (63.3%; $N = 49$).

DISCUSSION

Reservoir-type fall Chinook salmon juveniles moved downstream average distances of 26.6–76.3 km over average durations of 37–60 d during winter and large percentages of the fish passed one or more of the four lower Snake River dams during winter of each field season. Most winter studies on anadromous salmonid juveniles focus on localized movements of fish that stayed in relatively short study reaches (e.g., Roussel et al. 2004; Linnansaari et al. 2005; Enders et al. 2007). The most comparable study we are aware of involved tracking radio-tagged juvenile Atlantic salmon *Salmo salar* over several kilometers in a Newfoundland stream as they moved back and forth between streams and lakes over average distances of only 0.3–0.5 km and over an average duration of 3 wks (Robertson et al. 2003). These short distances reinforce the idea that anadromous salmonid parr in natural systems show little downstream movement during

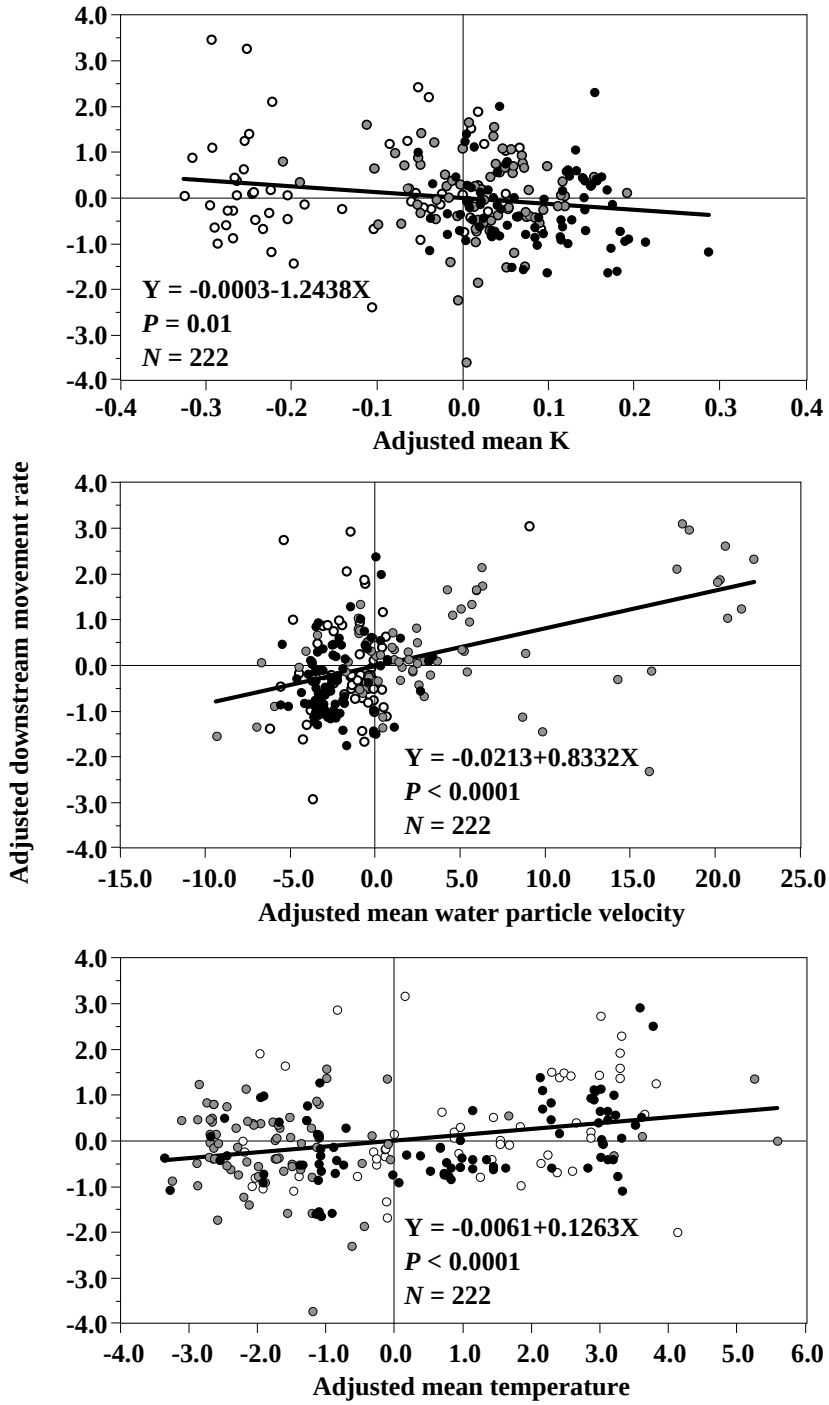

Figure 3. Partial regression plots showing the relations between adjusted downstream migration rate of reservoir-type juveniles and adjusted mean condition factor, adjusted mean water particle velocity, and adjusted mean temperature pooled across field seasons, seasons, and reservoirs. The white, grey, and black circles represent field seasons 1–3, respectively.

winter. Consequently, we question whether the strategy of restricting winter movement by anadromous salmonid parr to conserve energy and promote survival in unperturbed fluvial systems applies to reservoir-type juveniles, which moved long distances in large reservoirs. Because habitats and environmental conditions that act to restrict fish movement in winter in natural systems are fundamentally different from those found in the reservoir habitats we studied, we suggest that in altered habitats, such as reservoirs, the need for cover, protection from predators, and energy conservation are met in different ways that allow fish more unrestricted movement at lower energetic costs.

The need for cover by reservoir-type juveniles is likely met by depth rather than structure in lower Snake River reservoirs, which lacked the structural complexity and variability found in low-order natural streams. The use of depth as cover has been shown for brook trout *Salvelinus fontinalis* and Atlantic salmon parr (Gibson and Power 1975). Use of depth by pelagically-oriented (as inferred from our sampling both along shore and off shore) reservoir-type juveniles would help them avoid predators such as mergansers *Mergus merganser*, double-crested cormorants *Phalacrocorax auritus*, mink *Mustela vison*, and river otters *Lutra canadensis* that would impose the highest predation risk along the reservoir shorelines. Pelagic orientation and use of depth to reduce predation threat has been shown in juvenile *O. nerka* (sockeye and kokanee salmon) in deep lakes (Eggers 1978; Hardiman et al. 2004), and is consistent with prevailing theory on predator avoidance (Werner et al. 1983). The homogenous distribution of low water velocities in reservoirs compared to rivers (e.g., Tiffan et al. 2009b) and the use of pelagic habitats that lack cues for holding might facilitate (either actively or passively) fish movement downstream during winter at reduced energetic costs. Finally, the relatively warm winter environment (mean, approximately 5°C) in the lower Snake River reservoirs would also promote downstream movement because temperatures never fall to the 1-°C threshold for inactivity (Brown et al. 2011).

Condition factor of reservoir-type juveniles at tagging explained a large portion of the annual variability in downstream movement rate, whereas water particle velocity and temperature explained the seasonal variability in downstream movement rate. Further, there was evidence for a spatial effect on the migrational disposition of the fish. The influence of condition factor might be linked to food availability and search behavior if food requirements are not being met (e.g., coho salmon, Giannico and Healey 1998; Atlantic salmon, Huntingford et al. 1992, Robertson et al. 2003). Fish with low condition factors entering autumn and winter may be more prone to move in search of food than fish with higher condition factors. Laboratory and field studies have shown that seasonal increases in water velocity can stimulate downstream movement (coho salmon; McMahon and Hartman 1989; Giannico and Healey 1998) and also increase downstream movement rate (fall Chinook salmon; Tiffan et al. 2009b). Water temperature can influence autumn and winter movement rates of juvenile salmon by either precipitating it or retarding it. Movements of juvenile Chinook and Atlantic salmon and

steelhead tend to be least when temperatures are coldest during winter (Bjornn 1971; Youngson et al. 1983; Roberston et al. 2003), but downstream movement increases in the spring as temperatures increase (Bjornn 1971; Whalen et al. 1999; Miller and Sadro 2003). The increase in fast movers as fish moved from reservoir to reservoir might reflect their in-river experience. Both Connor et al. (2003) and Smith et al. (2003) showed that the downstream movement rate of juvenile fall Chinook salmon increased three- to four-fold with increased distance traveled through Snake and Columbia river reservoirs. As in our study, Connor et al. (2003) and Smith et al. (2003) could not correlate the spatial trend observed in downstream movement rate with annual or seasonal variation in the reservoir environment. We suggest that spatially affected increases in migratory disposition and downstream movement rate that cannot be tangibly linked to annual or seasonal variations in the environment constitute a type of "migratory momentum."

This study is the first to report on winter movements of reservoir-type fall Chinook salmon in large impoundments with emphasis on detecting and explaining annual, seasonal, and spatial variation in downstream movement rate. We acknowledge that we were not able to determine if sampling fish by angling fully represented the population of reservoir-type juveniles in the population. We also selected the factors studied *a priori* because they were convenient to measure and biologically supportable. We fully agree with Huusko et al. (2007) who concluded that a multitude of physical and biological factors affect winter behavior of salmonids making observed patterns difficult to interpret and generalize. A fuller understanding of winter movement by reservoir-type juveniles will require the integration of a more diverse set of factors measured at a finer scale than was done in our study (e.g., local water velocities, food availability, and predator abundance). As such, we hypothesize that (1) long-distance downstream movement during winter is a behavioral and physiological response to habitat alteration caused by anthropogenic activity, and (2) reservoir-type juveniles increase their downstream movement rate in response to annual differences in food availability and needs, seasonal differences in water particle velocity and temperature, and spatially dependent migratory momentum. Regardless of cause, reservoir-type juveniles move far and fast enough to pass dams during winter when the only route of passage is through turbines that may increase mortality. First-year wintering in reservoirs is an important tactic and contributes to adult returns in the Snake River basin population listed for protection under the U.S. Endangered Species Act (NMFS 1992). Gaining a better understanding of the dam passage survival of winter migrants and how to maximize it might further protect Snake River basin fall Chinook salmon and provide information to help recover imperiled populations of anadromous salmonids elsewhere.

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CHAPTER TWO

Gene Expression and Physiological Development of Natural Subyearling Fall Chinook Salmon in the Snake and Clearwater Rivers

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INTRODUCTION

Historically, natural juvenile fall Chinook salmon *Oncorhynchus tshawytscha* (hereafter, subyearlings) in the Snake River basin exhibited an "ocean-type" life history strategy and migrated to the ocean in early to mid summer as subyearlings (Connor et al. 2002). However, with the impoundment of the lower Snake River and water management strategies intended to improve migratory conditions for juvenile salmonids, some subyearlings have adopted an alternative life history strategy. These "reservoir-type" juveniles delay seaward migration, overwinter in reservoirs created by impoundments on the Snake River, and then migrate seaward the following spring as yearlings (Connor et al. 2002). The cause of this life history variant is currently unknown but may be related to rearing conditions that influence physiological development and migratory behavior.

The upper reach of the Snake River (from the mouth of the Salmon River to Hells Canyon Dam) is the warmest contemporary spawning area, and subyearlings produced there emerge the earliest. Nearly all subyearlings from the relatively warm upper reach of the Snake River grow rapidly, rear in the spring under an increasing photoperiod, and adopt an ocean-type life history strategy (Connor et al. 2002). In contrast, the lower Clearwater River is the coolest contemporary spawning area, and fish emerge about 40 d later than in the Snake River. Subyearlings in the Clearwater River experience cooler water temperatures during rearing, and most fish rear under a decreasing photoperiod after the summer solstice. Many of the reservoir-type juveniles come from cool spawning areas in the lower Clearwater River (Connor et al. 2002, 2003a).

Temperatures in the Clearwater River are cool during much of the subyearling rearing period due to a water management strategy called summer flow augmentation. Summer flows are augmented by the release of cool, hypolimnetic water from Dworshak Reservoir on the North Fork Clearwater River. Flow augmentation is intended to reduce water temperatures in the lower Snake River to improve survival of juvenile fall Chinook salmon (Connor et al. 1998). Temperatures in the Clearwater drop from about 14°C to 11°C in early July and remain at that temperature until mid September. However, the water temperature of the Snake River entering Lower Granite Reservoir at the confluence of the Snake and Clearwater rivers can be as high as 23°C during the summer. Consequently, temperatures in the reservoir vary greatly with the deepest waters being cool and the surface waters being warm. This thermal heterogeneity enables subyearlings that enter the reservoir from the Clearwater River to find suitable thermal conditions through behavioral thermoregulation (Tiffan et al. 2009a) and may facilitate the reservoir-type life history. Although flow augmentation improves survival of juvenile fall Chinook salmon in the Snake River reservoirs (Connor et al. 2003c for natural fish; Smith et al. 2003 for hatchery fish), it may have unexpected effects on juvenile life history strategies by providing thermal conditions in the reservoirs that permit migratory delay and overwintering behaviors.

The reservoir-type life history strategy is an apparently successful adaptation to altered river conditions. Juvenile fall Chinook salmon that overwinter in Snake River reservoirs and migrate out the following spring as yearlings are larger when they migrate than their ocean-type counterparts and are thought to have improved survival to seawater due to their large size and early spring migration (Connor et al. 2005). Of the wild adult fall Chinook salmon that return to spawn in the Snake and Clearwater rivers, 41% were reservoir-type juveniles (Connor et al. 2005), and studies of hatchery fall Chinook salmon indicated that the smolt-to-adult returns of juvenile fall Chinook salmon were ten times higher for those released as yearlings than for those released as subyearlings (Bugert et al. 1997). These data suggest that the reservoir-type life history strategy is an effective adaptation by juvenile fall Chinook salmon to contemporary conditions in the Snake and Clearwater rivers, but the mechanisms that contribute to migratory delay and adoption of this life history are not known.

Tagging studies have revealed many details of fall Chinook salmon life history in the Snake and Clearwater rivers (e.g., Tiffan et al. 2009b), but little work has been done to understand the physiology behind these life history decisions. In this study, we used both traditional measures of the parr-smolt transformation (smoltification) and gene expression microarrays to investigate the physiology of natural subyearlings from the Snake River that adopt the historical ocean-type life history strategy. We also collected subyearlings from the Clearwater River, where most reservoir-type juveniles originate, and compared their physiological status to Snake River ocean-type juveniles to explore the potential physiological basis for different life history strategies.

METHODS

Study area

We collected natural subyearlings in Lower Granite Reservoir at different times during their rearing and seaward migration. Lower Granite Reservoir is the first of eight reservoirs encountered during seaward migration. The reservoir extends from river kilometer 173 upstream to Asotin, Washington at rkm 234 (Figure 1). The Clearwater River enters the reservoir at rkm 224. The lower 6 km of the Clearwater River are characterized by a transition zone from the free-flowing river to impounded reservoir habitat, and this is where many subyearlings initially delay their seaward migration (Tiffan et al. 2009b). To describe flows and temperatures during the subyearling rearing and migration, we obtained flow and temperature data for the Snake River at Lower Granite Dam (DART 2012). We obtained flow and temperature data from the Clearwater River from the U.S. Geological Survey gage at Spalding, Idaho.

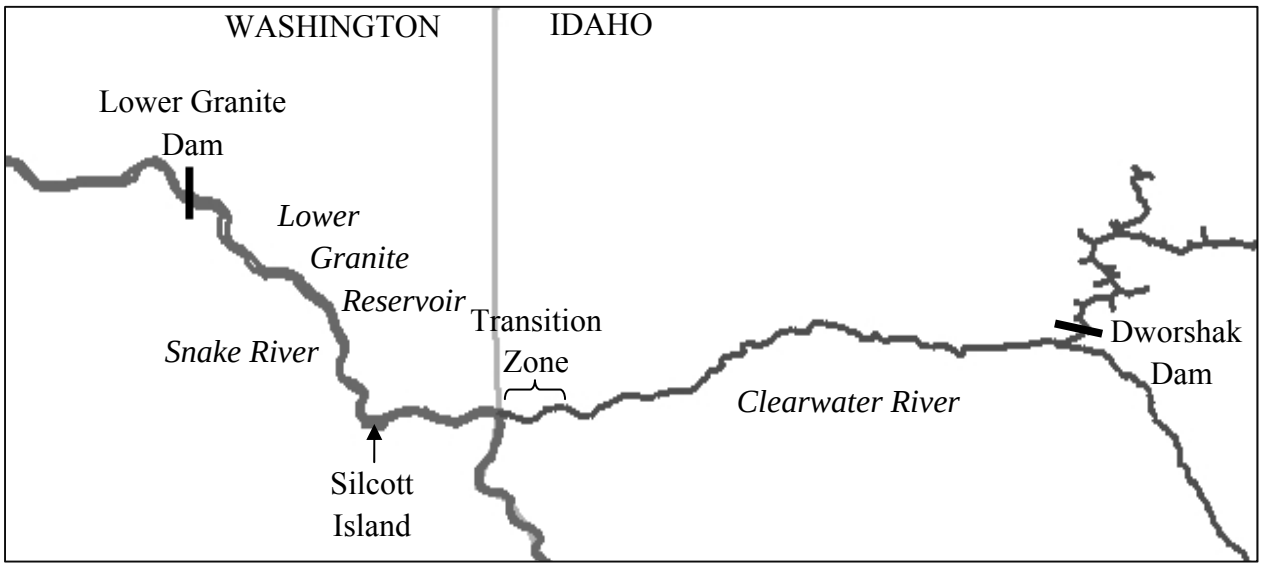

Figure 1. Map of the study area showing Lower Granite Dam where migrating subyearling fall Chinook salmon were collected, Silcott Island where rearing parr were collected, and the transition zone of the Clearwater River where juveniles of unknown physiological development were collected in 2011.

Fish and tissue collection

Snake River fall Chinook salmon are listed as "threatened" under the Endangered Species Act (NMFS 1992). This limited the number of fish we could sacrifice for collection of tissues and the number of comparisons we could make. We collected natural subyearling parr with a beach seine from a rearing site in the upper end of Lower Granite Reservoir (Silcott Island, rkm 211) on 10 May and 1 and 16 June 2011. We refer to this as the "rearing site" hereafter. We also collected presumed smolts from the Lower Granite Dam juvenile fish facility on 19 July, 23 August, and 12 October 2011 during normal sampling procedures. Finally, we collected natural juvenile fall Chinook salmon from the mouth of the Clearwater River near its confluence with the Snake River on 24 August 2011 using a lampara seine. On each sampling date, we collected 15 fish and held them in a cooler with aeration while fish were processed individually. We visually assessed each fish for pigmentation indicative of parr (i.e., dark vertical bars) or smolt (i.e., silver color) and measured fish fork length (FL) to the nearest mm and weight to the nearest 0.001 g. To collect blood, we severed the caudal peduncle and drained the blood into a heparinized capillary tube. Blood was transferred from the capillary tube into a clean microcentrifuge tube and placed on ice until all fish had been sampled. The blood was then centrifuged, and the plasma layer was transferred to a clean tube, which was frozen immediately on dry ice. We collected the first two gill arches on the left side and placed them in 1 ml ice-cold SEI buffer (250 mM sucrose, 10 mM Na_2EDTA, 50 mM imidazole, pH 7.3). Gill samples were placed on ice until sampling was complete and then transferred to dry ice. We collected up to 130 mg liver and placed it in 1.3 ml RNA*later* (Ambion, Inc., Austin, Texas). Liver samples were placed on ice and then refrigerated at 4°C overnight. The RNA*later* was then removed, and the samples were stored at -80°C until processed for RNA extraction.

Gill Na+/K+-ATPase assay

Na^+/K^+-ATPase activities were determined using the microassay method of McCormick (1993). Gill tissue was homogenized in 150 µl SEID (SEI buffer with 0.1% deoxycholic acid), then centrifuged at 3000g for 30 sec. Duplicate 10 µl homogenate samples were added to 200 µl assay mixture with and without 0.5 mM ouabain in 96 well microplates at 25°C and read at 340 nm for 10 min with intermittent mixing. Ouabain-sensitive ATPase activity was detected by enzymatic coupling of ATP dephosphorylation to NADH oxidation and expressed as µmol ADP/mg protein/hour. Protein concentration was determined using a Bicinchoninic Acid (BCA) Protein Assay (Pierce, Rockford, IL) with bovine albumin as standard. Both assays were run on a THERMOmax microplate reader using SOFTmax software (Molecular Devices, Menlo Park, CA).

Plasma T_4 and T_3 assays

A competitive binding radioimmunoassay specifically developed and validated for salmon plasma was used to measure thyroxine (T_4) and triiodothyronine (T_3) (Dickhoff et al. 1978). Five µl of plasma was run in duplicate for most samples, with the exception of a few samples from parr for which sample volume limited us to a single sample. The lower detection limit was 0.5 ng/ml (T_4) and 0.2 ng/ml (T_3). Intra-and inter-assay coefficients of variation for these assays were 4-11% and 3-6%, respectively.

Physiology data analysis

We calculated fish condition factor (*K*) using the following equation: $K = 10^5 \times (\text{weight}) / (\text{FL})^3$. Mean FL, mass, and *K* were compared between fish from each sampling date using one-way analysis of variance (ANOVA). We also compared mean gill Na^+–K^+ ATPase activity and plasma T4 and T3 concentrations using one-way ANOVA. Tukey's HSD test was used to identify differences between pair-wise comparisons of sampling dates. We performed statistical analyses with GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA), and the level of significance for all tests was 0.05.

RNA extraction

We extracted RNA from 12 fish collected on the following sampling dates: 1 June, 19 July, and 12 October from the Snake River and 24 August from the Clearwater River. Total RNA was extracted from 20–30 mg RNA*later*-preserved liver tissue using an RNeasy kit (Qiagen, Inc., Valencia, CA) with recommended on-column DNase digestion following the manufacturer's instructions. We checked RNA concentration and quality with a BioMate 3 UV spectrophotometer (Thermo Electron Corporation, Madison, WI). For each sampling date, four pools of RNA were generated containing RNA from three fish. We added equal amounts of RNA from each fish for a final concentration of 50 ng/µl.

Microarray processing

Microarray processing was performed at The Research Institute at Nationwide Children's Hospital Biomedical Genomics Core (Columbus, OH). Integrity of the pooled RNA samples was assessed using an Agilent 2100 Bioanalyzer Lab-On-A-Chip Agilent 6000 Series II chip (Agilent Technologies, Inc., Santa Clara, CA). Samples were labeled and hybridized to the Agilent Salmon Gene Expression Microarray, 4 x 44K following the manufacturer's protocol (One-Color Microarray-Based Gene Expression Analysis Manual [Low Input Quick Amp Version]). The arrays were hybridized overnight, washed, and then scanned with the Agilent G2505C

Microarray Scanner. The raw array data were extracted from the image data using Agilent Feature Extraction 10.10.

Microarray analysis

The raw array data were normalized using the Quantile Normalization package in R software (R Development Core Team, 2011; Bolstad et al. 2003). Genes with significant changes in expression between fish from different sampling dates were identified using significance analysis of microarrays (SAM; Tusher et al. 2001). For SAM analysis, the Bioconductor Siggenes package in R software (Gentleman et al. 2004) was used to calculate a false discovery rate (FDR) according to the q-value method (Storey and Tibshirani 2003). We compared gene expression for fish from different sampling dates, and genes with a fold change ≥ 2.0 and a FDR below 10% were considered significant. We $\log_2$ transformed and median centered the genes and arrays before performing hierarchical clustering using Pearson centered correlation and average linkage clustering with Cluster 3.0 (Hoon et al. 2004). We used the normalized data for all genes for unsupervised clustering and visualized the cluster results with Java TreeView (Saldanha 2004).

Annotation of microarray probes

To determine the identity of all probes on the Agilent salmon gene expression microarray, we searched for related sequences in the human genome using the National Center for Biotechnology Information (NCBI) standalone command line BLAST+ program (http://blast.ncbi.nlm.nih.gov). We performed BLASTX homology searches of the *Homo sapiens* RefSeq database. The top hit was selected for each sequence queried, and we used NCBI E-Utilities to identify the Entrez GeneID that corresponded to each blast hit accession number. Hits with an e-value ≤ $1e^{-5}$ and ≥50 bp were considered reliable homologs and used for gene functional analysis.

Gene ontology functional analysis

We used the Database for Annotation, Visualization, and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov; Huang et al. 2009a, 2009b) to identify gene ontology (GO) terms that were overrepresented in the lists of genes differentially expressed (fold change ≥ 2.0 and FDR <10%) between fish collected on different dates. Differentially expressed genes were uploaded as a list of Entrez Gene IDs and compared to a reference list consisting of all genes on the array that were annotated with a human homolog. We used the DAVID functional annotation tool to identify GO biological process and molecular function terms from the DAVID GOTERM_BP_FAT and GOTERM_MF_FAT categories that were overrepresented (i.e., there were more genes linked to that GO term than would be expected by chance alone). A modified

Fisher's exact p-value (EASE score) was calculated for each term, and terms with a p-value ≤ 0.05 were considered significant. The overrepresented GO terms were clustered into groups based on their number of shared genes as calculated using kappa statistics. Classification stringency was set to medium. An enrichment score was calculated for each cluster by determining the geometric mean of the p-values for all GO terms in the cluster. We used the Gene Ontology AmiGO visualization tool (http://www.geneontology.org/) to map the relationship of GO terms within each cluster and devised a short description for each cluster based on the main functional pathways of the GO terms included in the cluster. Complete lists of GO terms included in each enriched cluster are included in appendices.

Correlation analysis of gene expression and gill Na^+/K^+-ATPase activity

We calculated the mean gill Na^+/K^+-ATPase activity for each group of three fish used to generate the RNA pools for microarray analysis. The mean values for pools of fish from all sampling dates were assembled into a single data set and checked for normality using the D'Agostino and Pearson omnibus normality test. We used Pearson correlation to compare Na^+/K^+-ATPase activity to $\log_2$-transformed normalized gene expression values. A Pearson's *r* cutoff of 0.7 was used to identify probes that correlated highly with Na^+/K^+-ATPase activity. For each pair-wise comparison between sampling dates, we compared the list of probes correlated with Na^+/K^+-ATPase activity with the lists of probes differentially expressed ≥ 2.0 fold from our microarray analysis and determined the number of probes that both correlated with Na^+/K^+-ATPase activity and were differentially expressed. We divided this number by the total number of differentially expressed probes and multiplied by 100 to calculate the percentage of differentially expressed probes that correlated with Na^+/K^+-ATPase activity. The lists of probes both differentially expressed between two sampling dates and correlated with Na^+/K^+-ATPase activity were analyzed for overrepresented GO functional terms as described above.

RESULTS

Flows and temperatures

In 2011, flows in the Snake River increased from 124 kcfs (one thousand cubic feet per second) on 1 April to a peak of 211 kcfs on 8 June and then decreased to a low of 25 kcfs on 31 October (Figure 2). Water temperatures in the Snake River increased from 7.3°C on 1 April to a maximum of 19.8°C on 6 August and then decreased to 13.1°C by 31 October. In the Clearwater River, flows increased from 59 kcfs on 1 April to a peak of 77 kcfs on 8 June and then declined to 7 kcfs by 29 June. On 5 July, 13 kcfs of flow was released from Dworshak Reservoir (Figure 2), which was maintained through early September. On 15 September flow augmentation ended, and flows in the Clearwater River declined thereafter to a low of 4 kcfs by the end of October.

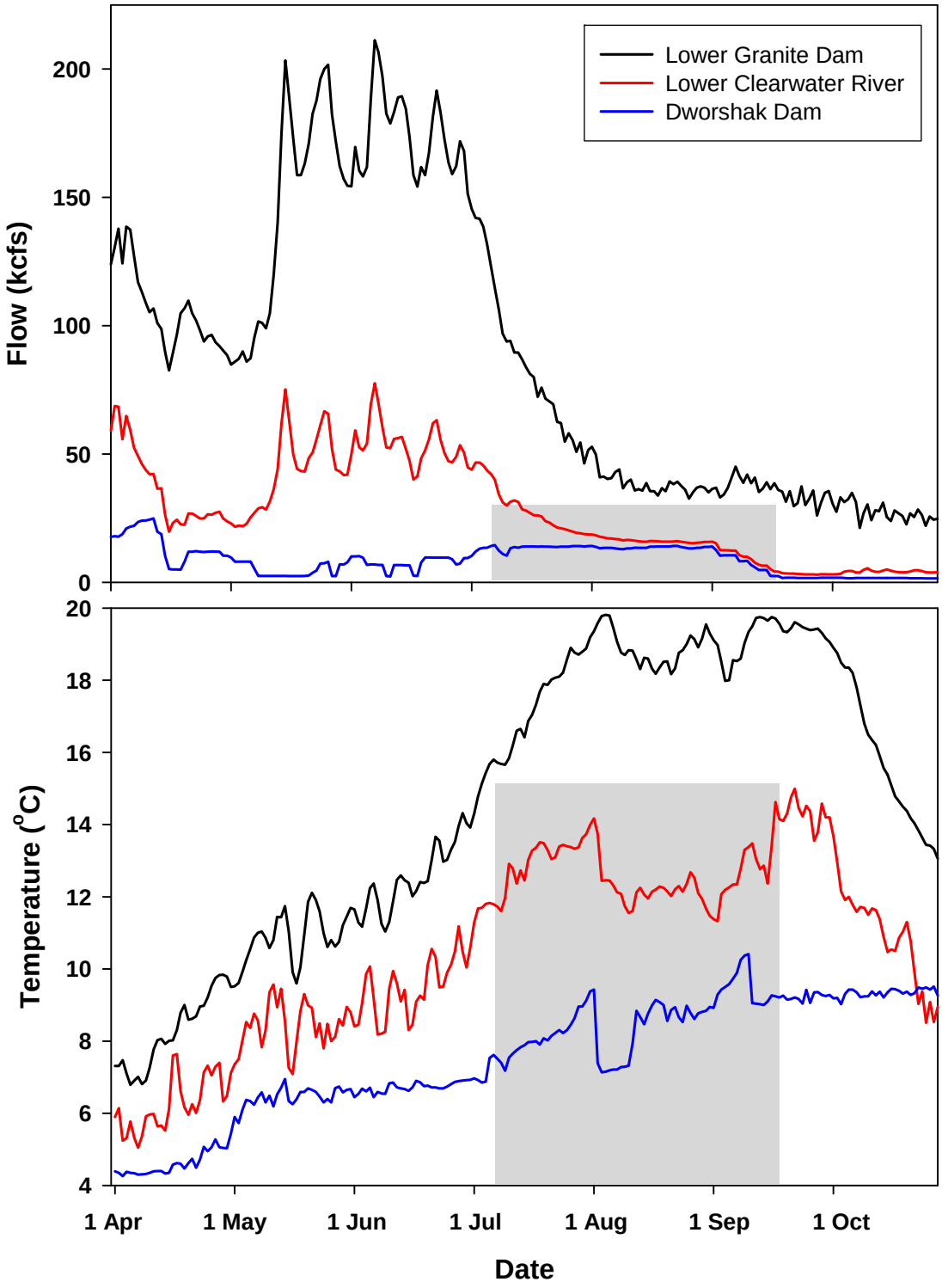

Figure 2. Seasonal trends in flow and temperature measured in 2011 at Lower Granite Dam on the Snake River (black line), the Lower Clearwater River (red line) and Dworshak Dam (blue line) on the North fork of the Clearwater River. The gray shaded area denotes the period of summer flow augmentation using water released from Dworshak Reservoir.

Temperatures in the Clearwater River increased form 5.9°C on 1 April to about 13°C by early July. Temperatures were maintained near 12–13°C by summer flow augmentation through early September. Thereafter, temperature increased slightly and then declined to 9°C by the end of October.

Physiological indicators

Natural subyearlings collected from the Snake River on 10 May and 1 June had dark vertical pigmented bars along their trunk characteristic of parr. Nine fish sampled on 16 June had early external signs of smoltification as indicated by the lightened appearance of vertical pigmented bars, and four of these fish had silver skin pigmentation. The remaining six fish had dark, pigmented bars. All fish sampled in the Snake River at Lower Granite Dam on19 July, 23 August, and 12 October had silver pigmentation, suggesting smolt status, except for two fish with light vertical pigmented bars sampled on 23 August. Fish sampled in the Clearwater River on 24 August all had light vertical pigmented bars visible, but all except one also showed silvering of the skin pigmentation.

There was no significant difference in length or weight of fish collected on 10 May through 16 June at a rearing site in the Snake River (Figure 3). After 16 June, length and mass of fish collected in the Snake River increased significantly on each sampling date (ANOVA: length: $F = 371.4$, df = 6, 98, $P < 0.0001$; mass: $F = 198.9$, df = 6, 98, $P < 0.0001$). Fish sampled from the Clearwater River were not significantly different in size from migrating Snake River fish collected at Lower Granite Dam on 19 July (Figure 3). Condition factor did not show any consistent seasonal trends but was near or above 1.0 for all sampling dates (Figure 3).

Gill Na^+/K^+-ATPase activity was not significantly different between parr collected on different sampling dates at Snake River rearing site (Figure 4). Gill Na^+/K^+-ATPase activity increased significantly, however, in smolts migrating past Lower Granite Dam although it was significantly lower in fish migrating later in the year (August and October) than in fish migrating early (July; ANOVA: $F = 32.97$, df = 6, 98, $P < 0.0001$). Gill Na^+/K^+-ATPase activity in fish collected from the Clearwater River was not significantly different from that of parr collected from the Snake River (Figure 4).

Plasma T_4 levels were highest in subyearling parr collected at the Snake River rearing site (Figure 4). They decreased significantly in smolts collected at Lower Granite Dam (ANOVA: $F = 18.92$, df = 6, 81, $P < 0.0001$). There was no significant difference between plasma T_4 levels in fish collected on different dates at the same site (i.e., rearing site or Lower Granite Dam). Fish collected from the Clearwater River had intermediate plasma T_4 levels with wide variation (Figure 4). They did not differ significantly from plasma T_4 levels of smolts collected from Lower Granite Dam or from parr collected on 10 May at the rearing site.

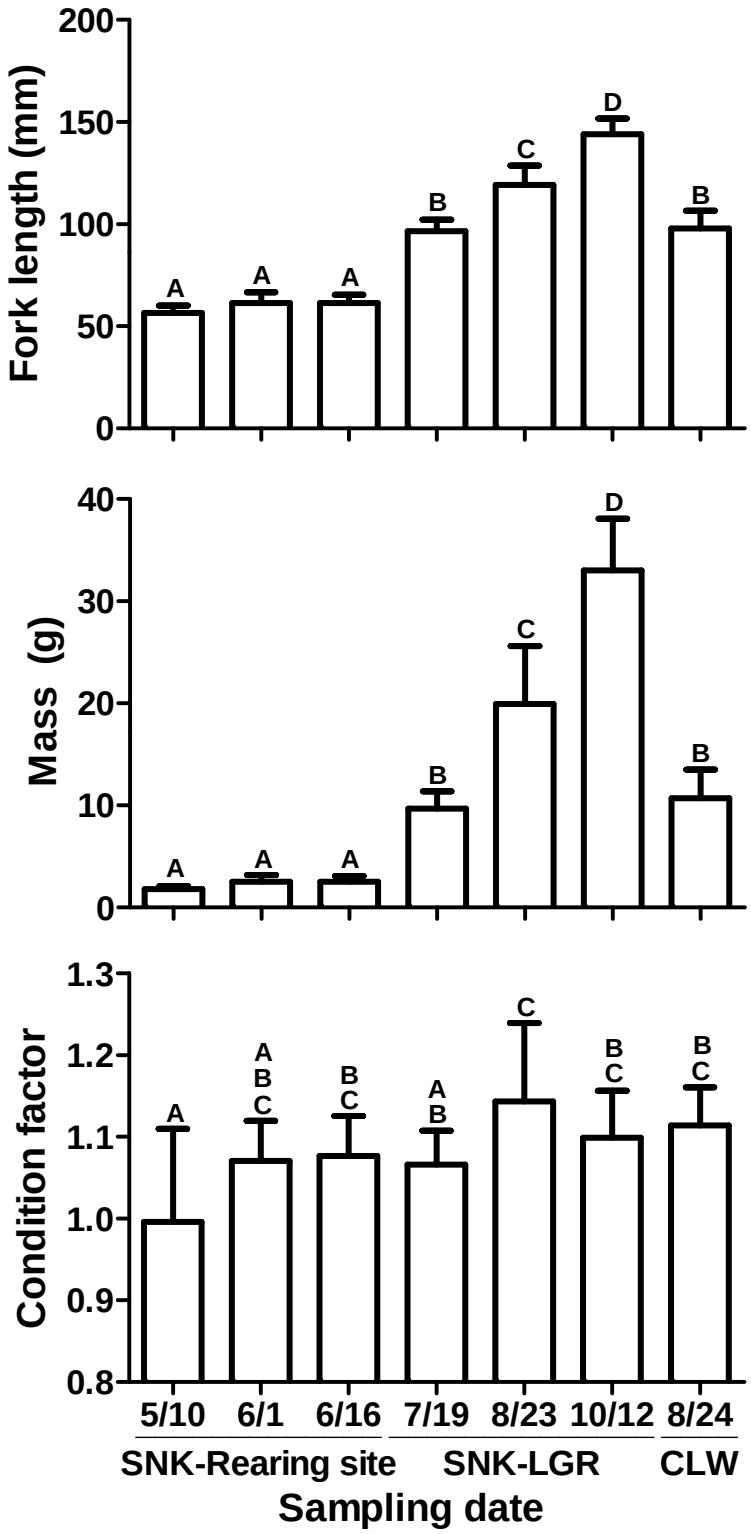

Figure 3. Fork length, weight, and condition factor of natural juvenile fall Chinook salmon from the Snake and Clearwater rivers. Samples without letters in common are statistically different as determined by one-way ANOVA.

Plasma T_3 levels were not significantly different between subyearlings collected from the Snake River on any sampling date (Figure 4). Fish collected from the Clearwater River had significantly higher plasma T_3 levels than parr collected from the Snake River rearing site on 1 and 16 June and from smolts collected at Lower Granite Dam on 12 October (ANOVA: $F = 3.77$, df = 6, 63, $P < 0.0001$; Figure 4).

Gene expression

For gene expression analysis, we chose subyearlings from four sampling dates that represented different stages of development or migration timing. Fish collected from the Snake River rearing site on 1 June were identified as parr whereas fish collected from Lower Granite Dam on 19 July and 12 October were identified as early and late-migrating smolts. Fish collected from the Clearwater River on 24 August were of unknown developmental status but had the potential to adopt the reservoir-type life history strategy along with the late-migrating fish collected on 12 October. We compared global gene expression between fish from the four sampling dates by hierarchical clustering. Fish from each sampling date clustered together, except one pool of Snake River early-migrating smolts, which clustered more closely with the Snake River late migrants (Figure 5). Comparison of parr with early-migrating smolts in the Snake River showed that over 500 probes were differentially expressed ≥ 2.0 fold, indicating that there were significant biological differences between parr and actively migrating smolts. Early and late-migrating smolts from the Snake River clustered together and had few probes differentially expressed ≥ 2.0 fold (Table 1, Figure 5). Parr collected from a rearing site in the Snake River and fish collected from the Clearwater River clustered together and had few differentially expressed probes, indicating that their gene expression profiles were similar (Table 1, Figure 5). Comparison of Clearwater River fish with early and late-migrating Snake River smolts showed that the number of differentially expressed genes between Clearwater and Snake River fish increased as sampling date became later in the Snake River (Table 1).

Functional characterization of differentially expressed genes

Analysis of GO terms overrepresented in the list of genes differentially expressed between Snake River parr and early-migrating smolts showed that genes involved in DNA repair and binding, the cell cycle, metabolism (steroid, fatty acid, and other metabolic pathways), iron homeostasis, heme and oxygen binding, the immune response, and male sexual development were highly enriched (Table 2 and Appendix 1). In genes differentially expressed between early and late-migrating Snake River smolts, however, only three related GO term clusters were enriched. All three clusters were related to mitosis and cell cycle processes (Table 2 and Appendix 1).

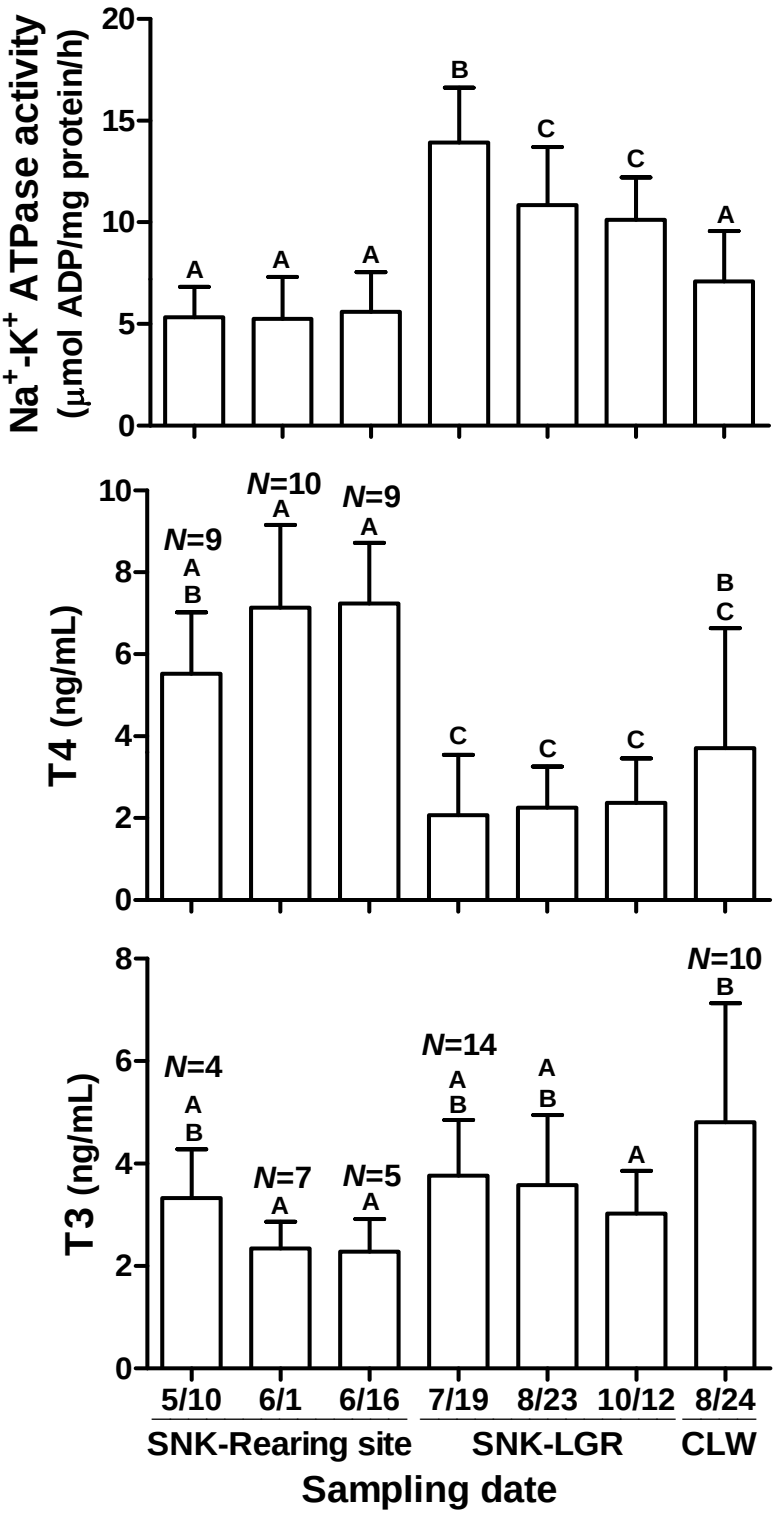

Figure 4. Gill Na^+/K^+-ATPase activity and plasma thyroid hormone levels in natural juvenile fall Chinook salmon from the Snake and Clearwater rivers. Sample size was 15 unless otherwise indicated. Samples without letters in common are statistically different as determined by one-way ANOVA.

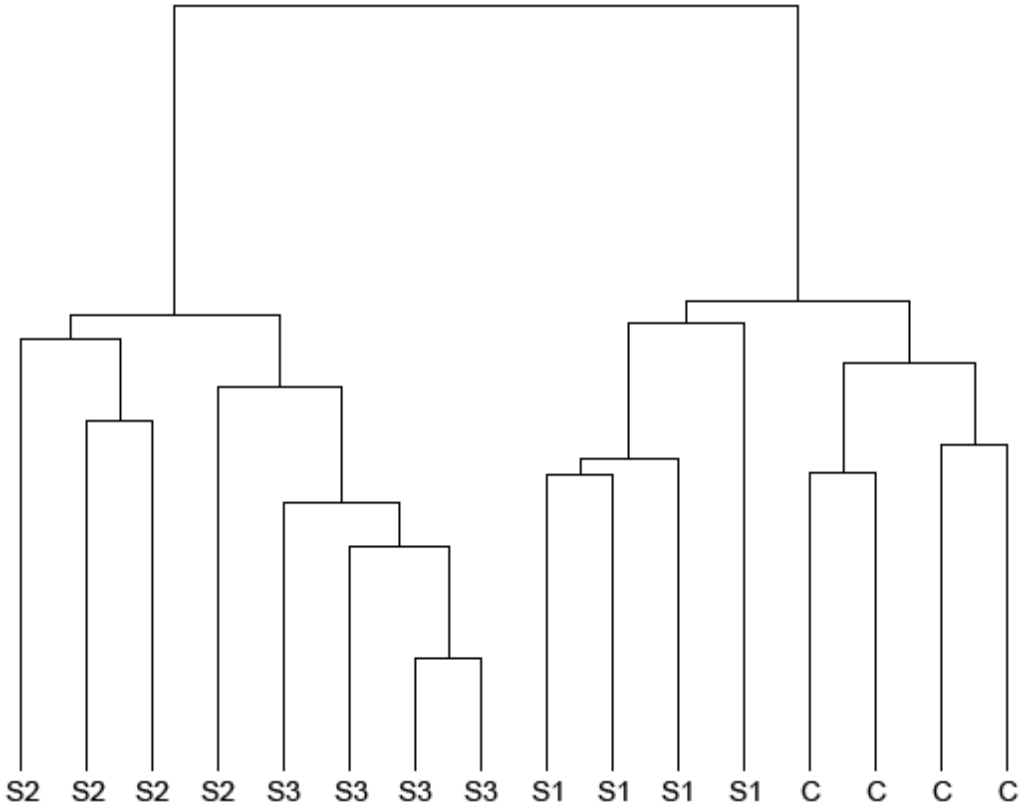

Figure 5. Hierarchical clustering of gene expression data from natural juvenile fall Chinook salmon from the Snake and Clearwater rivers (S1= Snake River parr, S2 = Snake River early-migrating smolt, S3= Snake River late-migrating smolt, and C = Clearwater River fish).

Table 1. Probes from salmon gene expression microarray differentially expressed (DE) in juvenile fall Chinook salmon from the Snake (SNK) and Clearwater (CLW) rivers.

Comparison	Total # of DE probes	DE probes with human homolog		DE probes correlated with Na^+/K^+-ATPase	
		Total #	%	Total #	%
SNK parr vs. SNK early-migrating smolt	586	355	60.6	326	55.6
SNK early-migrating smolt vs. SNK late-migrating smolt	160	83	51.9	4	2.5
CLW vs. SNK parr	157	99	63.1	15	9.6
CLW vs. SNK early-migrating smolt	608	358	58.9	252	41.4
CLW vs. SNK late-migrating smolt	1004	591	58.9	267	26.6

Table 2. Gene ontology functional categories enriched in the lists of genes differentially expressed (DE) between juvenile fall Chinook salmon collected from the Snake (SNK) River at different stages of development and with different migration timing.

Cluster description	Enrichment score	Gene number
SNK parr vs. SNK early-migrating smolts (all DE genes)		
DNA repair	5.61	45
Cell cycle/ Mitosis/ Spindle organization	5.32	49
Steroid metabolism	5.23	24
Chromosome organization/ DNA packaging	2.29	20
Tetrapyrrole, cofactor, and pigment metabolism	2.27	15
Iron homeostasis	1.97	17
Heme binding/ Oxygen binding and transport	1.94	17
Immune response	1.89	13
Isoprenoid, vitamin, and hormone metabolism	1.83	13
Fatty acid metabolism	1.79	15
Exodeoxyribonuclease activity	1.76	4
DNA binding/ Mismatch repair	1.74	10
Nucleotide biosynthesis	1.73	18
DNA damage response	1.65	7
Male sexual development	1.54	6
Adaptive immune response	1.49	10
ATP/ Nucleoside binding	1.41	62
SNK parr vs. SNK early-migrating smolts (DE genes correlated with ATPase)		
Steroid metabolism	3.85	15
DNA repair	3.69	26
Cell cycle/ Mitosis/ Centrosome and spindle organization	2.66	26
Tetrapyrrole, heterocycle, and pigment metabolism	2.20	12
Heme binding/ Oxygen binding and transport	2.18	7
Negative regulation of transcription	1.79	13
SNK early-migrating smolts vs. SNK late-migrating smolts (all DE genes)		
Cell cycle / Mitosis	2.86	11
Mitotic spindle organization	1.87	6
DNA packaging / Chromatin assembly	1.64	4

Table 3. Gene ontology functional categories enriched in the lists of genes differentially expressed (DE) between Snake (SNK) and Clearwater (CLW) river juvenile fall Chinook salmon.

Cluster description	Enrichment score	Gene number
CLW vs. SNK parr (all DE genes)		
Immune response	2.62	17
Vitamin B_6 binding	2.34	6
Negative regulation of apoptosis	1.71	7
CLW vs. SNK early-migrating smolts (all DE genes)		
Cell cycle/ Mitosis/ Spindle organization	7.26	61
DNA repair	4.48	41
Steroid and isoprenoid metabolism	3.86	22
DNA packaging/ Chromosome organization	3.31	33
DNA unwinding for replication	2.10	7
Centrosome organization	1.87	5
DNA mismatch repair	1.69	7
Vitamin metabolism and pyridine nucleotide biosynthesis	1.60	9
CLW vs. SNK early-migrating smolts (DE genes correlated with ATPase)		
DNA repair	4.14	25
Steroid metabolism	4.10	14
Cell cycle/ Mitosis/ Spindle and centrosome organization	2.53	25
Negative regulation of transcription/ Steroid hormone receptor activity	1.74	15
CLW vs. SNK late-migrating smolts (all DE genes)		
Cell cycle/ Mitosis/ Spindle organization	7.16	79
Steroid and isoprenoid metabolism	5.11	34
DNA repair	4.29	59
DNA packaging/ Chromatin assembly	4.11	31
Centrosome organization	3.17	19
Mitotic chromosome condensation and segregation	2.74	14
Microtubule motor activity	2.50	15
DNA mismatch repair binding	2.37	4
DNA unwinding for replication	2.36	9
Exodeoxyribonuclease activity	2.16	5
NAD or NADH binding	1.95	21
Coenzyme and glutathione metabolism	1.74	29

Table 3. Continued

Cluster description	Enrichment score	Gene number
ATP/ Nucleoside binding	1.38	71
CLW vs. SNK late-migrating smolts (DE genes correlated with ATPase)		
DNA replication and repair	5.65	22
Steroid metabolism	3.85	12
Protein polymerization/ Microtubule-based movement	2.70	17
Cell cycle/ Mitosis/ Centrosome and spindle organization	2.59	25
Chromatin/ Nucleosome assembly	2.05	11

When we compared genes differentially expressed between subyearlings collected in the Clearwater River and parr collected in the Snake River, the enriched GO terms were related to the immune response, vitamin B_6 binding, and regulation of programmed cell death (apoptosis; Table 3 and Appendix 2). Gene functional categories overrepresented in the list of genes differentially expressed between fish in the Clearwater River and early-migrating smolts in the Snake River were similar to those seen in the comparison between Snake River parr and smolts. Six of the enriched functional clusters related to the cell cycle, mitosis, DNA packaging, repair, and replication. The remaining two enriched clusters related to metabolic pathways for steroids, isoprenoids, vitamins, and pyridines (Table 3 and Appendix 2). Comparison of genes differentially expressed between fish from the Clearwater River and late-migrating smolts in the Snake River again showed enrichment of GO terms related to cell cycle, mitosis, and DNA repair, packaging, and replication. Genes involved in various metabolic pathways were also overrepresented, including those involved in steroid, isoprenoid, coenzyme, and glutathione metabolism (Table 3 and Appendix 2).

Correlation of gill Na^+/K^+-ATPase activity with differentially expressed genes

To identify gene functional pathways that may be involved in smoltification or related to migration status, we correlated gill Na^+/K^+-ATPase activity, a common indicator of smoltification, with gene expression. Of the 43,804 probes analyzed, expression of 1,883 probes correlated highly with gill Na^+/K^+-ATPase activity. Of these, 4–326 probes were also differentially expressed ≥ 2.0 fold in pair-wise comparisons between subyearlings collected on different sampling dates (Table 1). Only 2.5% of probes differentially expressed between early-migrating and late-migrating smolts in the Snake River correlated with Na^+/K^+-ATPase activity, whereas over one-half of probes differentially expressed between Snake River parr and early-migrating smolts correlated with Na^+/K^+-ATPase activity. When we compared Clearwater River to Snake River subyearlings, the percentage of differentially expressed probes that also correlated with Na^+/K^+-ATPase activity was highest in the comparison with early-migrating smolts and lowest in the comparison with parr (Table 1).

In comparisons between early and late-migrating smolts in the Snake River and between Clearwater River fish and Snake River parr, few of the genes differentially expressed were correlated with Na^+/K^+-ATPase activity. As such, no enriched GO term clusters were identified when the analysis was limited to genes that met both criteria. However, in the comparisons between Snake River parr and early-migrating smolts and between Clearwater River fish and Snake River smolts, analysis of genes that were both differentially expressed and whose expression pattern correlated with Na^+/K^+-ATPase activity showed that these genes belonged to the same functional categories that were overrepresented when all differentially expressed genes were analyzed. When we compared parr to early-migrating smolts in the Snake River, five of the top seven GO clusters enriched in the list of genes differentially expressed between the two

groups were still enriched when that list was limited to genes with expression patterns that also correlated with Na^+/K^+-ATPase activity (Table 2 and Appendix 1). In the comparison between Clearwater River fish and Snake River early-migrating smolts, the top three GO term clusters enriched in the list of differentially expressed genes were still enriched when the list was limited to those genes that correlated with Na^+/K^+-ATPase activity (Table 3 and Appendix 2). Finally, the top four GO term clusters enriched in the list of genes differentially expressed between Clearwater River fish and Snake River late-migrating smolts were still enriched when the list was limited to genes that correlated with gill Na^+/K^+-ATPase activity (Table 3 and Appendix 2).

DISCUSSION

Previously our understanding of natural juvenile fall Chinook salmon development has been limited to physical characteristics (i.e., size, condition factor, and pigmentation) and physiological markers (i.e., gill Na^+/K^+-ATPase activity and thyroid hormone levels). These can be good indicators of developmental status, specifically of smoltification, but they are limited in the information they provide. Here we combined an analysis of physical characteristics and physiological markers with gene expression analysis to provide a more complete understanding of juvenile fall Chinook salmon development in the Snake and Clearwater rivers. Our analysis of physical characteristics in fish from the Snake River showed the expected increase in size over time along with loss of parr marks and silvering of skin pigmentation as fish became smolts and began actively migrating. Fish from the Clearwater River were the same size as early-migrating smolts in the Snake River, but they retained light parr marks and did show complete silvering of the skin pigmentation, suggesting that they were progressing towards smoltification but had not completed the physical transformation associated with smoltification.

Gill Na^+/K^+-ATPase activity increases during smolt development and is causally related to the increase in salinity tolerance of smolts Hoar (Hoar 1988). For most salmon, gill Na^+/K^+-ATPase activity increases just prior to and during downstream migration. In the present study gill Na^+/K^+-ATPase activity was highest in fish captured during migration, consistent with their presumed smolt status and their external appearance. The highest gill Na^+/K^+-ATPase activity was observed early in migration (7/19) while in subsequent samples they were lower, suggesting that late migrants may be losing smolt characteristics. In steelhead *O. mykiss* and Atlantic salmon *Salmo salar* loss of smolt characteristics is a function of time and temperature, with substantial implications for marine survival (Zaugg 1981; McCormick et al. 2009). Fish sampled at the Snake River rearing site had relatively low gill Na^+/K^+-ATPase activity, consistent with their smaller size and non-migratory status. Fish sampled at the Clearwater site had intermediate and highly variable gill Na^+/K^+-ATPase activity. It is possible that fish from this region have a slower smolt development due to a cooler rearing environment. Alternatively, fish samples at the Clearwater site may represent a mixture of parr and smolts.

The observed differences in thyroid hormones, particularly plasma thyroxine levels, are somewhat surprising. Based on previous research on a number of salmonids we expected plasma thyroxine to be elevated in smolts relative to parr (Hoar 1988). In fact, the opposite pattern was observed. It should be noted, however, that parr and smolt were sampled at different times of the year; spring for Snake River parr and summer for Snake river smolts. The elevated levels of plasma thyroxine seen in parr may be due to a seasonal cycle of plasma thyroxine, with high levels in spring and lower levels in fall. Spring increases in plasma thyroxine have been observed in spring Chinook salmon smolts (Beckman et al. 1999; Beckman et al. 2000), though whether similar cycles exist in fall Chinook salmon and their relation to smolt development has not been established and is deserving of further research.

Clustering of gene expression data from all probes showed that in the Snake River, actively-migrating smolts, whether they were migrating early (July) or late (October), were physiologically distinct from parr. Some genes differentially expressed between Snake River parr and early-migrating smolts belonged to gene functional categories previously implicated in smoltification. For example, in a microarray comparison of Atlantic salmon parr versus smolts, Seear et al. (2010) identified differential expression of genes involved in growth, metabolism, and oxygen transport. Although we used a different tissue source (liver vs. gill, kidney, and brain), we identified genes from related functional groups that were differentially expressed between parr and smolts. Specifically, these were genes involved in the cell cycle related to mitotic cell division necessary for growth as well as genes involved in various metabolic pathways and oxygen binding and transport. Moreover, expression of genes in these functional categories also correlated with gill Na^+/K^+-ATPase activity, suggesting that these gene functions are important in the parr-smolt transformation of natural subyearlings.

DNA repair was one gene functional category that was highly enriched amongst all genes differentially expressed between parr and smolts and amongst genes with expression patterns that specifically correlated with Na^+/K^+-ATPase activity. Gallagher et al. (2008) hypothesized that salmon smolts would have low expression levels for genes involved in DNA damage repair and toxin biotransformation pathways compared to adult salmon, putting them at risk from exposure to water contaminants during their seaward migration. When they compared gene expression profiles of coho salmon *O. kisutch* smolts to that of adults, however, they did not identify differential expression of genes involved in these pathways. This was also true in the Seear et al. (2010) comparison of Atlantic salmon parr to smolts. Both studies, however, compared fish raised in captivity that were exposed to similar water, fed a similar diet, and faced no specific toxin or stress challenge. In this study, we compared natural fish, which face a myriad of stressors and potential water pollutants, especially during their seaward migration. Overrepresentation of genes involved in DNA repair in the comparison between parr and smolt suggested that smolts traveling past Lower Granite Dam were exposed to different contaminants and stressors than parr rearing upstream. Changes in expression of genes involved in DNA repair

may not be directly related to the physiological process of smoltification, but they demonstrate the important role that environment plays at each stage of development.

Besides comparing parr and smolts in the Snake River, we also compared smolts that migrated past Lower Granite Dam early and late in the year. Comparison of gene expression profiles between these two groups confirmed gill Na^+/K^+-ATPase and thyroid hormone data suggesting that active migrants in the Snake River were physiologically similar regardless of when they migrated. However, in spite of physiological similarities, the timing of seaward migration and the accompanying changes in migratory behavior may be more influential in determining life history decisions. The main difference between early and late migrants was size. Late-migrating smolts had increased opportunity for growth in the Snake River due to their delayed migration, and this was supported by overrepresentation of genes involved in the cell cycle, specifically mitosis and related DNA packaging and chromatin assembly. Our data did not suggest that gene expression differences control migration timing.

Direct physiological measurements along with gene expression data provided a baseline developmental pattern for ocean-type subyearlings in the Snake River. We compared gill Na^+/K^+-ATPase activity, thyroid hormone levels, and gene expression in subyearlings from the Clearwater River to data from ocean-type subyearlings in the Snake River to determine the developmental status of fish in the Clearwater River. Na^+/K^+-ATPase activity and gene expression suggested that Clearwater River fish were developmentally delayed compared to Snake River fish of the same size. It is difficult to separate growth rate and size, but based on studies in spring Chinook salmon, Ewing et al. (1980) suggested that a slow growth rate delays the increase in gill Na^+/K^+-ATPase activity associated with smoltification. Beckman and Dickhoff (1998) also showed that spring Chinook salmon could smolt either as subyearlings or as yearlings, and they correlated smoltification timing with growth rate. Subyearlings in the Clearwater River emerge later and grow more slowly than fish in the Snake River (Connor et al. 2002), and the cool water released for summer flow augmentation may contribute to this slower growth. Our data showed that fish collected in the Clearwater River were smaller than fish collected in the Snake River during the same time period (23 or 24 August). Photoperiod is important to physiological development (Clarke et al. 1981; Zaugg 1981; Muir et al. 1994). The later emergence in the Clearwater River means that many fish are rearing under a decreasing photoperiod which may retard physiological development. Smoltification may be delayed and migratory disposition may be reduced even though fish reach sizes similar to those of smolts in the Snake River.

Functional analysis of genes differentially expressed between Snake River parr and fish from the Clearwater River showed that the primary differences between these fish are likely environmental. The enriched functional clusters were immune response, vitamin B_6 binding, and negative regulation of apoptosis. Fish rearing in the Snake and Clearwater rivers may face

different disease challenges resulting in different immune responses, and vitamin B_6 is known to affect disease resistance in fish (Blazer 1992). Dietary variance between Snake River parr and Clearwater River fish due to environmental or size-related differences in diet composition could also alter vitamin B_6 availability. Finally, apoptosis or programmed cell death can be a normal part of tissue development, but often it signals disease or injury in the liver (Feldmann 1997). These data suggest that there could be health differences between subyearlings in the Snake and Clearwater Rivers but developmentally, they are at similar stages.

Comparison of gene functional categories enriched in the list of genes differentially expressed between subyearlings in the Clearwater River and early or late-migrating smolts in the Snake River showed that the majority of gene expression differences were similar to those seen in the comparison between Snake River parr and smolts. This again suggests that the Clearwater River fish have retained a parr-like developmental state in spite of their larger size. Monitoring of subyearling development in the Clearwater River over time will be necessary to better understand their smoltification and if there is a physiological basis for migratory delay and subsequent adoption of a reservoir-type life history.

Our study showed that physiological development of natural subyearlings can be effectively studied using gene expression microarrays. The microarray approach also revealed new gene functional pathways such as DNA repair that may be important in the life history experiences of subyearlings. Finally, using a combination of traditional smoltification indicators and gene expression microarrays, we showed that subyearlings from the Clearwater River retain parr-like physiological attributes longer than ocean-type juvenile fall Chinook salmon from the Snake River which may explain their propensity to delay seaward migration. Further microarray studies may be used to determine when Clearwater River subyearlings undergo smoltification, if they are physiologically distinct from ocean-type subyearlings when they begin active migration, and the implications of the reservoir-type life history strategy on long-term survival and fitness.

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CHAPTER THREE

Mortality and Severity of Gas Bubble Disease of Juvenile Fall Chinook Salmon Exposed to Supersaturated Gas Concentrations and Sudden Changes in Temperature

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INTRODUCTION

The Clearwater River in Idaho is home to one of the four major populations of threatened Snake River Basin fall Chinook salmon *Oncorhynchus tshawytscha* (Connor et al. 2002). This population is unique because juvenile fall Chinook salmon emerge from spawning gravels and emigrate later than other fall Chinook populations in the Snake River Basin (Connor et al. 2002). This late emergence and emigration is driven, in part, by water temperatures that are relatively cool compared to the warmer Snake River. In summer, water temperatures of the Clearwater River can be up to 15°C cooler than those of the Snake River into which it flows (Tiffan et al. 2009a, b, 2010). The cooling of the Clearwater River is artificial, resulting from hypolimnetic water released from Dworshak Dam, located about 55 km upstream of the confluence with the Snake River.

As a result of their later emigration during summer, juveniles are exposed to a thermally juxtaposed and potentially lethal area at the confluence where the relatively cool Clearwater River and relatively warm Snake River mix (Figure 1). For example, hatchery juvenile fall Chinook salmon emigrating through the lower Clearwater River and confluence (13-km reach) in 2007 and 2009 had very low probabilities of migrating and surviving (~0.30-0.60, Tiffan et al. 2009a; 0.30-0.35, Tiffan et al. 2010), which are considerably lower than relative survival proportions through Snake River dams (0.87-1.00; Muir et al. 2001). Also in 2009, emigrating juveniles reversed direction just upstream of the confluence and made repeated forays back upstream into the lower Clearwater River (Tiffan et al. 2010). Juveniles continued these upstream forays and delayed their emigration until the thermal difference between the rivers dissipated in mid-September when cold-water releases from Dworshak Dam ceased (Tiffan et al. 2009a, 2010). In addition, juvenile fall Chinook salmon captured in the lower Clearwater River in 2008 displayed visible signs of gas bubble disease (GBD; visible gas bubbles in fins and gills) despite relatively low concentrations of total dissolved gas (TDG) measured in the sampling area (101% to 109% TDG; Tiffan et al. 2009a). However, the cause of this GBD may have also been due to holding conditions during GBD evaluation (Tiffan et al. 2009a). The combination of low survival, migration delay, and incidence of GBD in emigrating juvenile salmon at the confluence suggest that confluence conditions are potentially dangerous through the cold-water release period (July through mid September) and may be causing lethal or sublethal GBD. However, the exact cause of the GBD and its direct or indirect relationship to mortality of emigrating juvenile salmon is not yet known.

In summer when cold, hypolimnetic water is released from Dworshak Dam, the confluence has a warm surface layer of Snake River water and a cold bottom layer of Clearwater River water. Consequently, juvenile salmon emigrating from the Clearwater River and passing through the confluence have the potential to be impacted by two factors that may cause GBD and direct or indirect (sublethal) mortality. First, an emigrating juvenile that is acclimated to

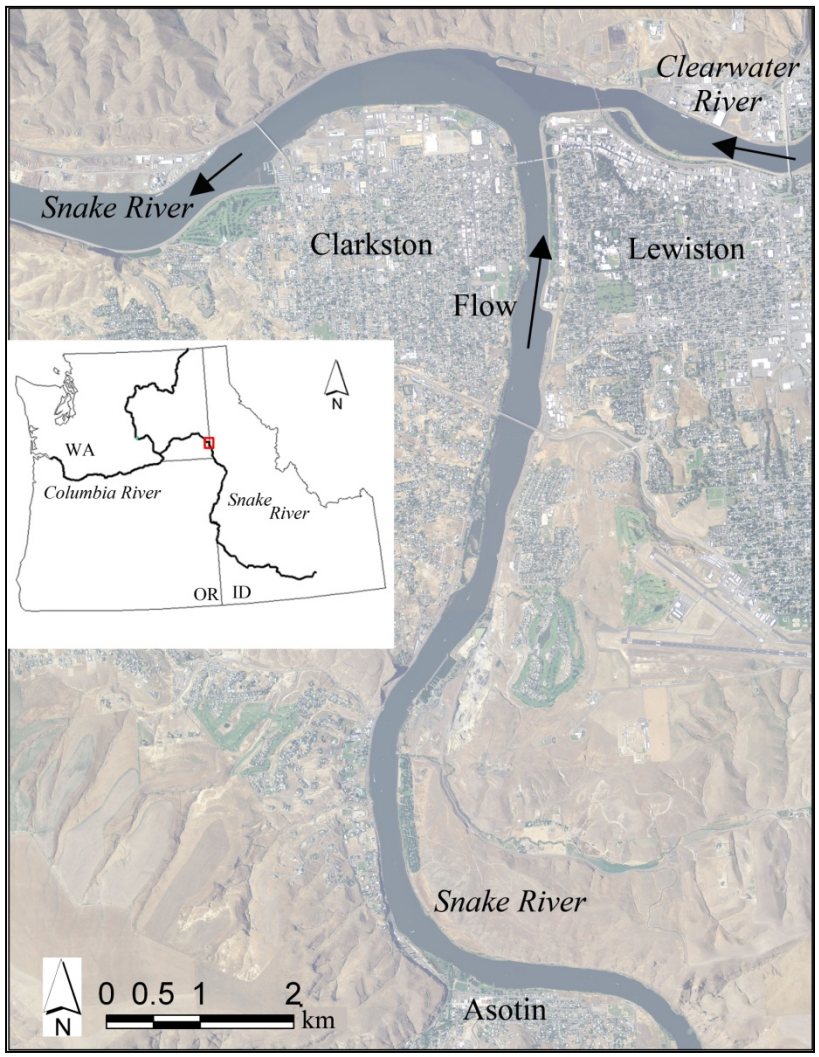

Figure 1.—Aerial view map of the Clearwater River and Snake River confluence on the border of southeastern Washington state and Idaho.

Clearwater River conditions in the summer (10-12°C and 100–110%TDG; Tiffan et al. 2010) would have a body temperature and gas saturation in equilibrium with the Clearwater River. If a juvenile salmon then passes quickly into the warm surface layer of the confluence (up to 25°C; Tiffan et al. 2009a, b, 2010), which would occur during known surface-feeding events at the confluence, it is possible that gas bubbles formed during residence in the slightly supersaturated Clearwater River may expand as a consequence of the temperature increase (up to 15°C), resulting in noticeable signs of GBD and direct or indirect GBD-induced mortality. This is reasonable because according to the physical properties of dissolved gas in water, water at 10°C and 100% TDG (Clearwater River conditions) that is heated to 25°C (Snake River conditions) would experience a difference in partial pressure (ΔP) of 250.2 mmHg, which is equivalent to approximately 133% TDG (assuming barometric pressure = 760 mmHg and salinity = 0.0 ppt; Colt 1984). If we assume that a juvenile salmon's body reacts similarly to water as it is heated, emigrants from the Clearwater River that swim into the warm surface layer of the confluence could similarly experience this change in partial pressure and equivalent high TDG concentration within their bodies. Depending on the gas saturation concentration of the confluence waters, dissolved gas levels within emigrating salmon that are created during the temperature transition would either decrease (if the confluence is at 100% TDG) or remain supersaturated (if the confluence has supersaturated TDG). Unfortunately, the dissolved gas saturation of the Snake River–Clearwater River confluence is currently unknown so we are unable to predict exactly how GBD will be expressed in migrating salmonids.

Second, it is possible that mixing of Clearwater River water at 10°C and more than 100% TDG and Snake River water at 25°C and more than 100% TDG (i.e., a worst-case-temperature scenario) could create supersaturated TDG concentrations at the confluence. This is so because a thorough mixing of equal flows of 10°C and 25°C water at 100% TDG would result in a TDG concentration of about 102% TDG (Colt 1984). Although 102% TDG is known to be safe for migrating juvenile salmon (Backman et al. 2002), the value could be higher because Colt (1984) does not provide an equation to account for supersaturated TDG of the premixed water, which is known to be as high as 110% TDG in the Clearwater River. In addition, baseline TDG levels of the Snake River during the summer are unknown, so estimating the actual super-saturated gas level of the confluence is difficult. However, if we assume a diel fluctuation of 100-110% TDG in the Snake River similar to that in the Clearwater River, the TDG concentration of mixed water at the confluence would be greater than 102% TDG, which may be a concentration of concern for creating GBD. Consequently, under some combinations of river temperatures and baseline TDG concentrations of the two rivers during summer, these two scenarios have the potential to cause GBD and direct or indirect mortality of emigrating juvenile fall Chinook salmon through the Clearwater–Snake confluence. Field measurement of TDG concentrations at the confluence during the summer are needed to quantify TDG concentrations and help elucidate potential impacts of TDG on emigrating juvenile salmon.

Temperature increases alone, depending on the magnitude, are known to cause mortality of juvenile Chinook salmon. However, no known studies have evaluated GBD as the mortality mechanism in juvenile salmon following an increase in temperature. In a previous study, groups of juvenile spring Chinook salmon acclimated to 10°C and supersaturated dissolved gas (125-130% TDG) and then immediately exposed to 25°C had mean times to death of 7.5 min (Ebel et al. 1971); however, no results of GBD and its effect on mortality were reported or discussed. Thus, the causative mechanism of death is uncertain for fish acclimated to supersaturated gas conditions and exposed to increased temperatures. Further, spring-run Chinook salmon were used in Ebel et al. (1971) and may have differential thermal and GBD tolerance than those of fall-run Chinook salmon (Sauter et al. 2001); this potential difference should be evaluated to understand the probability of mortality related to GBD of Snake River juvenile fall Chinook salmon from the Clearwater River.

Previous studies have described GBD symptoms in juvenile salmon and the potential relationship between symptoms and mortality risk. In one study, GBD symptoms in juvenile Chinook salmon did not correlate well with mortality at either low (110-115%) or high (120-125%) levels of TDG (Dawley and Ebel 1975). However, in another study relating GBD symptoms to mortality, yearling spring Chinook were examined for bubbles in the lateral line, fins, external body surface, and gills after exposure to 110, 120, and 130% TDG (Mesa et al. 2000). Mortality did not occur with exposure to 110% TDG, and a weak relationship was found between most symptoms and mortality at 120% TDG (Mesa et al. 2000). However, occurrence of gas bubbles in the lateral line was highly correlated with mortality at 120% TDG (Mesa et al. 2000). Severity of bubbles in the fins was highly correlated with mortality at 130% TDG; however, no mortality occurred at 110% TDG despite very severe fin bubbles. Based on this result, Mesa et al. (2000) recommend that fin bubbles may be a limited indicator of potential mortality. Other studies have shown that bubbles in the gills are often correlated with mortality. For example, Dawley et al. (1976) found that bubbles and emboli in gill filaments were prevalent in dead fish, but they rarely observed these symptoms in live fish. Similarly, occluded gill filaments were observed in almost all dead or moribund fish after exposure to high TDG levels in Mesa at al. (2000). Although gill bubbles may be a proximate cause of death, this and other symptoms are likely related to mortality only at higher levels of TDG (120-130%), which may occur to fish migrating through the Clearwater–Snake confluence.

To evaluate the potential for emigrating juvenile fall Chinook salmon from the Clearwater River to incur GBD symptoms and direct mortality from GBD while migrating into the warm surface layer of the Clearwater–Snake River confluence in summer, we performed a controlled laboratory study to simulate acclimation (Clearwater River) and challenge (Snake River) conditions potentially experienced by fish in the wild. The specific objective of this laboratory study was to evaluate mortality rate as well as incidence and severity of GBD of juvenile fall Chinook salmon that undergo this change in acclimation and challenge conditions. Our null hypothesis can thus be stated as

H_o: Mortality rate and incidence and severity of GBD do not differ between groups of juvenile fall Chinook salmon acclimated to Clearwater River conditions that swim into the warm surface layer at the Clearwater–Snake confluence.

For the challenge (Snake River) condition, we assumed a TDG concentration of 100% because Snake River TDG concentrations are unknown. To corroborate this assumption, we measured field TDG concentrations of the Snake River in 2011. These data were used not only to corroborate our laboratory study assumptions post-hoc (the data were collected concurrently) but also to record TDG concentrations at the Clearwater–Snake confluence to determine if supersaturated conditions exist during the juvenile salmon emigration season.

METHODS

Laboratory Setup

Fish acquisition and rearing.—Fall Chinook salmon eggs were obtained from the Priest Rapids Hatchery in Mattawa, Washington, in fall 2010 and transported to the Aquatic Research Laboratory at the Pacific Northwest National Laboratory in Richland, Washington. Fish were reared in 600-L circular fiberglass tanks in a combination of well water and heated river water varying from 4.4°C to 18.8°C and up to 100% TDG for approximately 7 months prior to the experiment. Temperature was manipulated to achieve experimental fish lengths from 60 to 100 mm fork length (FL; hereafter, all fish lengths referenced are fork length) with a mean of about 80 mm. These lengths represent the approximate mean length and range of wild juveniles emigrating from the Clearwater River in July through September. One week prior to experimental trials, all fish were measured and divided into groups based on their length: small (≤80 mm) or large (>80 mm). We used larger fish in earlier experimental replications and allowed smaller fish to grow for later replications in order to minimize length differences between replications, which occurred within a 15-d period. During rearing, fish were kept at a photoperiod that mimicked natural conditions and were fed a commercial feed to satiation (Bio-Vita Fry 1.5 mm, Bio-Oregon, Longview, Washington).

Juvenile fall Chinook salmon from the Hanford Reach Columbia River population were used as surrogates of Snake River fall Chinook salmon from the Clearwater River due to the threatened status of the Snake River stock and the potential difficulties associated with obtaining eggs. However, we assumed that Columbia River eggs would be suitable surrogates based on the proximal geographic location and similar temperature tolerance of juveniles from both populations. Juvenile Columbia River fall Chinook salmon acclimated to 10°C experienced 100% mortality after 37 minutes of exposure to 26.7°C (Snyder and Blahm 1970). Similarly, Geist et al. (2010) calculated the upper lethal temperature of juvenile Snake River fall Chinook salmon to be 26.8°C. Although the experimental methods used to calculate these temperature-related mortality rates differ, we believe these mortality estimates indicate similar temperature

tolerances between juvenile Snake and Columbia River fall Chinook salmon and that Columbia River stock fish are a suitable surrogate.

Experimental system setup.—Acclimation and challenge conditions of 100-110% TDG and temperatures varying from 10-25°C were obtained using a managed system consisting of a water supply, water heater and chiller, and sensors and an apparatus to control gas-saturation. During the experiment, ambient Columbia River water at 15.6 ± 0.3°C and up to 100% TDG was divided into two head tanks and chilled or heated to 8°C and 30°C. Both tanks were aerated after heating or cooling to maintain TDG at approximately 100%. To obtain supersaturated water for experimental conditions, a third head tank with 10°C water was used to supply a gas supersaturation column (Specific Mechanical Systems Ltd., Saanichton, British Columbia, Canada; Figure 2) that consisted of a 2-m-high × 20-cm-diameter stainless steel column with inflow lines for both compressed air and water. Water to the supersaturation column was delivered by a centrifugal pump, while a gate valve at the bottom of the tank controlled the flow rate and internal tank pressure. Compressed air, regulated with a sensor and flow control valve, was injected to the top of the column to increase the total dissolved gas pressure in the water entering the tank. A sight glass at the side of the column with a water-level float switch maintained a constant air-to-water volume ratio inside the column. The supersaturation level of the outflow from the column was maintained at approximately 130% TDG by manipulating the water inflow, water outflow, and column water level. These three head tanks were then mixed using an integrated TDG and temperature sensing and control platform to obtain acclimation and challenge conditions in each experimental chamber (i.e., tank).

Figure 2. Gas supersaturation column that produced elevated TDG levels.

Acclimation and challenge conditions were controlled within six circular 600-L tanks. Four acclimation tanks were maintained at 10°C and 100% TDG (two tanks) or 10°C and 110% TDG (two tanks). Two challenge tanks were programmed to be 10, 15, 20, or 25°C and either 100% or 110% TDG, depending on the challenge conditions needed for an experimental trial on a given day. Three solenoid valves (Irritrol Utra Flow 700-1, Irritrol Systems, Riverside, California; Figure 3) controlled the proportion of water from each of the three head tanks entering the acclimation and challenge tanks. The TDG levels were monitored by a TDG sensor (Model T507, In-Situ Inc., Fort Collins, Colorado) at the outlet of each tank. A barometric pressure sensor (Model CS100, Campbell Scientific Inc., Logan, Utah), data logger (Model CR1000, Campbell Scientific Inc.), and controller (model SDM-CD16AC, Campbell Scientific Inc.) maintained the TDG level within each tank (± SD ≤ 4.8 mmHg; Figure 4). The TDG sensors were calibrated using a pressure calibrator (Fluke-719-30G, Fluke Corporation, Everett, Washington). The gas sensors had an accuracy of ± 2 mmHg over the range of 400 to 1400 mmHg. Gas levels of each tank were controlled by a computer program (written in CRBasic and implemented via LoggerNet; Campbell Scientific Inc, Logan, Utah) that operated three solenoid valves for each tank (Figure 4). The valves controlled delivery of the gas supersaturated water and the heated and chilled saturated water as needed to maintain target gas and temperature levels. Gas and temperature level data were checked against the target value every second and recorded to the data logger every minute. Data loggers were downloaded approximately once daily.

Figure 3. Solenoid valves used to control levels of heated, cooled, and cooled supersaturated water entering each tank.

Figure 4. Data logger, relay controller, and power supply used to control and maintain conditions in acclimation and challenge tanks.

Gas Bubble Disease Evaluation

Experimental procedure.—The experimental procedure included an acclimation phase followed by a challenge phase chosen to emulate conditions encountered by fish migrating from the cooler water of the Clearwater River into the warmer water of the Snake River. Fish were acclimated at 10°C in either 100% TDG or 110% TDG for 6 to 7 d prior to the challenge exposure. This acclimation period simulated the approximate water conditions and migratory period that a hatchery-released or wild migrating juvenile salmon might experience during emigration from the Clearwater River. After acclimation, cohorts of six fish were placed into one of nine challenge conditions: one of four challenge conditions for fish acclimated at 100% TDG or one of five challenge conditions for fish acclimated at 110% TDG (Table 1). A cohort remained in the challenge conditions for either 15, 45 or 75 min; the 15-min period represented the minimum amount of time required for a fish to incur GBD symptoms caused by the temperature increase, and the 75-min period was set as the maximum time that GBD symptoms would remain due to equilibration that would occur with fish in 100% TDG in the challenge condition. Three replications of each acclimation–challenge–time exposure combination were performed. Challenge tanks with 10°C water and 100% or 110% TDG were used to control against temperature and differentiate the effect of increased TDG acclimation on severity of GBD. The order of challenge tests was randomized within each replicate.

Table 1. Number of fish exposed to each acclimation and challenge combination per time group in the experimental matrix. The asterisks denote control treatments.

Acclimation Condition (TDG %)	Challenge Condition (°C/TDG %)	Number of Replicates	Exposure Time (min)			Total
			15	45	75	*N*
100	10/100*	3	6	6	6	54
	15/100	3	6	6	6	54
	20/100	3	6	6	6	54
	25/100	3	6	6	6	54
110	10/100	3	6	6	6	54
	10/110*	3	6	6	6	54
	15/100	3	6	6	6	54
	20/100	3	6	6	6	54
	25/100	3	6	6	6	54

The day prior to moving fish from acclimation to challenge conditions, challenge conditions were initiated in challenge tanks and water quality parameters were monitored overnight to ensure conditions were stable within ± 0.4°C for temperature and ± 1.0% for TDG. Fish were prepared for the next day's challenge by randomly collecting three sets (one per time group) of cohorts of six fish from the acclimation tank with a net and placing half of each cohort in a cylindrical mesh basket (45 cm high × 20 cm diameter, 7-mm × 7-mm mesh size; Figure 5). Cohorts were separated into two baskets to stagger the timing of entry and removal from the challenge conditions so that all fish could be immediately and simultaneously evaluated for GBD. Fish were held within the acclimation tank overnight to allow the fish time to acclimate to the baskets prior to the challenge. Styrofoam floats were attached to each basket to maintain their maximum depth at 30 cm, which constrained fish to the water surface so they could not depth-compensate for supersaturated TDG conditions. Baskets were arranged randomly in the acclimation and challenge tanks to avoid any confounding tank effects. Baskets were transferred between acclimation and challenge tanks in water baths to reduce stress to fish.

Fish examination.—Fish were removed from each challenge after 15, 45, or 75 min. During the challenges, fish were periodically monitored to check for dead or moribund fish. Mortalities that occurred prior to the end of a trial were removed when found and evaluated within 15 min of death. Fish were euthanized in a lethal dose of 200 mg tricaine methanesulfonate (MS-222)/L of water buffered with an equal part of sodium bicarbonate to a pH of 7. Anesthetic was prepared in normally saturated water for fish challenged at 100% TDG and in supersaturated water for fish challenged at 110% TDG. Fish were removed from the anesthetic, measured (fork length, mm) and weighed (g), and placed left side up on a dissection tray for examination. Three examiners, who did not know the treatment, performed the physical examinations, and each fish was examined for 15 min. Each fish was examined by only one of the three examiners.

Figure 5. Acclimation tank containing baskets of cohorts. Styrofoam noodles held baskets in a random fixed location.

The methods used to determine the incidence and severity of GBD in fish were derived from Mesa et al. (2000). Fish were examined both externally and internally for incidence and severity of GBD using dissecting microscopes with 10-60x zoom magnification (Carl Zeiss Miscroscopy, LLC, stemi 2000-CS, Thornwood, New York). First, the lateral line was examined to determine the percentage occluded with bubbles. A clear ruler with 1-mm-spaced hatch marks was used to measure the length of the lateral line from the operculum to the caudal peduncle; the number of hatches containing bubbles was noted to determine the percentage of lateral line occlusion. Each unpaired and paired fin was also measured using a transparent graph paper with 1-mm × 1-mm squares for the percentage of surface area covered by bubbles. Fish were then examined for presence or absence of bubbles in eyes, mouth/jaw, head, right and left flank, and caudal peduncle. The external examination was completed by holding open the opercle and examining the first gill arch for the presence of bubbles. If bubbles were present, they were quantified by percentage of surface area covered using the transparent graph paper. Internal examination included recording the presence of bubbles in the stomach, swim bladder, heart, intestines, kidneys, and caudal vein.

Data Analyses

Fish size variability.—Mean length and weight of juvenile salmon varied 3.5 mm and 0.5 g between replicates, respectively (Table 2); however, this difference is likely not biologically significant. Juvenile salmon in the first and second replications had mean fork lengths of 84.0 mm and 84.9 mm and mean weights of 6.5 g and 6.6 g, respectively. Juveniles in the third replication were slightly smaller, with a mean length of 81.4 mm and a mean weight of 6.1 g. The 3.5-mm variability in mean length among replicates in our study was well within mean size differences of 10.8 mm (Mesa et al. 2000) and 10.3 mm (Hans et al. 1999). Thus, we considered fish size to be biologically equivalent between replicates and proceeded with our statistical analyses.

Tank conditions.—Several tests were performed to determine if conditions were statistically similar among the three replicates. Water quality variability within acclimation and challenge tanks was compared across replications using ANOVA with a Tukey–Kramer HSD post-hoc test after data were truncated to the period of time when fish were present. This was done to determine if fish experienced the same conditions per replicate. All data were tested for normality and equal variances prior to testing, and a nonparametric Kruskal–Wallis ANOVA was performed on non-normally distributed data.

Although mean temperature and TDG differed significantly between replicates in both the 100% TDG and 110% TDG acclimation tanks (Table 3), we believe these differences are not meaningful in relation to biological response. In the 100% TDG tank, mean temperature varied from a high of 10.03°C to a low of 9.95°C ($H = 1066.9$, $df = 2$, $p < 0.001$). Total dissolved gas

Table 2. Average fish length and weight by acclimation and challenge groups per replication. All acclimations occurred at 10°C. Abbreviations are fork length (FL) and total dissolved gas (TDG). Variance is reported as one standard deviation of the mean.

		Replication 1		Replication 2		Replication 3	
Acclimation (TDG %)	Challenge (°C/TDG %)	Mean FL (mm ± SD)	Mean Weight (g ± SD)	Mean FL (mm ± SD)	Mean Weight (g ± SD)	Mean FL (mm ± SD)	Mean Weight (g ± SD)
100	10/100	85.3±3.9	6.3±0.9	85.9±4.4	6.8±1.3	82.8±3.1	6.3±0.8
110	10/100	84.7±3.5	6.5±0.9	85.1±3.6	6.8±1.2	80.9±4.2	5.9±1.0
110	10/110	83.0±3.1	6.4±0.9	84.3±3.5	6.6±1.0	80.9±2.9	5.9±0.8
100	15/100	84.9±3.9	6.8±0.9	85.2±2.6	6.2±0.8	81.8±4.1	6.1±1.1
110	15/100	82.9±3.0	6.2±0.8	87.7±4.8	6.9±1.2	81.9±2.9	6.2±0.7
100	20/100	84.7±4.3	6.7±1.1	82.4±2.6	6.0±0.7	80.8±4.4	6.0±1.2
110	20/100	83.4±3.5	6.5±0.9	82.6±4.2	6.4±1.1	81.6±5.2	6.3±1.3
100	25/100	83.7±4.0	6.3±1.0	86.1±4.7	7.0±1.1	80.0±2.9	5.7±0.8
110	25/100	83.0±3.5	6.6±1.0	84.4±2.8	6.7±0.9	81.8±3.9	6.3±1.0

Table 3. Mean temperature and total dissolved gas (TDG) during acclimation periods per replication. Error values are one standard deviation (SD) of the mean. Asterisks denote significant differences in mean temperature or mean TDG between replications for each acclimation condition.

Acclimation (°C/TDG%)	Replication	Mean Temperature (°C±SD)	Mean TDG (%±SD)
10/100	1	10.03±0.19*	100.5±1.3*
	2	9.96±0.21*	100.1±0.9*
	3	9.95±0.14*	99.9±1.0*
10/110	1	10.02±0.19*	109.4±3.5*
	2	10.32±0.87*	109.1±0.5*
	3	9.87±0.09*	109.1±0.6*

varied from a high of 100.5% to a low of 99.9% (H = 2157.6, df = 2, p < 0.001). In the 110% TDG acclimation tank, mean temperature varied from 9.87°C to 10.32°C, and TDG varied from 109.1% to 109.4%; statistically significant differences were noted between replicates (temperature: H = 2476.3, df = 2, p < 0.001; TDG: H = 6066.2, df = 2, p < 0.001). Overall, despite these statistical differences, actual mean values differed only slightly, and the effects of these differences on the experimental replicates are likely negligible.

Small differences in mean temperature and TDG were observed in the challenge tanks between the three replications and sometimes were significantly different; however, the differences were negligible and likely would not have biased our experiment (Table 4). The significant differences were primarily due to very large sample sizes (i.e., physical conditions were measured about once every 60 s during the experiment). Challenge 10/100 was not statistically different in mean temperature (H = 1.812, df = 2, P = 0.404) nor mean TDG (H = 2.938, df = 2, P = 0.230) between replications. Replications for challenge 10/110 were also not significantly different in mean temperature (H = 4.559, df = 2, P = 0.102), but were significantly different in mean TDG (H= 17.468, df = 2, p < 0.001). TDG in the 10/110 challenge varied from 107.8% to 110.6% during the three replications. Challenge 15/100 replications were not statistically different in mean temperature (H = 3.740, df = 2, P = 0.154) but had statistically different mean TDG concentrations (H = 113.383, df = 2, p < 0.001); TDG varied from 98.7% to 102.4%. Challenge 20/100 replications did not differ in mean temperature (H = 4.391, df = 2, P = 0.111), but mean TDG did differ (H = 20.701, df = 2, p < 0.001). TDG levels varied from 99.8% to 102.6% for the 20/100 challenge. Challenge 25/100 had significant differences between replications for both temperature (H = 11.087, df = 2, P = 0.004) and TDG (H = 154.254, df = 2, p < 0.001). Temperature during challenge 25/100 varied from 23.7°C to 25.7°C, and TDG varied from 99.1% to 103.5%. Although statistical differences were observed in water quality conditions between replications, these differences are likely not physiologically detectable by fish. Because these differences were small, we considered it acceptable to group replications in GBD analyses to make inferences about physiological fish response to each challenge condition.

Total Dissolved Gas Field Measurements

Total dissolved gas and the distribution of water temperature near the confluence of the Clearwater and Snake Rivers was monitored from July through September 2011. The purpose was to determine if sharp increases in water temperature cause TDG to increase above levels that could harm juvenile Chinook salmon as they travel from the lower Clearwater River into the Snake River. TDG was monitored at two fixed sites on the Snake River. One station was downstream of the Snake River–Clearwater River confluence at the Red Wolf Bridge to monitor "mixed" conditions (Figure 6). The second station was near the town of Asotin (Figure 6) to record Snake River TDG upstream of the confluence with the Clearwater River. Additional

Table 4. Mean temperature and total dissolved gas during challenge trials for each replication. Error values are one standard deviation (SD) of the mean. Asterisks denote significant differences in mean temperature or mean TDG between replications for each challenge condition.

Challenge Trial (°C/TDG %)	Replication	Mean Temperature (°C ± SD)	Mean TGD (% ± SD)
10/100	1	10.23±0.21	100.4±0.9
	2	10.21±0.23	100.4±0.9
	3	10.19±0.20	100.3±0.9
15/100	1	15.06±0.19	100.4±1.0*
	2	15.10±0.31	99.7±0.7*
	3	15.10±0.39	99.8±0.6*
20/100	1	19.89±0.21	101.1±0.8*
	2	19.89±0.31	100.8±0.6*
	3	19.81±0.39	100.7±0.6*
25/100	1	24.91±0.29*	99.6±0.1*
	2	24.88±0.29*	100.3±0.7*
	3	24.80±0.39*	101.0±1.3*
10/110	1	10.18±0.15	109.4±0.7*
	2	10.13±0.12	109.3±0.6*
	3	10.17±0.21	109.6±0.7*

Figure 6. Locations of total dissolved gas and temperature monitoring sites near the Snake River–Clearwater River confluence. Locations of long-term TDG monitoring stations are signified by red dots, temperature monitoring locations are shown by small black dots, and vertical temperature and TDG profile locations are labeled (T10, T11, and T3.5) and shown by black triangles.

U.S. Army Corps of Engineers (USACE) TDG monitoring stations were used. One USACE station was on the Clearwater River about 2 mi upstream of the confluence, near the east end of Lewiston, Idaho (LEWI), and the second was farther upstream near Peck, Idaho (PEKI). Mobile sensors were deployed in efforts to determine the location of the thermocline at the confluence of the Clearwater and Snake Rivers and vertical TDG profiles in this location were also taken (Figure 6).

Fixed site measurements.—Hydrolab Model 5 MiniSonde sensors (MS5; Hach Environmental, Loveland, Colorado) were used to monitor water quality. Each MS5 sensor had five individual sensors that monitored dissolved oxygen, specific conductance, depth, TDG, and temperature. At each long-term monitoring location, one or two MS5 sensors were programmed to record data using a 1-h interval from July through September 2011. Sensors were attached to a 0.5-in.-diameter wire that connected a buoy to a 70-lb anchor. One sensor was deployed at the

Asotin site in July and was placed in approximately 2 to 3 m of water until its removal in September. One sensor was deployed in July at the Red Wolf Bridge at a 10- to 13-m depth. An additional sensor was added at the Red Wolf Bridge in August at a depth of 7 m. In September, one sensor at the Red Wolf Bridge was removed, leaving one sensor at a depth of 7 m. Detailed specifications, including the accuracy and resolution of each sensor, are listed in Table 5.

Table 5.—MiniSonde 5 water quality sensor specifications (Hach Environmental 2006).

Sensor	Range	Accuracy	Resolution
Total dissolved gas	400 to 1400 mmHg	±1.5 mmHg	1.0 mmHg
Specific conductance	0 to 100 mS/cm	±2 µS/cm	0.001 µS/cm
Depth	0 to 25 m	±0.05 m	0.01 m
Temperature	−5 to 50ºC	±0.10ºC	0.01ºC
Luminescent dissolved oxygen	0 to 60 mg/L	0.1 mg/L at < 8 mg/L; ±0.2 mg/L at > 8 mg/L	0.01 mg/L

Prior to each deployment, all water quality sensors on each MS5 sensor were calibrated and new batteries were installed. The accuracy of TDG readings was ensured by collecting post-deployment data adjacent to a freshly calibrated unit (hereafter termed a side-by-side test). To collect the side-by-side test data, the laboratory-calibrated MiniSonde and the recovered MiniSondes were placed in the river at an approximate depth of 2 m immediately following each deployment. For side-by-side deployments, we logged depth, temperature, TDG, dissolved oxygen, and specific conductance every 3 min for 60 min. Following side-by-side tests, each TDG membrane was removed and later transported back to PNNL facilities in Richland, Washington, for quality assurance checks. Following membrane removal, the TDG pressure sensor on the Minisonde was attached to a Druck pressure calibrator (Druck Inc., Houston, Texas) that was certified to 0.0015-psi accuracy. The TDG sensor was checked for accuracy at 0, 100, 200, and 300 mmHg. If the pressure reading was off by more than 1 mmHg, the unit was recalibrated. A barometric pressure reading was obtained from a Brutnon ADC Pro (Brunton Outdoor Group, Riverton, Wyoming) and used to reset the pressure sensor, if needed. After recalibration, the sensor was checked once more for accuracy at 100, 200, and 300 mmHg. The dissolved oxygen sensor was calibrated by attaching a calibration cap filled with air-saturated water to the MiniSonde. The MiniSonde was held upright for the duration of the calibration by a clamping laboratory stand. The specific conductance sensor was calibrated using 100-µS/cm NaCl standard. The depth sensor was calibrated by holding the sensor out of the water, pointing down, and setting the depth value to zero. After all calibrations were completed, a laboratory-

tested TDG membrane was attached to the pressure sensor. Following deployments, the membranes were brought back to Richland and were tested for functionality before they were reused in the field. These methods are outlined in Tanner and Johnston (2001) as well as by Arntzen et al. (2008). For quality assurance, we looked at both the membrane test results and the side-by-side data. No membranes failed during our study.

Mobile measurements.—Several vertical temperature profiles were recorded between the mouth of the Clearwater and Clarkston on the Snake River in order to locate areas where temperature stratification was maximized and vertical TDG profiles should be conducted. The location of each profile site was recorded by a Global Positioning System (GPS; Figure 6). MS5 sensors were attached to a 0.25-in. poly rope with a 10-lb anchor attached. Sensors were quickly lowered to the river bottom, then programmed to log data every 1 s while slowly being raised to the surface. Temperature data were collected this way from several different positions each month.

Vertical TDG profiles were collected at one location each trip where the largest temperature differences were found via vertical temperature profiles near the confluence of the Snake and Clearwater Rivers (Figure 6); locations were recorded by GPS. At locations where vertical TDG profiles were recorded, the boat was anchored and a downrigger was used (Figure 7). The TDG sensor was allowed to equilibrate at the bottom and then raised incrementally and allowed to equilibrate at each interval (approximately every 1 m). TDG readings were recorded every 30 s during vertical profiling. Data were viewed in real time, and files were saved electronically.

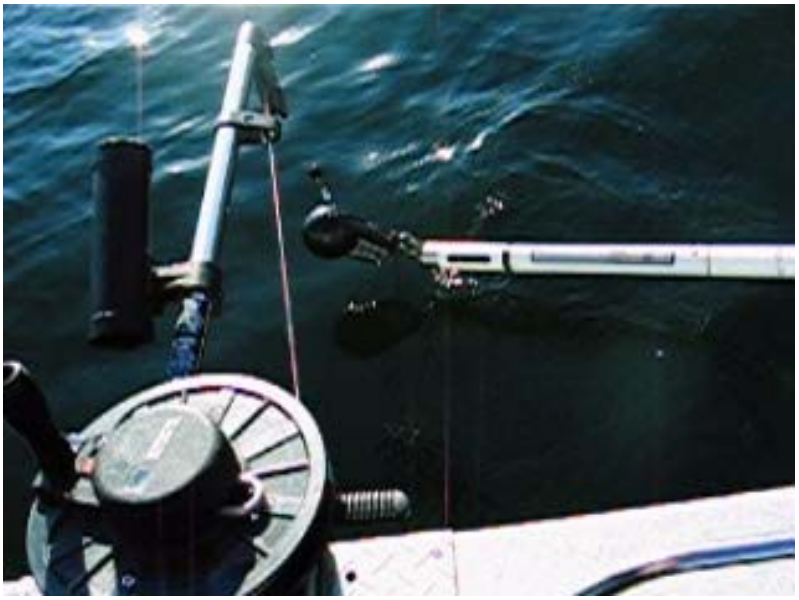

Figure 7. MiniSonde 5 attached to 10-lb weight and downrigger for vertical TDG profiling.

Analysis of Laboratory Results

We combined results from related anatomical regions within each fish for seven distinct groups for analysis—lateral line, unpaired fins, paired fins, gills, eyes, body, and internal areas. For the lateral line, bubbles were noted as either present (value = 1) or absent (value = 0), and the proportion occluded was calculated. For unpaired and paired fins, the presence or absence of bubbles was noted, and the proportion of fin area affected by bubbles was summed across all four fins in each group. Thus, the bubble-affected area of unpaired or paired fins could vary from 0% to 400%. For gills, bubbles were noted as either present or absent in addition to the proportion of the first gill arch occluded. For eyes, body, and internal organs, the presence or absence was summed across eyes, all five body areas, and all six internal areas, respectively.

Analyses of GBD found in the seven anatomical groups in relation to acclimation, challenge, and time exposure were performed using experimental units, which consisted of six fish exposed to the same acclimation and challenge conditions per period of time in each replication. The mean proportion of GBD incidence per anatomical group was calculated for the experimental unit for each treatment replicate. The mean proportion of GBD severity was also calculated by experimental unit for lateral line occlusion and unpaired and paired fin bubbles. Because these percentages had a high degree of variation, severity values were standardized by rank by graphing data and examining percentiles. For lateral line severity, percentages of 0 to 13 were given a rank of 0; and percentages higher than 13 were given a rank of 1. For unpaired fins, fish with 0% to 20% of the fin area affected by bubbles were given a rank of 0; fish with more than 20% of the summed fin area affected by bubbles were given a rank of 1. For paired fins, fish with 0% to 10% of the summed fin area affected by bubbles were given a rank of 0, and fish with more than10% were given a rank of 1.

To elucidate any potential relationships between conditions and exposure time with GBD, a general linear model was run with acclimation condition, test condition nested within acclimation condition, and exposure time as a covariate, with a Tukey HSD post-hoc test. If residuals did not meet assumptions, all mean responses of the experimental unit were $\log_{10}$transformed. If residuals still did not meet assumptions, data were divided and run by acclimation condition. In some cases of non-normal data, nonparametric Kruskal–Wallis tests were used. All tests were significant at $p < 0.05$ and statistics were performed in Minitab Version 16 (Minitab, Inc., State College, Pennsylvania) or SigmaPlot 12.0 (Systat Software Inc., Chicago, Illinois).

RESULTS

Dissolved Gas and Temperature Effects on Gas Bubble Disease

Mortality.—Mortality by challenge condition differed significantly for the 100% TDG acclimation group (H = 58.8806, df = 1, p < 0.001) and also for the 110% TDG acclimation group (H = 74.429, df = 1, p < 0.001); 27 fish died during the experiment and all after exposure to 25°C (Figure 8). In addition, the time spent in the 25°C water was correlated with mortality (H = 134.976, df = 1, p < 0.001), as fish died only when exposed for 45 or 75 min. Of the 27 mortalities, 41% had lateral line bubbles, 30% had fin bubbles, 11% had body bubbles, and 7% had bubbles in gills and internal organs. There was no significant difference in mortality between fish acclimated at 100% TDG versus 110% TDG (H = 0.122, df = 1, *P* = 0.727).

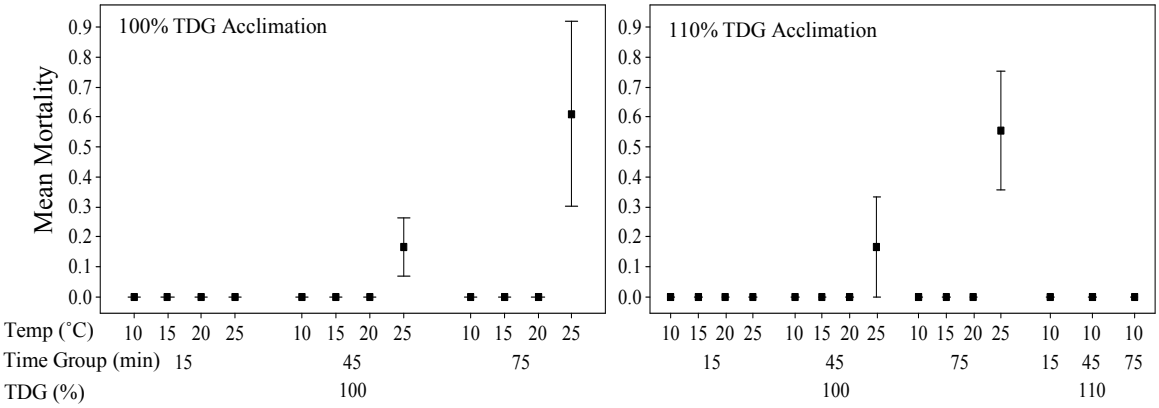

Figure 8. Mean (±SE) proportion of mortalities within each time group for fish acclimated at either 100% or 110% total dissolved gas (TDG) and exposed to challenge conditions.

Lateral line occlusion incidence and severity.—The proportion of fish with incidence of bubbles in the lateral line did not differ significantly between acclimation conditions (F = 3.28, df =1, *P* = 0.08); however, there were differences in the proportion of fish with lateral line bubbles within the 100% TDG acclimation group by challenge temperature (F = 3.61, df = 3, *P* = 0.02). Pairwise comparisons (Tukey's HSD) within the 100% TDG acclimation group (observation periods grouped) revealed a significantly greater mean proportion of fish with lateral line occlusion in the 10/100 challenge control group (mean = 0.6) than in the 25/100 challenge (mean = 0.3, Figure 9). There was no difference in the mean proportion of fish with lateral line occlusion between challenges within the 110% TDG acclimation group (F = 1.09, df = 4, *P* = 0.38). Further, the mean proportion of fish with lateral line occlusion did not change (increase or decrease) with challenge exposure time (F = 0.83, df = 1, *P* = 0.37).

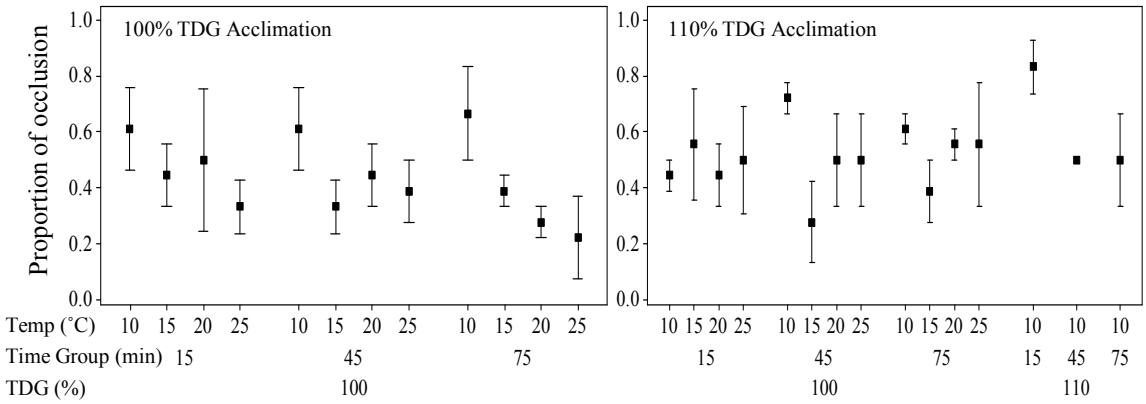

Figure 9. Mean (±SE) proportion of fish with lateral line occlusion observed after challenge exposure for fish acclimated at either 100% or 110% total dissolved gas (TDG).

The lateral line occlusion severity rank was significantly higher for fish acclimated in 110% TDG (mean = 0.28) than in 100% TDG (mean = 0.1; F = 24.03, df = 1, p < 0.01; Figure 10). There were no significant differences between challenge conditions for fish acclimated at 100% TDG (F = 1.02, df = 3, *P* = 0.40), nor for fish acclimated at 110% TDG (F = 0.76, df = 4, *P* = 0.56). Also, there was no significant effect of exposure time to challenge conditions on the severity of lateral line occlusion (F = 0.79, df = 1, *P* = 0.38).

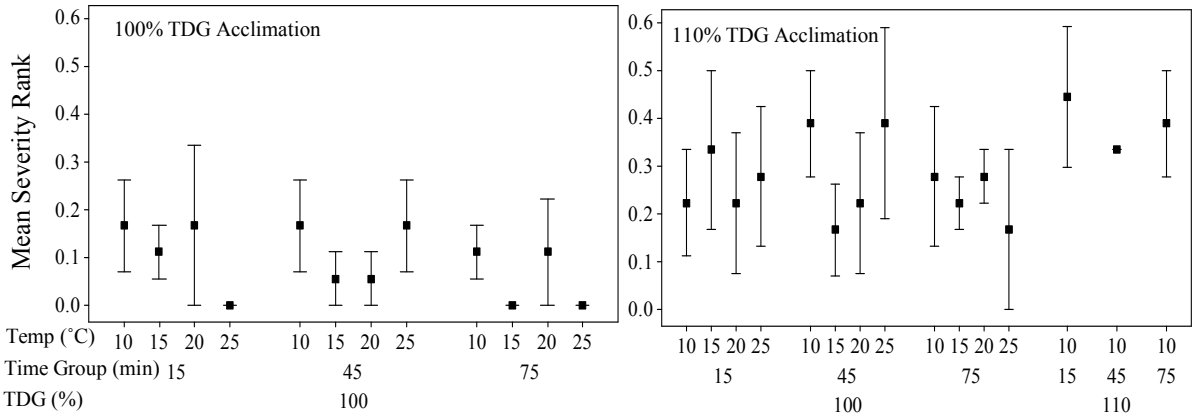

Figure 10. Mean (±SE) rank of lateral line bubble severity observed after challenge exposure for fish acclimated at either 100% or 110% total dissolved gas (TDG).

Unpaired fins bubble incidence and severity.—A greater proportion of unpaired fins with gas bubble incidence was observed on fish acclimated at 110% TDG than on those acclimated at 100% TDG (F = 23.73, df = 1, p < 0.01; Figure 11). The mean number of unpaired fins with bubbles did not differ between challenge groups for fish acclimated at 100% TDG (H = 3.90, df = 11, *P* = 0.97), or for fish acclimated at 110% TDG (F = 1.26, df = 4, *P* = 0.30). The time spent in the challenge conditions was not significantly related to the number of unpaired fins with bubbles (F = 0.20, df = 1, *P* = 0.66).

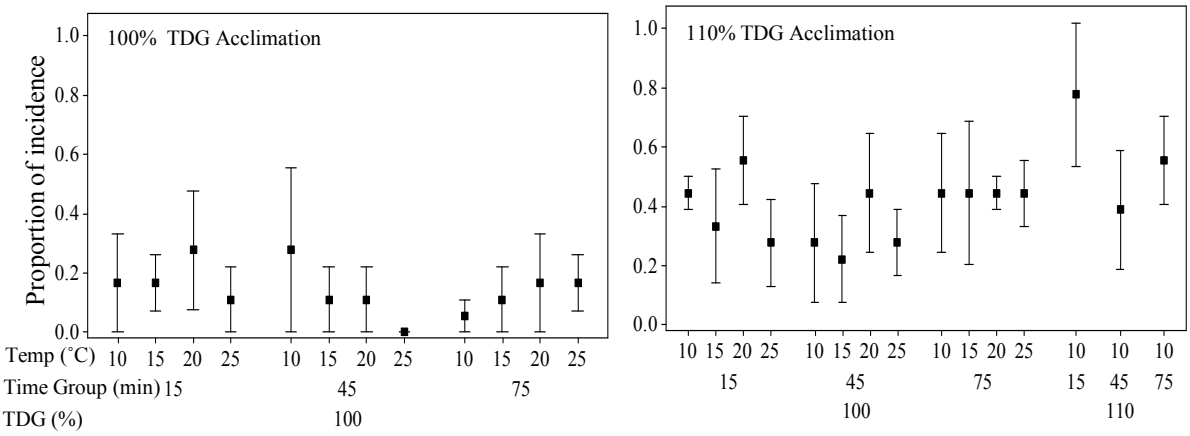

Figure11. Mean (±SE) proportion of unpaired fins with bubbles as observed after challenge exposure for fish acclimated at either 100% or 110% total dissolved gas (TDG).

Similarly, the severity of bubbles in unpaired fins was greater in fish acclimated at 110% TDG than fish acclimated at 100% TDG (F = 12.05, df = 1, *P* = 0.001; Figure 12). There were no differences between challenge conditions for fish acclimated at 100% TDG (H = 8.75, df = 11, *P* = 0.65) or 110% TDG (H = 6.41, df = 14, *P* = 0.96). Exposure time to the challenge conditions did not affect bubble severity in the unpaired fins (F = 0.00, df = 1, *P* = 0.97).

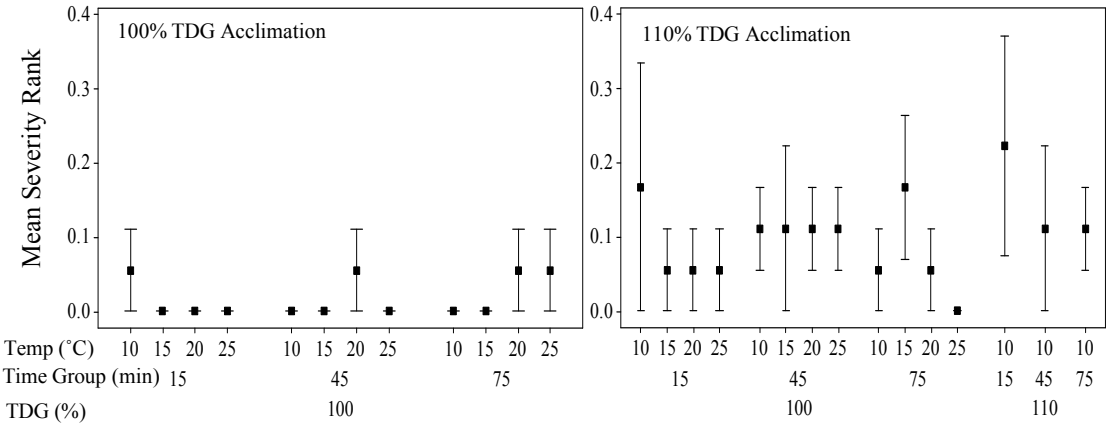

Figure 12. Mean (±SE) severity rank of unpaired fin gas bubble disease observed after challenge exposure for fish acclimated at either 100% or 110% total dissolved gas (TDG).

Paired fins bubble incidence and severity.—Bubbles in paired fins occurred five times less frequently than bubbles in unpaired fins (4.7 and 24.2 experimental units with bubbles, respectively) and were present approximately equally on pectoral and pelvic fins. Similar to unpaired fins, more bubbles were observed in paired fins on fish acclimated at 110% TDG than on those acclimated at 100% TDG (F = 6.07, df = 1, P = 0.016; Tables 5 and 6). The incidence of bubbles on paired fins did not vary between challenge groups for fish acclimated at 100% TDG (H = 12.16, df = 11, P = 0.351) or 110% TDG (H = 10.56, df = 14, P = 0.720). Time spent in challenge conditions also did not affect the observed number of bubbles in paired fins (F = 0.57, df = 1, P = 0.45). There was no significant difference in the severity of bubbles between acclimation conditions (H = 2.79, df = 1, P = 0.095).

Table 5. Mean proportion (±SE) of fish from the 100% total dissolved gas (TDG) acclimation group showing gas bubble disease (GBD) symptoms by anatomical region.

Challenge	Paired Fin Bubbles	Eye Bubbles	Body Bubbles	Gill Bubbles	Internal Bubbles
10°C, 100%	0.0±0.1	0.0±0.1	0.0±0.1	0.0±0.0	0.0±0.0
15°C, 100%	0.0±0.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
20°C, 100%	0.1±0.1	0.0±0.0	0.0±0.0	0.0±0.1	0.0±0.0
25°C, 100%	0.0±0.1	0.0±0.1	0.0±0.0	0.0±0.1	0.0±0.1

Table 6. Mean proportion (±SE) of fish from the 110% total dissolved gas (TDG) acclimation group showing gas bubble disease (GBD) symptoms by anatomical region.

Challenge	Paired Fin Bubbles	Eye Bubbles	Body Bubbles	Gill Bubbles	Internal Bubbles
10°C, 100%	0.0±0.0	0.2±0.3	0.1±0.1	0.0±0.1	0.0±0.1
10°C, 110%	0.1±0.1	0.1±0.1	0.1±0.1	0.0±0.1	0.0±0.0
15°C, 100%	0.1±0.1	0.3±0.4	0.1±0.1	0.0±0.1	0.0±0.1
20°C, 100%	0.1±0.2	0.2±0.3	0.1±0.1	0.0±0.0	0.0±0.0
25°C, 100%	0.1±0.1	0.1±0.2	0.1±0.1	0.0±0.1	0.0±0.1

Eye bubbles.—Fish acclimated in 110% TDG had significantly more bubbles in eyes than fish acclimated at 100% TDG (H = 13.95, df = 1, $p < 0.01$). Only three fish had eye bubbles in the 100% TDG acclimation, and the low frequency precluded any formal statistical analysis on the effect of challenge condition or exposure time for that group. Within fish acclimated at 110% TDG, there was no significant difference in the number of fish with bubbles in the eyes between any challenge condition (H = 5.54, df = 14, $P = 0.977$). Challenge exposure time did not affect the number of fish with bubbles in the eye for fish acclimated at 110% TDG (F = 0.84, df = 1, $P = 0.36$).

Body, gill, and internal bubbles.—Observations of GBD on the body surface, gills, or internal organs were low and precluded any formal analysis. Bubbles were found on all body parts to varying degrees. Two percent of fish had bubbles present on their mouth/jaw or head. Two percent of fish also had bubbles on either flanks or caudal peduncle. Of the fish exhibiting one or more body bubbles, 92% came from the 110% TDG acclimation group. Only eight fish (2%) had bubbles in their gills, and six of those fish were from the 110% TDG acclimation group. Four fish (1%) had bubbles in internal organs and three of these fish were from the 110% TDG acclimation group. Two fish (0.4%) had bubbles in their hearts, one in the caudal vein, and one in the swim bladder.

Field Measurements of Total Dissolved Gas

Fixed site measurements.—The maximum hourly TDG at a fixed site during this study was 109.7% on the Clearwater River (at the LEWI gauge), 107.9% on the Snake River upstream of the confluence (at the Asotin gauge), and 109% in Clarkston downstream of the confluence of the Clearwater and Snake Rivers (at the Red Wolf Bridge gauge). TDG values were averaged between the two sensors at the Red Wolf monitoring station because results were very similar. TDG at the Red Wolf monitoring location was similar to that found at the LEWI gauge (Figure 13), while TDG at the Asotin monitoring location was typically lower than that at the LEWI gauge and Red Wolf site (Figure 14). TDG fluctuated daily, up to 8% during August and early September, and was highest late in the afternoon and lowest in the early morning.

Mobile measurements.—Temperature stratification caused by layering of colder Clearwater River water with warmer Snake River water was observed on all trips; larger temperature differences occurred later in the summer. On July 5, there was a maximum temperature difference of 4°C between the bottom and the surface at site T10. At site T11, the largest temperature differences were 7°C on August 2 and 10°C on August 31. On September 29, the largest temperature difference found at T3.5 was 6°C.

Vertical TDG profiling was conducted at sites where the maximum temperature differences were observed (T10 on July 5, T11 on August 2 and 31, and T3.5 on September 29). On July 5, TDG increased slightly as the sensor traveled from the bottom of the river to the

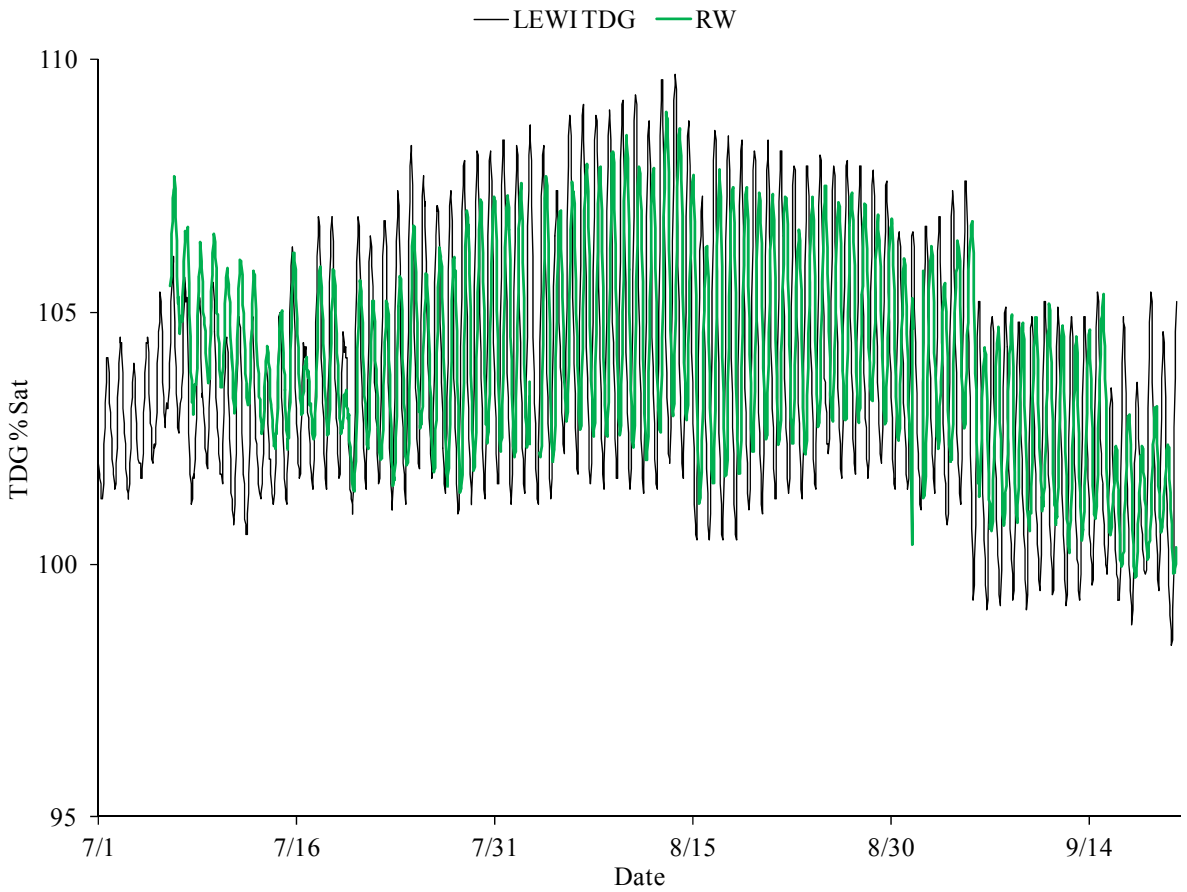

Figure 13. Hourly total dissolved gas (TDG) recorded at the USACE monitoring station (LEWI; black line) on the Clearwater River in Lewiston (2 mi upstream of the confluence) and at the Red Wolf Monitoring site (RW; green line; downstream of the confluence) from July through September 2011. The Red Wolf value is an average from the Red Wolf 1 and the Red Wolf 2 sensors, which were deployed at depths of 10 to 13 m and 7 m, respectively.

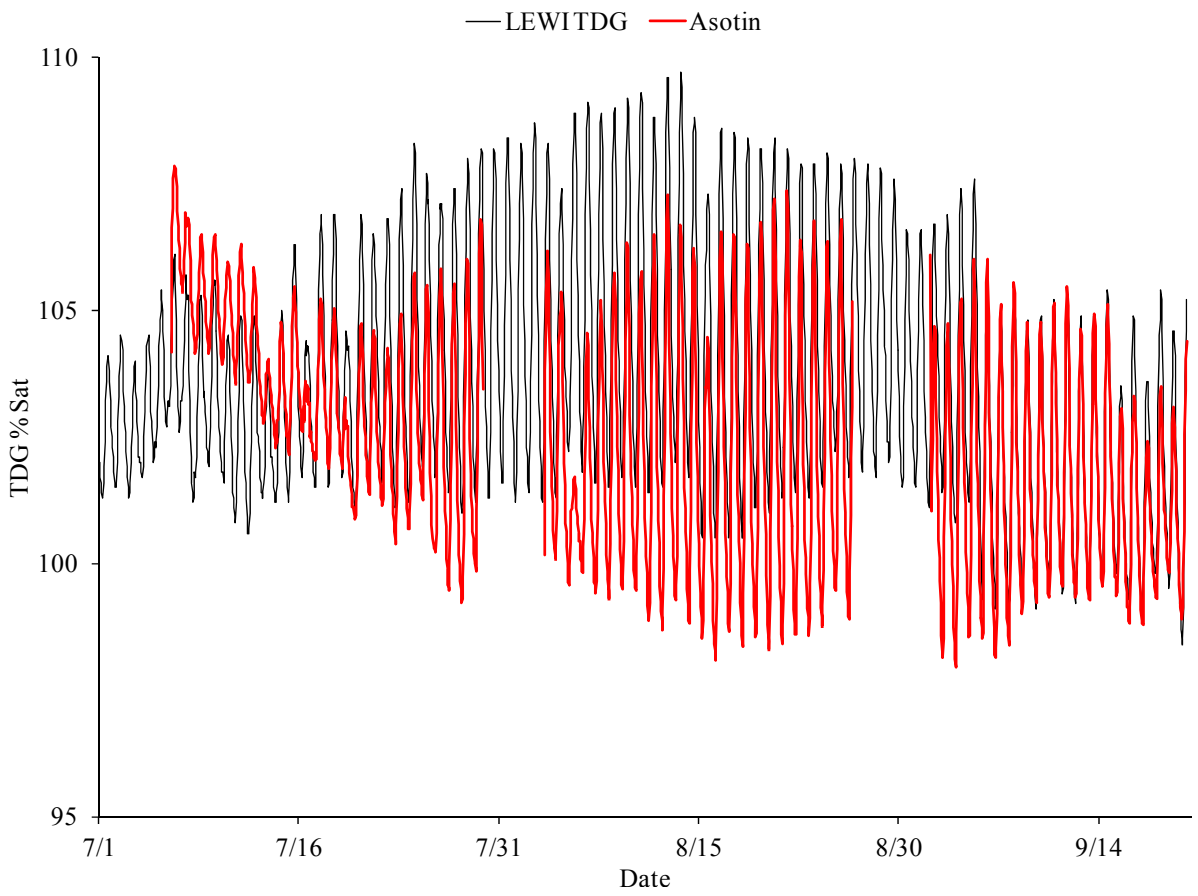

Figure 14. Hourly total dissolved gas (TDG) recorded at the USACE monitoring station (LEWI; black line) on the Clearwater River in Lewiston (2 mi upstream of the confluence) and on the Snake River near Asotin (red line) from July 2011 through September 2011. The Asotin sensor was approximately 2 to 3 m below the water surface.

surface. Temperatures near the bottom were indicative of the Clearwater River while temperatures near the surface were similar to those of the Snake River. The difference in water temperature between river bottom and river surface was approximately 4°C during this profile, while TDG increased approximately 2% (Figure 15). On August 2, we saw the largest change in TDG at a confluence vertical profile. At the river bottom, TDG was approximately 110% and exceeded 122.5% at 5-6 m depth; temperature varied by about 8°C (Figure 16). In comparison, the fixed LEWI station (upstream in the Clearwater River) recorded an average TDG of 107.8%. In addition, the Snake River monitoring station at Anatone (about 40 km upstream of the confluence) recorded an average of 103.4% during our vertical profile.

On August 31, there was a temperature difference of 10°C between the river bottom and the surface of the water, but there was less than 5% change in TDG. TDG increased slightly as the sensor traveled from the bottom of the river through the warmer water of the Snake River, then decreased as the sensor approached the surface (Figure 17). On September 29, barometric and TDG data were not available from the LEWI station for this period, so TDG was computed using barometric data from the PEKI gauge near Peck, Idaho, and TDG values from the PEKI station are shown for reference. TDG near the bottom was similar to data at the PEKI gauge and then increased slightly (up to 3%) as the sensor traveled through warmer water at a depth of approximately 3 to 4 m (Figure 18). The difference in water temperature between river bottom and surface approached 6°C.

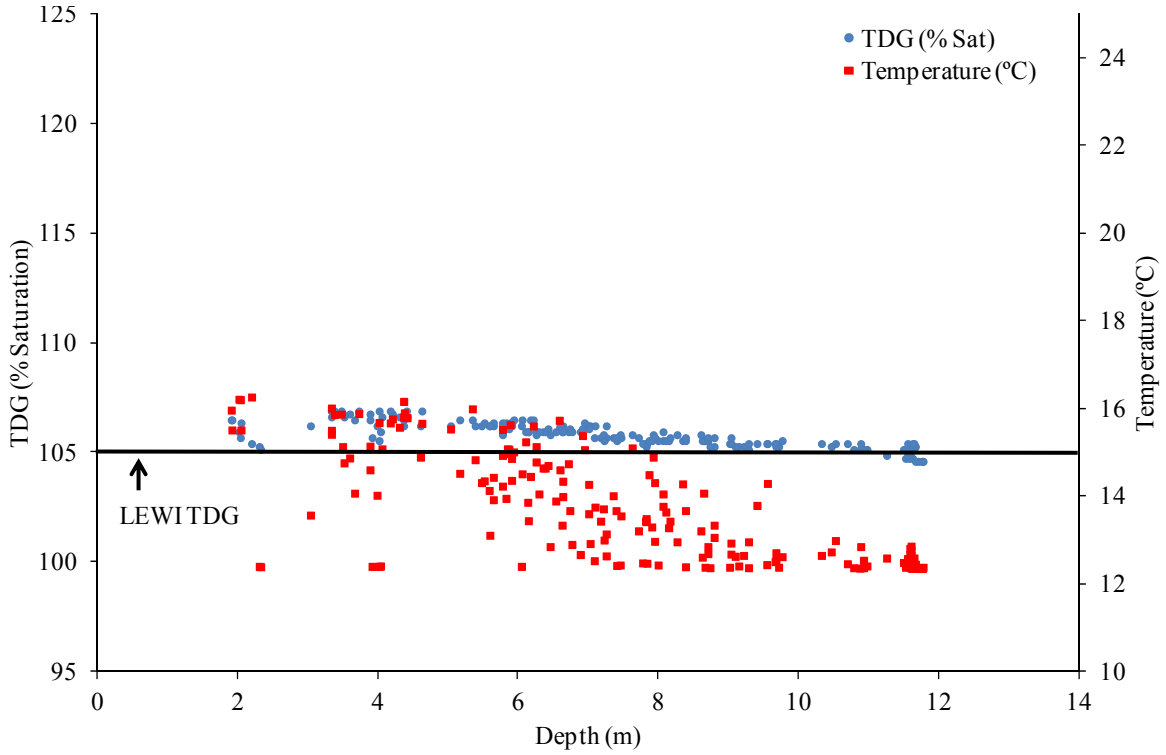

Figure 15. Vertical total dissolved gas (TDG; % saturation) and temperature profiles collected near the mouth of the Clearwater River on July 5, 2011, at site T10. The black horizontal line indicates the total dissolved gas concentration recorded at the USACE-owned sensor (LEWI) in the Clearwater River near Lewiston, Idaho.

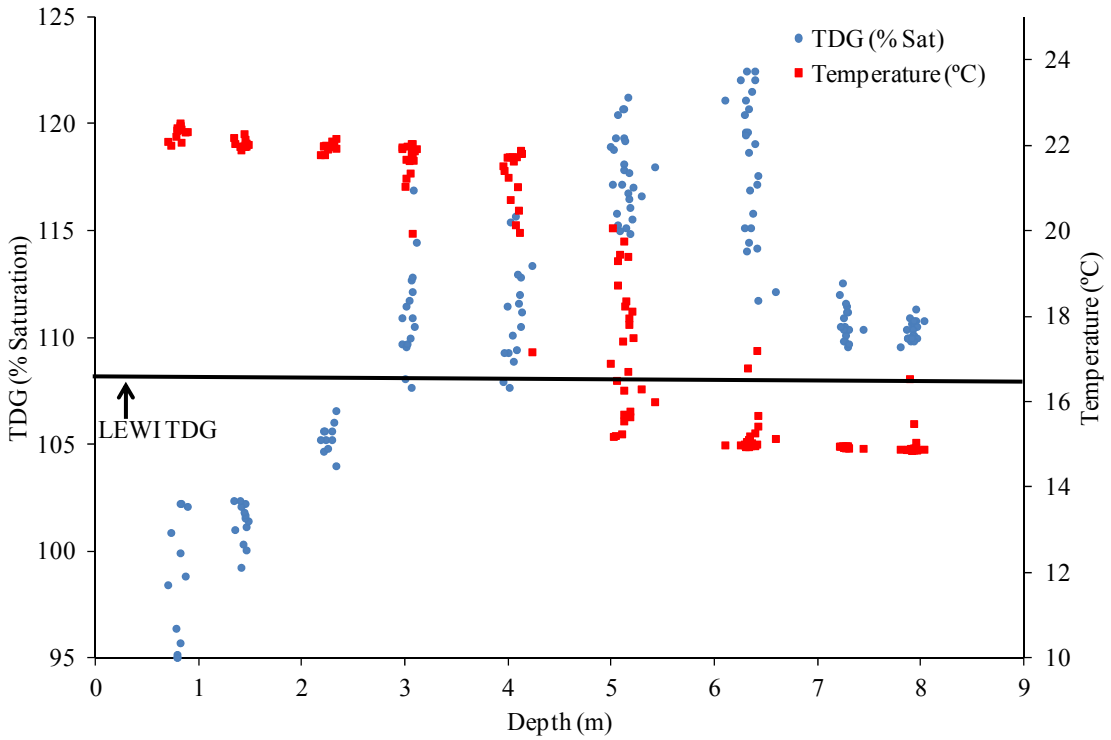

Figure 16. Vertical total dissolved gas (TDG; % saturation) and temperature profiles collected near the mouth of the Clearwater River on August 2, 2011, at site T11. The black horizontal line indicates the total dissolved gas concentration recorded at the USACE-owned sensor (LEWI) in the Clearwater River near Lewiston, Idaho.

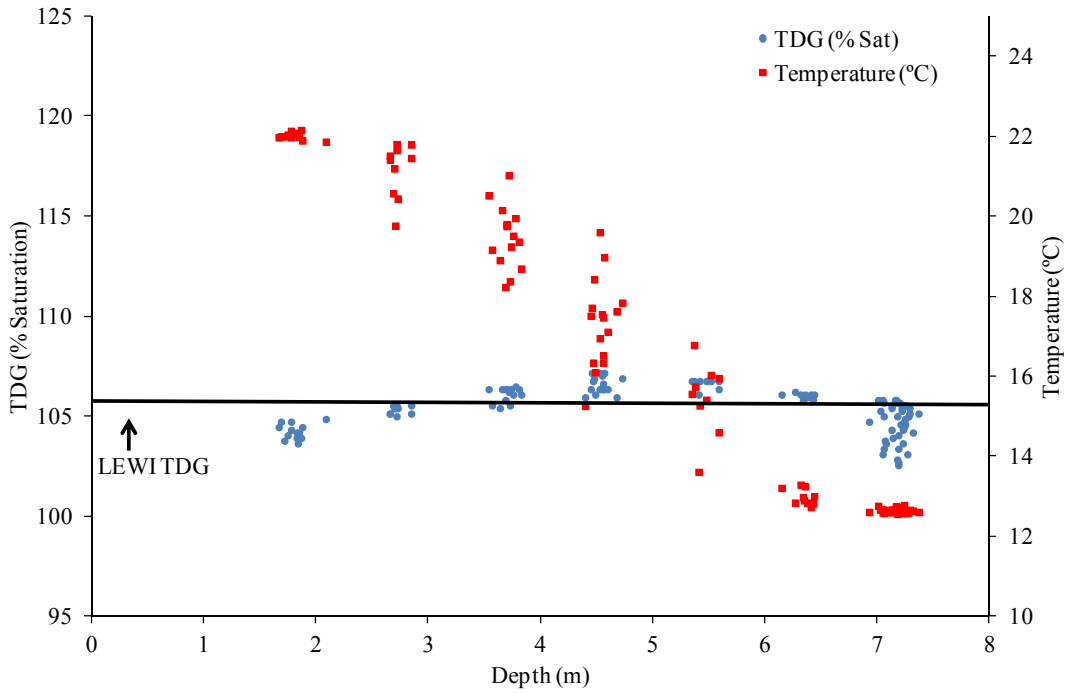

Figure 17. Vertical total dissolved gas (TDG; % saturation) and temperature profiles collected near the mouth of the Clearwater River on August 31, 2011, at site T11. The black horizontal line indicates the total dissolved gas concentration recorded at the USACE-owned sensor (LEWI) in the Clearwater River near Lewiston, Idaho.

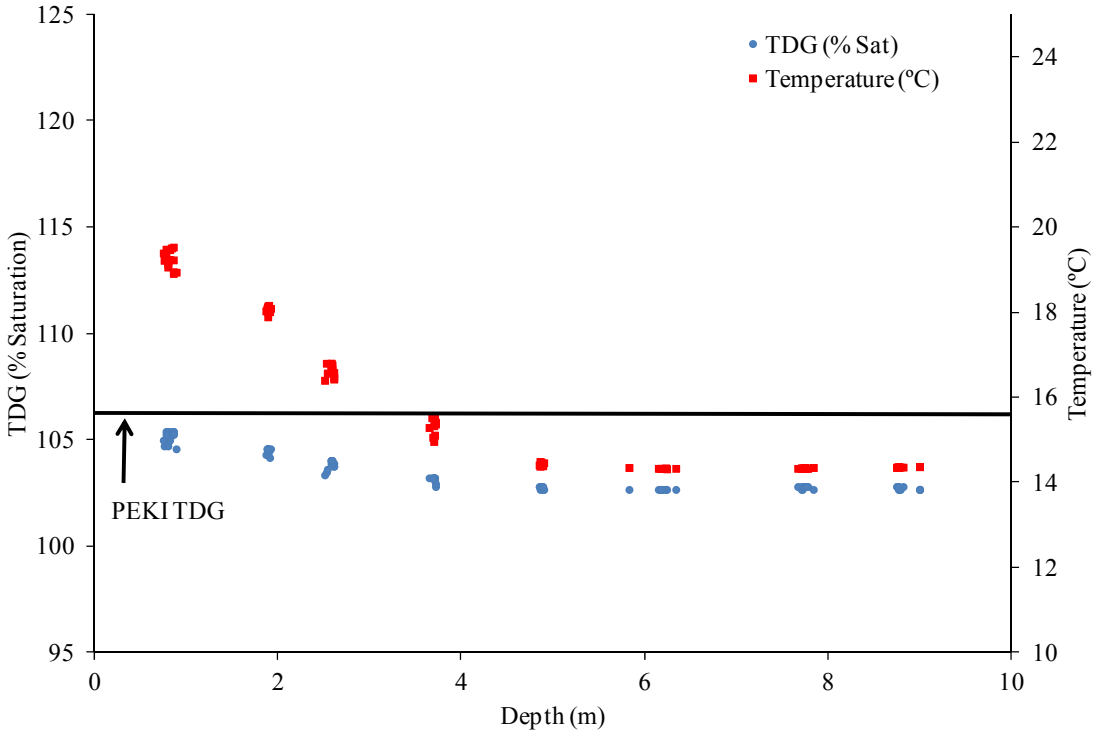

Figure 18. Vertical total dissolved gas (TDG; % saturation) and temperature profiles collected near the mouth of the Clearwater River on September 29, 2011, at site T3.5. The black horizontal line indicates the total dissolved gas concentration recorded at the USACE-owned sensor (PEKI) in the Clearwater River near Peck, Idaho.

DISCUSSION

Mortality occurred only in fish exposed to the 25°C challenge; however, relatively mild GBD symptoms of these fish suggest that the temperature difference may have been the mortality mechanism rather than GBD. Of the 27 mortalities (25% of those exposed to 25°C), only 7% ($N = 2$) had bubbles in the gills or internal organs. Significantly fewer fish had lateral line occlusion at 25°C than the control group, and no statistical differences in occlusion severity were found between the four challenge-temperature groups. Further, we found no significant differences in incidence or severity of GBD in unpaired fins between challenge-temperature groups. In a previous study, juvenile Hanford Reach fall Chinook salmon from the Columbia River acclimated to 12°C and heated at a rate of 4.7°C/h to 26°C experienced 25% mortality within 2-2.5 h (Mesa et al. 2002). Similarly, groups of juvenile Snake River fall Chinook salmon acclimated to 10°C and heated at a rate of 1.5°C/h to mean temperatures of 26.9°C and 27.5°C began dying and experienced 50% mortality, respectively (Geist et al. 2010). Relatively similar mortality-at-temperature rates between juvenile Hanford Reach and Snake River fall Chinook salmon in the current and previous studies suggest that these stocks may be appropriate surrogates, despite the differences in experimental warming rate (i.e., sudden up to 4.7°C/h).

Bubbles in the lateral line were the most commonly observed symptom of GBD, although the mortality risk due to lateral line occlusion is likely minimal. Fish acclimated at 110% had significantly greater severity of lateral line occlusion than fish acclimated at 100% TDG. Similar severity of GBD symptoms have been noted with chronic exposure to slightly supersaturated TDG concentrations of 110% to 115% (Dawley and Ebel 1975; Weitkamp and Katz 1980; Mesa et al. 2000). These results are not surprising, as Mesa and Warren (1997) and Mesa et al. (2000) also noted bubbles in the lateral lines of yearling spring Chinook salmon acclimated at 110% to 112% TDG. However, it was somewhat unexpected that control fish in 100% TDG and 10°C water had bubbles in their lateral lines; other studies both confirm or refute this finding. Dawley and Ebel (1975) detected external signs of GBD in steelhead that had been in saturated (100% TDG) water for 3 d, whereas Mesa and Warren (1997) did not see any symptoms of GBD in fish exposed to gas-saturated (100% TDG) water. However, the Mesa and Warren study used larger fish than used in our study (104-106 mm). It is possible that smaller fish (~81-85 mm) with smaller scales (and smaller lateral line pore size) may allow bubbles to be trapped. Beeman et al. (2003) and Morris et al. (2003) showed an inverse relationship between lateral line occlusion and lateral line pore size. As in the Beeman and Morris papers, it is possible that the relatively small pore size in juvenile Chinook salmon in our study allowed gas to be trapped easily and resulted in an increased rate of lateral line occlusion. Other authors have cautioned against relating presence of external bubbles to severity of GBD due to unpredictable gas-bubble formation (Hans et al. 1999). Although our control fish did exhibit bubbles in the lateral line, the severity of this symptom was low, and their presence alone does not indicate GBD.

The observation that control fish exposed to 10°C had greater lateral line occlusion than fish exposed to 25°C after acclimation at 100% TDG is likely due to the properties of gas during warming. It is plausible that warmer temperatures cause the lateral line bubbles, which were present from acclimation, to expand and diffuse out of the lateral line, whereas they remain at the lateral line in control fish exposed to 10°C. Charles's Law states that the volume of a gas under constant pressure will increase linearly as temperature increases (Laugier 2007). This theory could explain why we observed a general reduction in lateral line bubbles with increasing temperature.

We hypothesized that GBD symptoms might be most prevalent at the first 15-minute observation due to expansion of any acclimation-incurred bubbles caused by the temperature increase and that bubbles would be less prevalent at the 45- and 75-min observations due to gas equilibration at 100% TDG; however, this trend was not observed. Similarly, other studies have found that gas bubbles formed by TDG supersaturation disappear relatively quickly upon transfer to water with little or no supersaturation. Weitkamp (2008) stated that gill bubbles can disappear within minutes, lateral line bubbles in about half an hour, but that the rate of disappearance from gills is on a scale from hours to days. Hans et al. (1999) showed that GBD symptoms in the gills, lateral line, and external surfaces that developed after being held in high (120-130%) TDG return to normal after 2, 5, and 48 h, respectively, after returning to "normal" (104%) conditions. Elston et al. (1997) showed that bubbles in the gills of young Chinook dissipated in less than 10 min, bubbles in the lateral line were nearly absent at 30 min, and fins were free of bubbles after 120 min.

Field monitoring documented supersaturated TDG concentrations at all sampling locations in summer and fall 2011, which likely impact emigrating juvenile salmon albeit to an unknown extent. Slightly supersaturated concentrations (110-110%) occurred in the Clearwater and Snake rivers upstream of the confluence and peaked each day during late afternoon when photosynthetic activity was highest. However, the highest TDG concentrations were found at the confluence (up to 122.5% in August), which exceeded the "safe" threshold of 120% suggested by Backman et al. (2002). Saturation at the Clearwater River monitoring location remained below 110%, which was similar to levels observed in the lower Clearwater River in summer 2008 by Tiffan et al. (2009a). Dissolved gas concentrations recorded at the confluence, however, are the highest known recorded levels of TDG at this location and have the potential to impact migrating juvenile salmon located shallower than the compensation depth (~1-3 m, depending on TDG and atmospheric pressure). Although juvenile Chinook salmon typically migrate at depths sufficient to provide hydrostatic compensation for short-term exposure to TDG levels from 115% to 120% (Beeman et al. 1999; Beeman and Maule 2006), it is possible that short forays to the water surface would expose juveniles to highly supersaturated conditions and potentially impact fitness.

As predicted, a relatively high TDG concentration of 122.5% was measured at the river confluence when the 15°C Clearwater River mixed with the 22°C Snake River on 2 August. This increase in TDG agrees with the general rule that a 1°C temperature increase results in a 2.0-2.5% TDG increase (Nebeker et al. 1978; Schneider et al. 2003; Arntzen et al. 2009); however, we did not see this magnitude of TDG increase with temperature at the confluence on most sampling trips. On July 5, August 31, and September 29, there were water-column temperature differences at the confluence of 4°C, 10°C, and 6°C, respectively, and TDG levels varied less than 5%. It is unclear why the same increase of 2-2.5% TDG per 1°C was not observed during these sampling events; however, the variability may be due to the amount of mixing or water-temperature stratification that occurs at the confluence.

Temperature and TDG levels used for acclimation periods and challenge trials in the laboratory study were chosen based on known conditions of the Clearwater and Snake rivers. We used 100% TDG for challenge conditions, but field measurements show that baseline TDG in the Snake River is very similar to that of the Clearwater River and fluctuates daily between 100 and 110%. Thus, we may have underestimated the effect of TDG on fish migrating through the Snake–Clearwater confluence as simulated by the laboratory experiment. If gas bubbles are formed in juvenile salmon emigrating from the Clearwater River, they may not dissipate upon entrance to the Snake River based on environmental TDG alone, whereas the 110% TDG acclimation to 100% TDG challenge in our experiment likely caused gas equilibration through time. Potentially higher levels of TDG at the confluence, which were documented in 2011 (i.e., 122.5% TDG) would not allow gas bubbles in Clearwater River-origin fish to equilibrate, which may result in mortality related to GBD. The TDG peak of 122.5% was caused by a temperature difference of only 7°C between the Clearwater and Snake rivers. This difference is less than in previous years when the rivers can differ by as much as 15°C, which may lead to even higher TDG concentrations than those measured in summer 2011. Although direct mortality was unlikely from the 122.5% TDG measured in 2011, this elevated concentration may be high enough to indirectly affect fish mortality (e.g., predation susceptibility). However, further investigation is needed to fully understand the interacting and indirect effects of TDG and temperature on juvenile salmon migrating through the Clearwater-Snake confluence.

Although chronic exposure to low levels of TDG and potentially short-term exposure to high concentrations of TDG (>120%) caused by mixing of the Clearwater and Snake rivers may not produce GBD that would lead directly to mortality, it may lead to increased stress and decreased fitness. Weber and Schiewe (1976) reported that bubbles in the lateral line reduced the sensitivity of that tissue, possibly reducing the affected fish's ability to avoid predators. Further, Mesa (1994) found that juvenile Chinook salmon were more susceptible to predation for 1 h after exposure to multiple stressors. Mesa and Warren (1997) found no increased susceptibility to predation in subyearling Chinook salmon exposed to 112% TDG despite the fact that these fish had severely blistered fins; however, this study did not manipulate temperature. They also found no increased susceptibility to predation in these same fish when exposed to

120% TDG despite an average lateral line occlusion of 20%. That study suggested that the severity of the signs of GBT, not the specific exposure history, was the determining factor in the reduced predator avoidance observed in juvenile spring Chinook exposed to 130% TDG. Multiple stressors likely play a role in predation rates on juvenile Chinook salmon; however, the combined effect of TDG and temperature on predation susceptibility has not yet been studied and is the goal of the next year of study.

Management Implications and Future Research

Baseline environmental conditions of the Snake River measured in 2011 should be considered in future experiments. The diel cycle of TDG in the Snake River is similar to the daily cycle in the Clearwater River and reaches its highest saturation during the late afternoon hours and lowest saturation during the early morning hours (driven by photosynthesis).

The confluence of the Clearwater and Snake rivers is likely a physiological challenge to emigrating juvenile fall Chinook salmon due to large temperature differentials and potentially high TDG levels in excess of 120%. While taken separately, acute exposure to high temperatures (Mesa et al. 2002) and supersaturated conditions of 120% TDG and below (Mesa and Warren 1997) may not increase predation of juvenile Chinook salmon. However, when combined, elevated temperatures and supersaturated TDG could increase prey vulnerability (Mesa 1994) and predator activity (Tabor et al. 2007), leaving migrating fish more susceptible to predation. Therefore, we propose to study the impact of elevated temperatures and supersaturated conditions on predator avoidance in juvenile Chinook salmon in a laboratory setting.

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CHAPTER FOUR

Distribution and abundance of potential invertebrate prey for juvenile fall Chinook salmon in the Snake River

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INTRODUCTION

Many Snake River fall Chinook salmon *Oncorhynchus tshawytscha* use lower Snake River reservoirs, particularly Lower Granite Reservoir, for rearing before migrating seaward as subyearlings (Connor et al. 2002). The impoundments in which subyearlings rear are fundamentally different than the riverine Hells Canyon Reach where most natural subyearlings are produced. In Hells Canyon, subyearlings rely on lotic food webs that are mainly composed of aquatic macroinvertebrates. By contrast impounded habitats are characterized by a lentic food web composed of phytoplankton, zooplankton, and other invertebrates that are more adapted to low velocities. These reservoir food webs have changed over the last 20 years with the colonization of introduced species that may influence subyearling productivity.

One non-native invertebrate that has recently become very abundant in Lower Snake River reservoirs is the estuarine opossum shrimp, *Neomysis mercedis* (hereafter, *Neomysis*; Figure 1). Subyearlings prey heavily on this relatively large (~15 mm, total length) species when they are present in shallow water (Tiffan et al. 2012). Although *Neomysis* is omnivorous, it can prey heavily on zooplankton (Murtaugh 1981a; Cooper et al. 1992; Haskell and Stanford 2006), which subyearlings also prey upon in reservoir habitats. The consumptive demand that *Neomysis* exerts on lower trophic levels is unknown, but warrants investigation given their relatively high biomass. Whether this species is an energetically profitable food for subyearlings also is unknown at this time.

Another recent invasive species that has colonized the lower Snake River reservoirs is the Siberian prawn *Exopalaemon modestus* (hereafter, prawns; Figure 2). This species was first documented in the Snake River at fish collection facilities at main-stem dams in the late 1990s and has since increased exponentially in abundance (Haskell et al. 2006). However, virtually nothing is known about this species' distribution, abundance, reproductive biology, food habits, and role in the food web in the lower Snake River reservoirs. The Siberian prawn is a large (~60 mm, total length) benthic organism that is omnivorous (Bell and Coull 1978). Given that *Neomysis* is also a benthic species, it is probable that prawns prey upon *Neomysis* and other taxa such as *Corophium* sp. (hereafter, *Corophium*; Figure 3), oligochaetes, and benthic aquatic insects. The role of Siberian prawns in the food web that ultimately supports subyearlings is unknown but should be cause for concern given that so little is known about them.

Both *Neomysis* and Siberian prawns have the potential to both directly and indirectly impact subyearling feeding and growth opportunity if their proliferation causes changes to the food web. In 2011, we began sampling Lower Granite and Little Goose reservoirs to collect basic information on these species given their abundance and potential for altering the food web. In addition, we also collected data on primary productivity, zooplankton, and surface drift. For this report, our objectives were to 1) describe the seasonal abundance and spatial distribution of

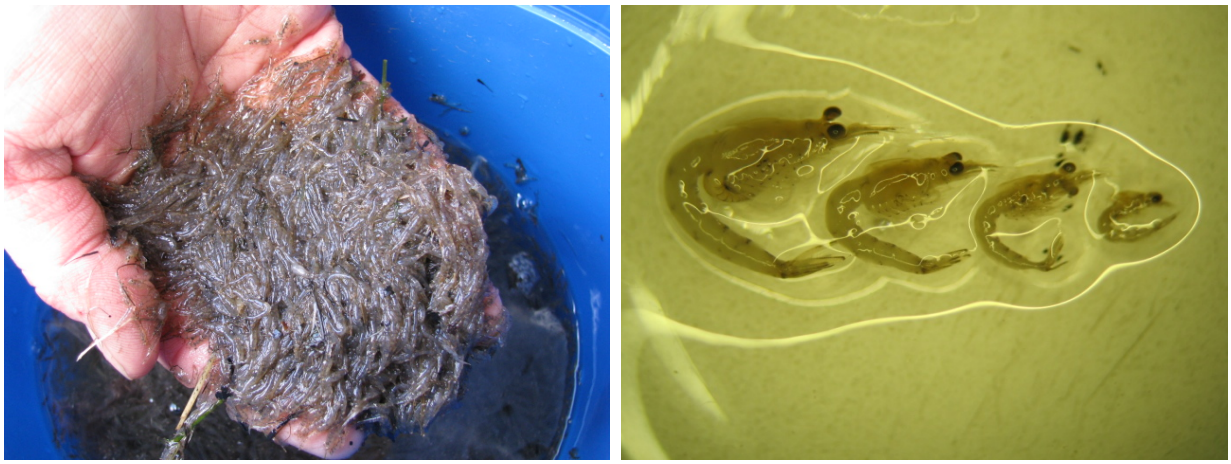

Figure 1. Pictures of *Neomysis mercedis*.

Figure 2. Pictures of Siberian prawns *Exopalaemon modestus*.

Figure 3. Pictures of *Corophium* sp.

Neomysis and Siberian prawns, 2) describe the seasonal changes in prawn population size structure, and 3) describe the reproductive timing of prawns.

METHODS

Sampling design and locations

We collected monthly samples in both Lower Granite and Little Goose reservoirs from May 2011 to April 2012. Each reservoir was divided into an upper, middle, and lower reach to ensure that samples were collected over a broad spatial area. Within each reach, we established systematic sampling sites that were sampled each month. At these systematic sites, 100-m transects were initially selected at random and then became fixed sampling transects. Roughly one-half of the transects was located in shallow water (<12 m) and one-half was located in deep water (>12 m). The locations of sampling sites are provided in Table 1.

Sample collection

We collected epibenthic invertebrates along 100-m transects using a beam trawl (Figure 4). The trawl had a rectangular opening that measured 2 m × 0.5 m and a net that was 3.7 m long, which tapered to a cod end. The trawl was constructed of 6.3 mm nylon delta mesh. The last 1.2 m of the cod end contained an internal liner constructed of 1.6-mm nylon delta mesh. Heavy nylon mesh was attached around the outside of the cod end to reduce chafing. The trawl frame had curved skids that allowed it to ride more easily along the reservoir bottom without getting snagged. A tickle chain was attached across the inside of the frame at the bottom in front of the trawl lead line to move benthic organism off the bottom during trawling so they could more easily be captured by the net.

The trawl was fished from an 8-m prop-driven boat equipped with two hydraulic winches, spools containing metered cable, and an A-frame and stantion support structure for deploying and retrieving the trawl (Figure 5). Beginning and ending waypoints for each transect were input in a GPS, which was used for navigation and to record the distance trawled. The trawl was deployed until it reached the bottom, which was determined based on the depth of the water, the amount of cable deployed, and the angle of cable. The trawl was then towed a distance of 100 m along the bottom and then retrieved. The trawl sample was poured through a 600 μm sieve to remove silt and debris and then preserved in 90% ethanol. On many occasions when a large trawl sample was collected, a random subsample was preserved. In these instances, both the subsample and the remainder of the total sample were weighed (wet).

In each reservoir reach (upper, middle, lower) a water sample from the surface was collected monthly to measure turbidity in nephelometric turbidity units (NTU) with a portable

Table 1. Sampling locations and frequency of sampling for epibenthic invertebrates in Lower Granite (LGR) and Little Goose (LGO) reservoirs in 2011. Samples were collected along 100-m transects with a beam trawl in shallow (<12 m) and deep (>12 m) water.

Reservoir	Location	River kilometer	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
LGR	Port Of Wilma	216.5		X	X	X	X	X	X	X
LGR	Wildlife Management Area	212.5			X	X	X	X	X	X
LGR	Silcott Island	211.0		X	X	X	X	X	X	X
LGR	Alpowa Creek	210.0		X	X					
LGR	Carp Flats	208.5		X	X	X	X	X	X	X
LGR	Centennial Island	193.0	X	X	X	X	X	X	X	X
LGR	Wawawai	178.5	X	X	X	X	X	X	X	X
LGR	Offield Landing	174.5	X	X	X	X	X	X	X	X
LGO	Illia	163.0	X	X	X	X	X	X	X	X
LGO	Wades Bar	158.0		X	X	X				
LGO	Swift Bar	152.0				X	X	X	X	X
LGO	Tucker Bar	145.5	X	X	X	X	X	X	X	X
LGO	Central Ferry	133.5	X	X	X	X	X	X	X	X
LGO	New York Island	126.5	X	X	X	X	X	X		X
LGO	Little Goose Bay	117.5		X	X	X	X	X		X
LGO	Little Goose Forebay	114.0		X	X	X	X	X		X

Figure 4. Pictures of the 2-m beam trawl used to collect benthic invertebrates in Lower Granite and Little Goose reservoirs in 2011-2012.

Figure 5. Picture of the boat used to deploy a beam trawl to sample benthic invertebrates in Lower Granite and Little Goose reservoirs in 2011-2012.

turbidity meter. We also measured water clarity with a 20-cm Secchi disk. We pooled all data between the two reservoirs and expressed mean (±SE) turbidity and Secchi depth by month.

We measured chlorophyll-a concentrations in the water during each month of sampling as an indicator of primary productivity of pelagic habitats. Monthly water samples were collected in each reach of each reservoir. A volume of 100 ml of water was collected, stored in a light-proof container, and stored on ice until processing at the end of each day. Each water sample was concentrated onto a glass fiber filter paper by low vacuum filtration then frozen. In the laboratory, pigments were extracted in 90% acetone, for 24 hours at -20°C, then the extracted slurry was removed and its fluorescence was measured with a fluorometer. The concentration of chlorophyll-a was then calculated in μg/L.

Sample processing and analysis

Prawns.—All prawns were blotted to remove excess ethanol and then weighed (±0.001 g, wet) individually. A portion of the prawns, encompassing a broad range in sizes, were selected to develop a carapace length-weight regression. These prawns were weighed and measured (±0.001 mm) from the tip of the rostrum to the back of the carapace at the dorsal midline. The carapace lengths of the remaining prawns were estimated using this regression. The weights of all individuals within a sample were summed to determine the total weight of prawns for each sample. If the processed sample was a subsample, then its weight was multiplied by the subsampling rate to estimate a total sample weight. We pooled data within reach (i.e., upper, middle, or lower), depth strata (i.e., deep or shallow), and by reservoir. We calculated mean monthly prawn density (#/m^2) and biomass (g wet/m^2) and graphically summarized results. We then pooled prawn carapace length data into 1 mm intervals for the two reservoirs and constructed monthly length-frequency histograms by depth strata to examine trends in size distributions.

We randomly selected 30 ovigerous prawns collected from July through December to estimate fecundity. We removed eggs from individual ovigerous prawns, counted them, and weighed the total number (g, wet). We also weighed each prawn after the eggs had been removed to determine the percentage of the total prawn weight (prawn + eggs) that the eggs represented.

We examined the diet of 10 randomly selected adult prawns from our collections. Individual prawns were weighed, measured, and had their stomach contents removed. Individual taxa were identified under a dissecting microscope to the lowest practical level for the purpose of drawing general conclusions about the types of prey consumed by prawns. Additional prawns will be analyzed for diet composition in the future.

Neomysis and Corophium.—All *Neomysis* from a given sample were blotted to remove excess ethanol and then weighed (±0.001 g, wet) collectively. If the processed sample was a subsample, then its weight was multiplied by the subsampling rate to arrive at a total sample weight. From each sample, a random subsample was removed and weighed and then the *Neomysis* in the subsample were counted. The number of *Neomysis* per gram of subsample was then multiplied by the total sample weight to determine the number of individuals in the sample. The same procedure was used to process *Corophium* in each sample. Densities and biomass of *Neomysis* and *Corophium* were calculated the same way as for prawns. Other invertebrate taxa that were collected were removed from each sample, identified to the lowest practical taxon, and weighed.

To determine the energetic profitability of prawns, *Neomysis*, and *Corophium* as prey for salmonids, we analyzed samples to determine their caloric content (Kcal). Prawns and *Neomysis* collected for this were frozen whereas Corophium were aggregated from samples stored in ethanol. Sample analysis required 0.5 g (dry weight) of each prey type to run a sample. This often necessitated pooling samples to achieve the minimum weight for analysis. Samples were dried and ground at the University of Idaho and then sent to the Hagerman Fish Culture Experiment Station for final analysis using bomb calorimetry.

RESULTS

Flows and temperatures were similar in Lower Granite and Little Goose reservoirs during our sampling (Figure 6). Flows were highest (~200kcfs) from May through July, decreased to about 25 kcfs during winter, and then increased again the following spring. Temperatures increased from about 10°C in early May to a peak of around 20°C by August and then declined to a low of about 2.5°C in late January 2012 (Figure 7). Turbidity was highest (17.8±0.25 NTU) in June coincident with high flows and then declined thereafter to a low of 2.28±0.11 NTU in December (Figure 7). Similarly, water clarity was lowest (0.67±0.065 m) in June and highest (3.61±0.17 m) in December (Figure 7).

Chlorophyll-a concentrations showed seasonal trends by sampling sites and reservoir. In Lower Granite Reservoir, chlorophyll-a concentrations increased from low levels in spring and early summer to peaks (depending on the site) in early autumn and then declined thereafter (Figure 8). Chlorophyll-a concentrations were generally highest at Offield (lower reach) and lowest at Silcott Island (upper reach; Figure 8). Chlorophyll-a concentrations peaked at Silcott (7.0 μg/L) and Centennial Island (13.0 μg/L; middle reach) in September, and peaked at Offield (16.3 μg/L) in October. In Little Goose Reservoir, chlorophyll-a results were less clear and often counterintuitive. Chlorophyll-a concentrations increased from low levels in spring and early summer to peaks in October (~9.0 μg/L) at both Illia (upper reach) and Tucker Bar (middle

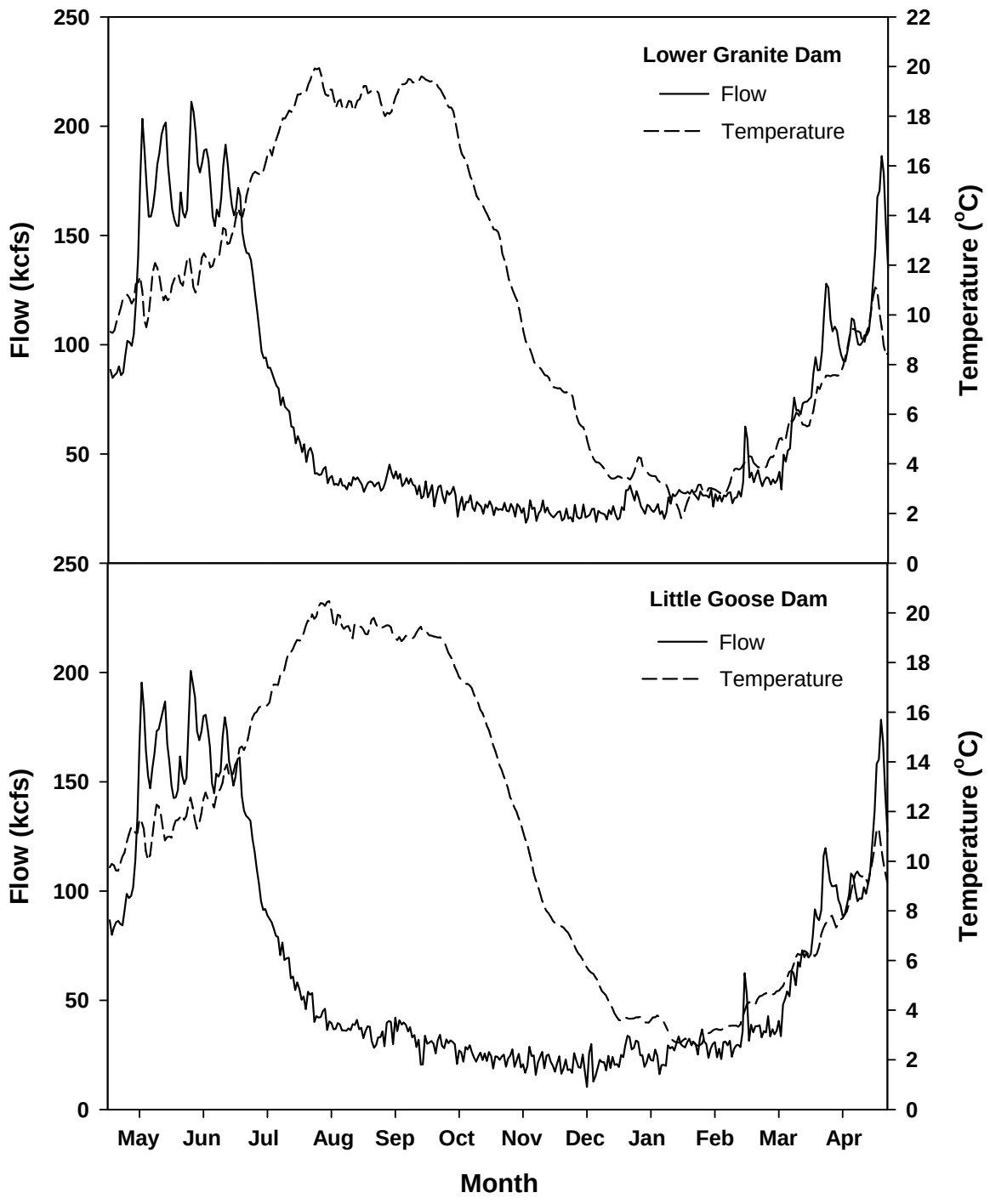

Figure 6. Flows and temperatures recorded at Lower Granite Dam (top panel) and Little Goose Dam (bottom panel) from May 2011 through April 2012.

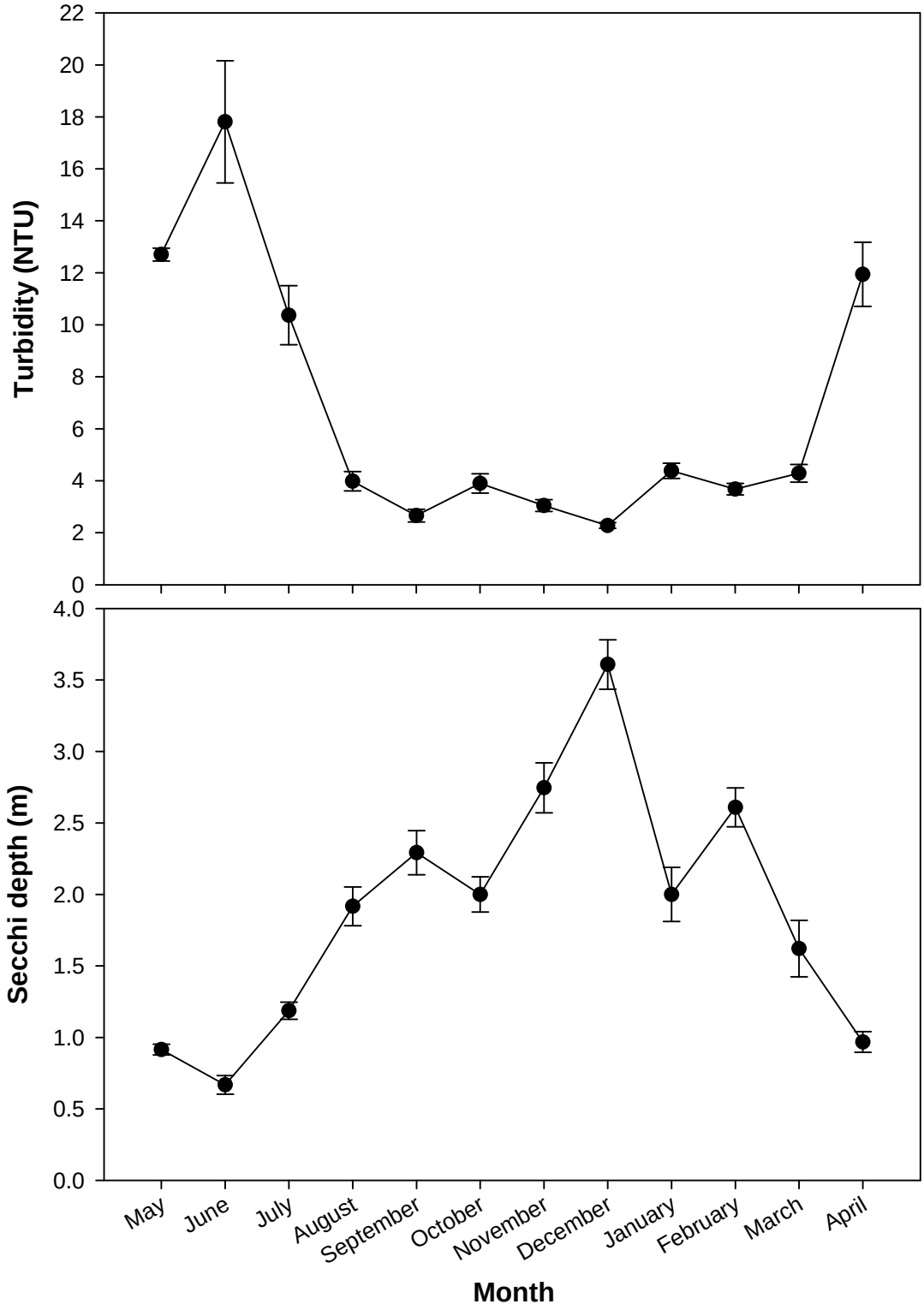

Figure 7. Monthly turbidity (±SE; top panel) and Secchi depth (±SE; bottom panel) measured in Lower Granite and Little Goose reservoirs (data pooled) from May 2011 through April 2012.

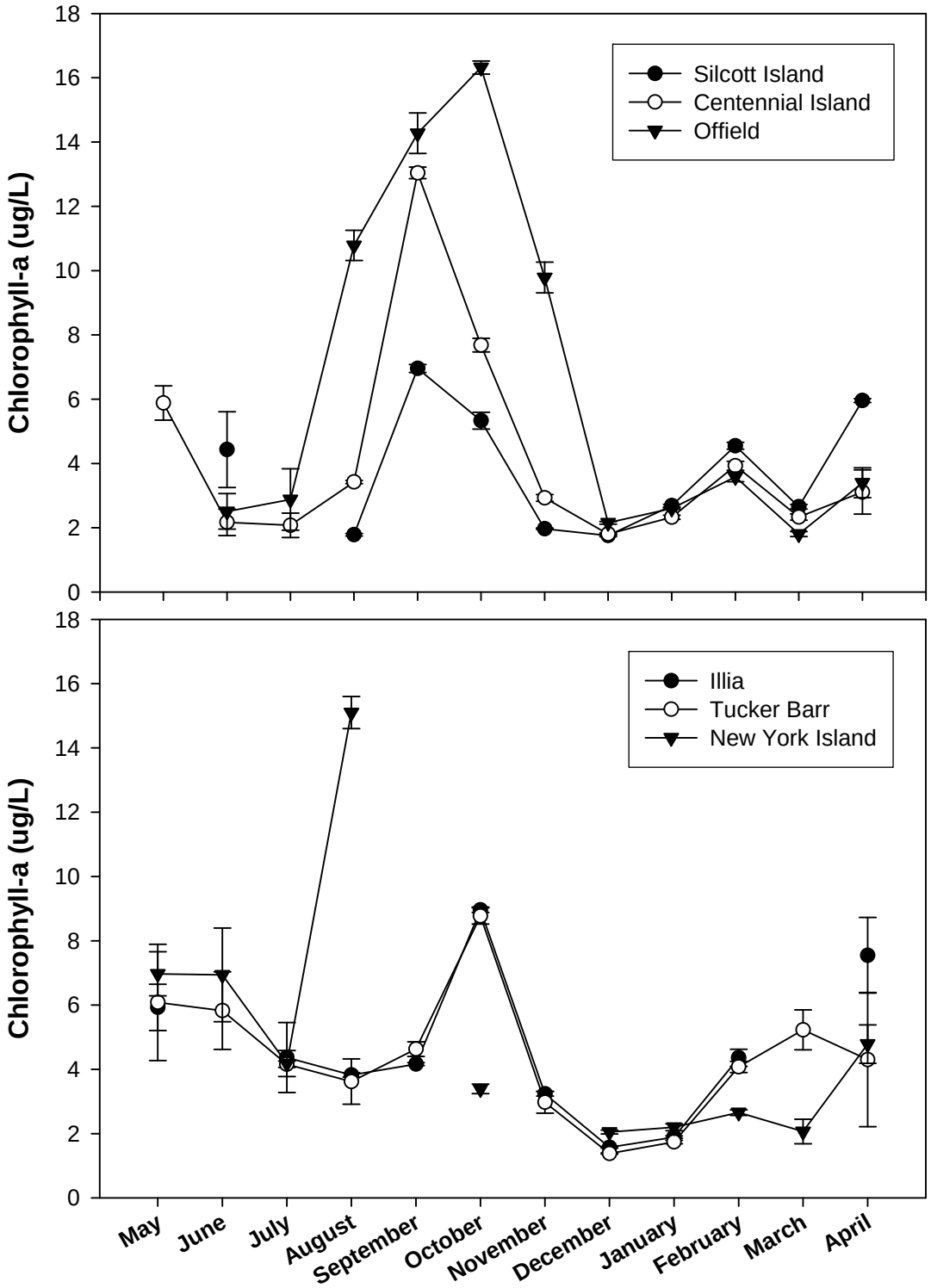

Figure 8. Monthly mean (±SD) chlorophyll concentrations at three sampling sites in Lower Granite (top panel) and Little Goose (bottom panel) reservoirs in 2011-2012.

reach) and then declined thereafter (Figure 8). However, there was no peak in chlorophyll-a concentration at New York Island (lower reach), the downstream-most sampling site in Little Goose Reservoir. In addition, we recorded a high (15.1 μg/L) concentration at New York Island in August, but did not obtain samples at this site in September or November that may have helped to clarify trends.

We collected epibenthic invertebrates in 454 trawl samples from May through December. Depth information on sample sites in Lower Granite and Little Goose reservoirs is shown in Table 2. *Neomysis* dominated the catch, composing 78-99% of samples in terms of both density and biomass (Table 3). At times we were not able to sample all sites within a month due to high winds, inclement weather, and unsafe trawling conditions.

Prawns

The presence of prawns was documented in all reaches of Lower Granite Reservoir. Mean prawn densities in shallow sample sites were generally low in all reaches except in June in the lower reach and in September in the middle reach (Figure 9). Prawn densities increased slightly in December in shallow sites in the lower reach. Prawns were generally more abundant at deep locations in the middle and lower reaches of Lower Granite Reservoir with peaks in densities occurring in August, November, and December (Figure 9). Prawns were never abundant at shallow or deep sites in the upper reach of Lower Granite Reservoir during any month sampled (Figure 9).

Trends in prawn abundance in Little Goose Reservoir were similar to those observed in Lower Granite Reservoir (Figure 9) and prawn presence was also documented in all reaches. Abundances were generally highest in the middle reach (Figure 9). Relatively high abundances were observed in June in the middle and lower reaches of the reservoir and declined thereafter. There was large increase in prawn abundance in the lower reach in October and slight increases in December in both the middle and lower reaches (Figure 9). As in Lower Granite Reservoir, prawns were more abundant at deep locations in Little Goose Reservoir (Figure 9) with the exception of June. Densities increased from spring to late autumn in the middle and lower reaches, but were consistently low in the upper reach.

Trends in prawn biomass generally followed those of prawn densities in both Lower Granite and Little Goose reservoirs (Figure 10). Prawn biomass was substantially higher at deep sample sites compared to shallow sample sites in both reservoirs. In Lower Granite Reservoir shallow sites, biomass was highest in the lower reach in June, and at deep sites biomass was highest in the lower reach in August and highest in the middle reach in November. In Little Goose Reservoir shallow sites, biomass was highest in the middle reach in June and declined

Table 2. Depth information on shallow and deep sites in Lower Granite (LGR) and Little Goose (LGO) reservoirs that were sampled with a beam trawl in 2011 to collect epibenthic invertebrates.

Site	N	Mean depth (m)	Standard deviation (m)	Minimum (m)	Maximum (m)
LGR Shallow	137	5.1	4.7	1.5	50.9
LGR Deep	79	22.5	6.1	3.8	32.9
LGO Shallow	145	7.8	3.3	2.4	14.8
LGO Deep	93	21.8	8.9	5.5	37.2

Table 3. Percent of catch of different epibenthic invertebrates based on density and biomass at shallow and deep sample sites in Lower Granite (LGR) and Little Goose (LGO) reservoirs from May through December, 2011.

Location	Prawns	Neomysis	Corophium	Corbicula	Other
		Based on density (#/m^2)			
LGR Shallow	<1%	99%	<1%	<1%	<1%
LGR Deep	<1%	98%	1%	<1%	<1%
LGO Shallow	<1%	99%	<1%	<1%	<1%
LGO Deep	3%	78%	16%	2%	<1%
		Based on biomass (g/m^2)			
LGR Shallow	<1%	98%	<1%	<1%	<1%
LGR Deep	9%	88%	<1%	2%	<1%
LGO Shallow	6%	90%	<1%	2%	<1%
LGO Deep	45%	27%	2%	26%	<1%

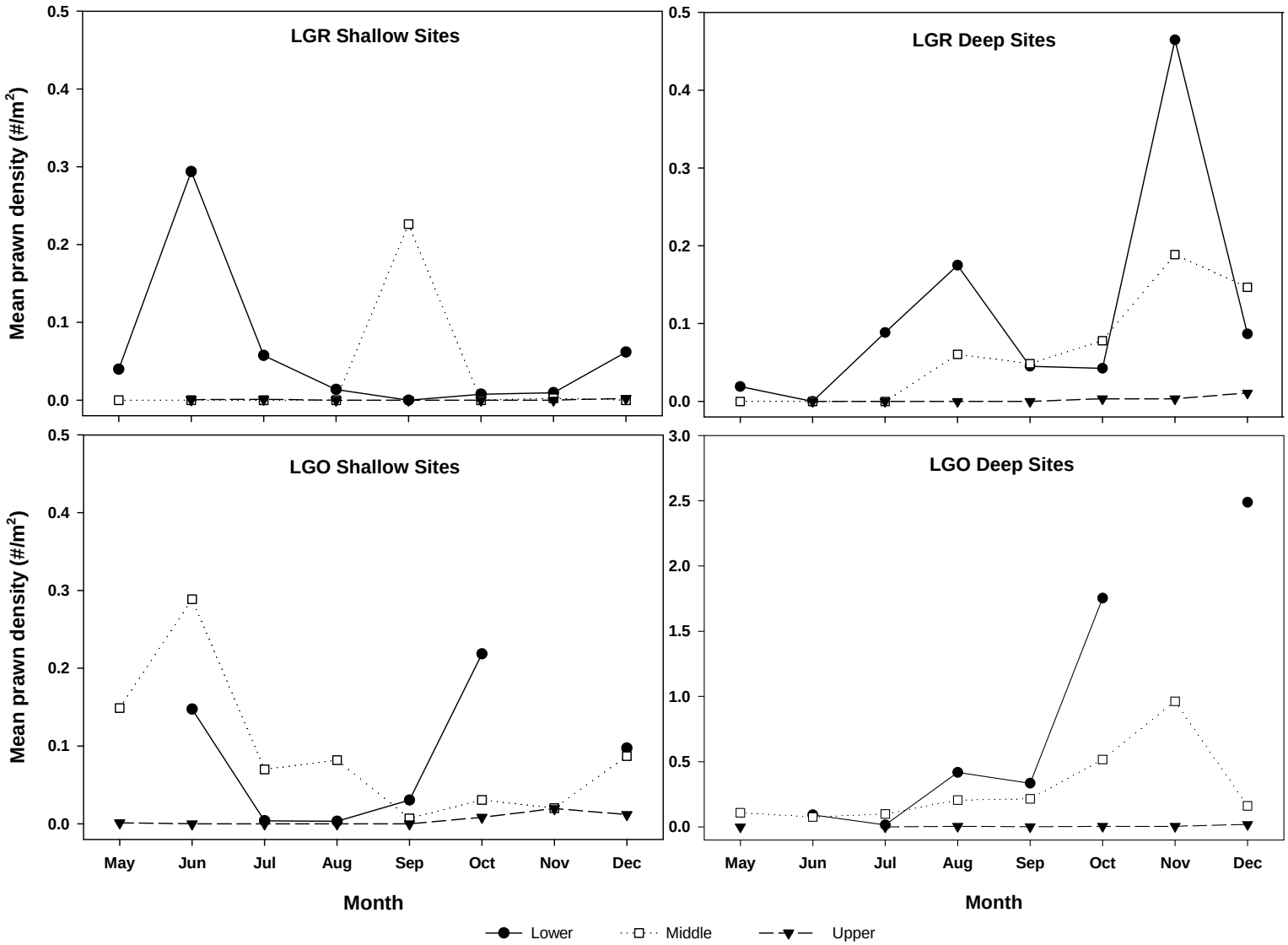

Figure 9. Monthly prawn densities at shallow and deep sampling sites in Lower Granite (LGR) and Little Goose (LGO) reservoirs, 2011. Densities are summarized for the lower (solid line), middle (dotted line), and upper (dashed line) reaches.

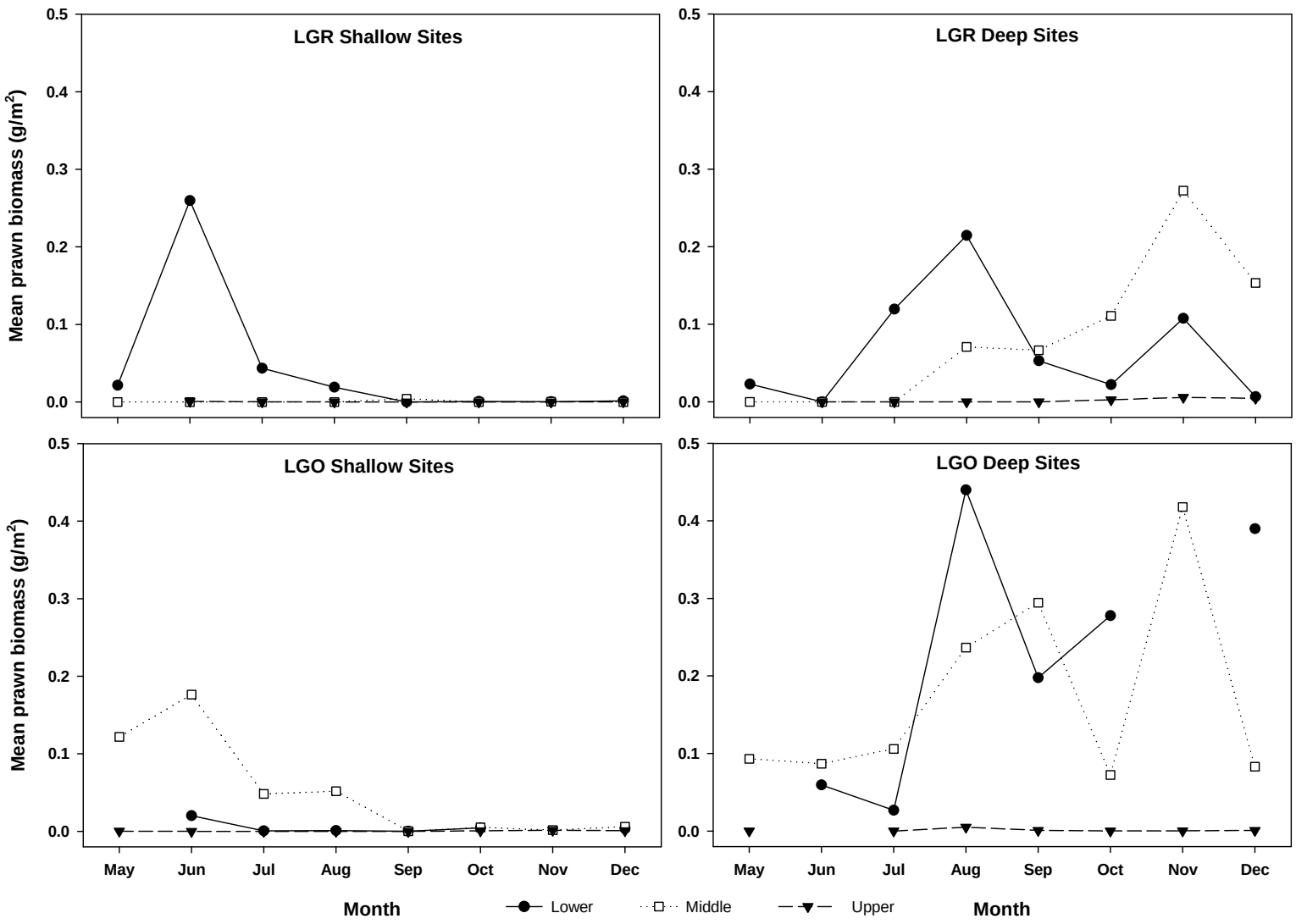

Figure 10. Monthly prawn biomass at shallow and deep sampling sites in Lower Granite (LGR) and Little Goose (LGO) reservoirs, 2011. Biomasses are summarized for the lower (solid line), middle (dotted line), and upper (dashed line) reaches.

thereafter. At deep sites, biomass showed more monthly variation but was relatively high in both the lower and middle reaches from August to December (Figure 10).

Results of the carapace length frequency analysis depicted variability in the size distribution both monthly and by depth strata. In May, two apparent size classes of prawns were present in both shallow and deep sites (Figure 11). By June, a broader range of prawn sizes was evident in the shallow sites (Figure 10), when prawns were also substantially more abundant (Figure 9), whereas the deep sites were mainly represented by larger individuals (20.8% were 22mm). In July, the size distribution of prawns at shallow sites was more compressed and fewer small prawns were collected. At deep sites, the size distribution continued to shift toward larger individuals as adult prawns dominated collections (Figure 11). The overall size distribution continued to increase into August and a few small juvenile prawns began appearing in shallow sites, indicating that a possible third size class (young-of-year) was developing (Figure 12). In September, adult prawns were absent from samples collected at shallow sites, but dominated catches from deep water. The abundance of young-of-year prawns increased in the shallow sites and began appearing at deep sites. From October to December, the young-of-year size distribution shifted towards the right at both shallow and deep sites. The adult prawns remained absent at shallow sites while the size distribution slowly increased (right shifted) at the deep sites (Figure 13).

Ovigerous female prawns were first collected in July and were present in collections through December. More ovigerous prawns were collected in Little Goose Reservoir than in Lower Granite Reservoir and the greatest number were collected in September (Figure 14). Ovigerous females ranged in size from 19.8 mm to 28.3mm (carapace length) and weighed from 0.8 g to 2.4 g (total weight). The mean number of eggs per female was 171 (range, 91 to 281) and constituted 11.5% of total body weight (range, 5.0 to 19.5%).

A cursory examination of the stomach contents of 10 adult prawns showed that diet consisted of *Corophium*, *Neomysis*, and aquatic insects. *Corophium* was the most common prey consumed.

Neomysis

Neomysis abundance was considerably higher in Lower Granite Reservoir than in Little Goose Reservoir and varied by depth and seasonality (Figure 15). In Lower Granite Reservoir, *Neomysis* were generally more abundant at shallow sites than deep sites. Densities were lowest in the spring and early summer and then began increasing in September, particularly in the upper and middle reaches at shallow sites but to a lesser degree at deep sites (Figure 15). In Lower Granite Reservoir, the highest mean density of 550/m^2 was observed in October in the middle reach. In Little Goose Reservoir, *Neomysis* densities were generally less than 100/m^2 at both

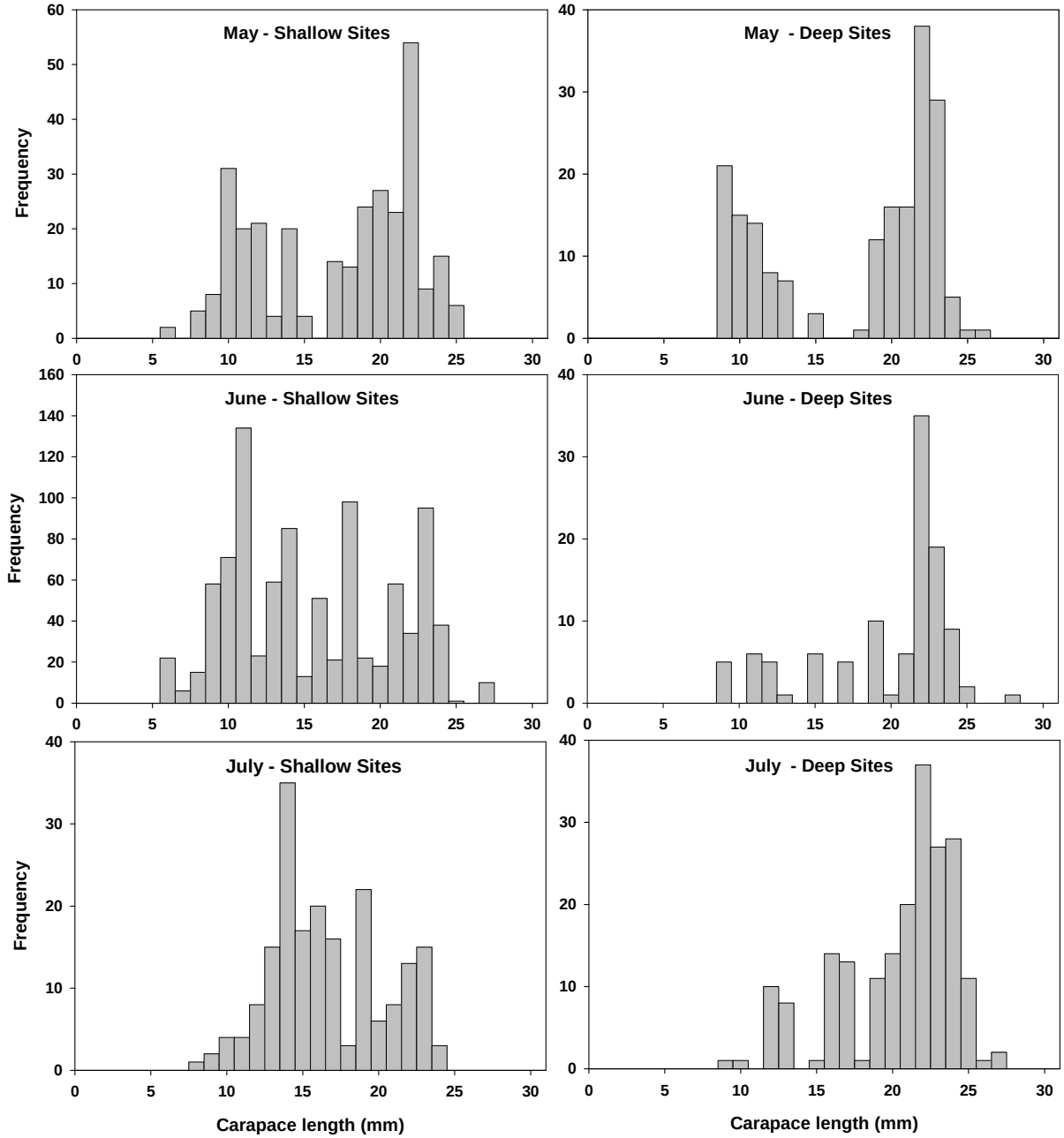

Figure 11. Length-frequency plots of prawns collected from shallow and deep sites from Lower Granite and Little Goose reservoirs (combined) from May through July, 2011.

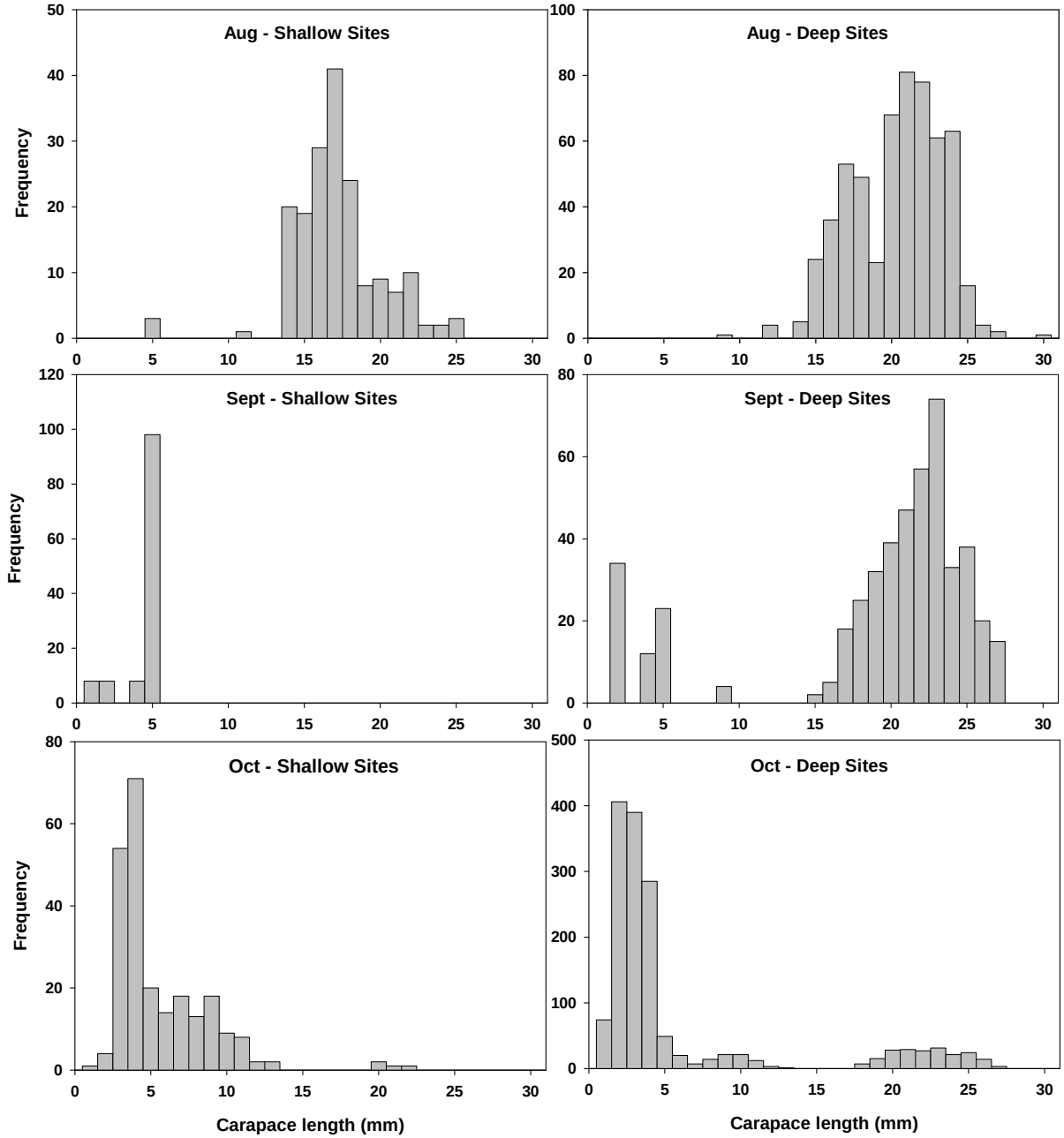

Figure 12. Length-frequency plots of prawns collected from shallow and deep sites from Lower Granite and Little Goose reservoirs (combined) from August through October, 2011.

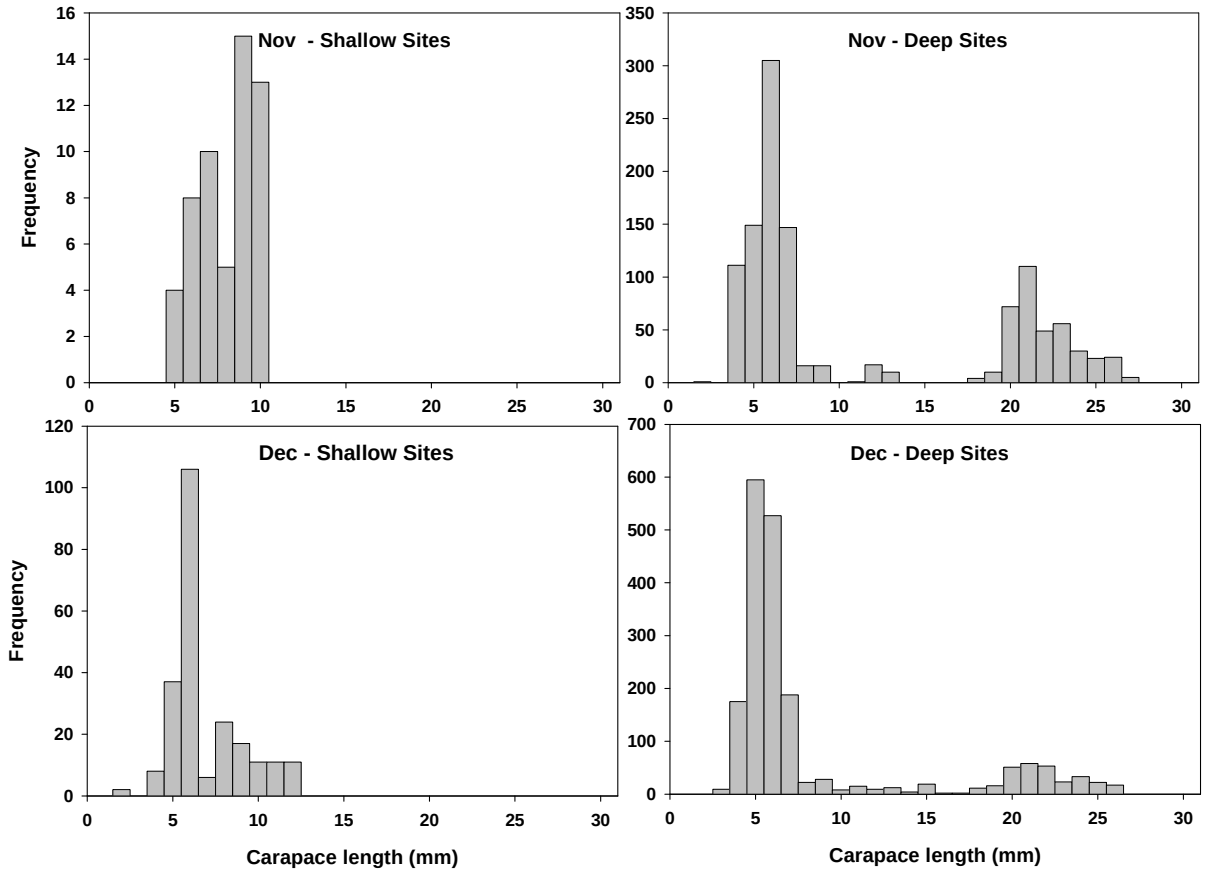

Figure 13. Length-frequency plots of prawns collected from shallow and deep sites from Lower Granite and Little Goose reservoirs (combined) in November and December, 2011.

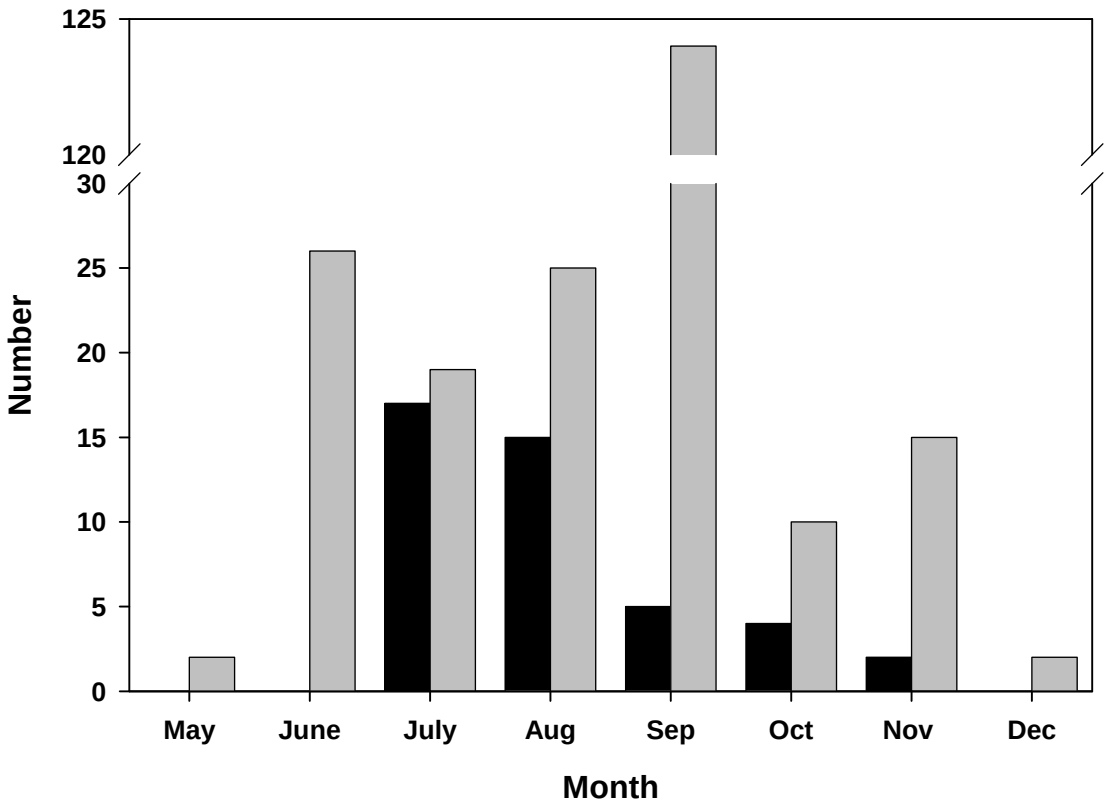

Figure 14. Number of ovigerous prawns collected in Lower Granite Reservoir (black bars) and Little Goose Reservoir (gray bars) from May to December, 2011.

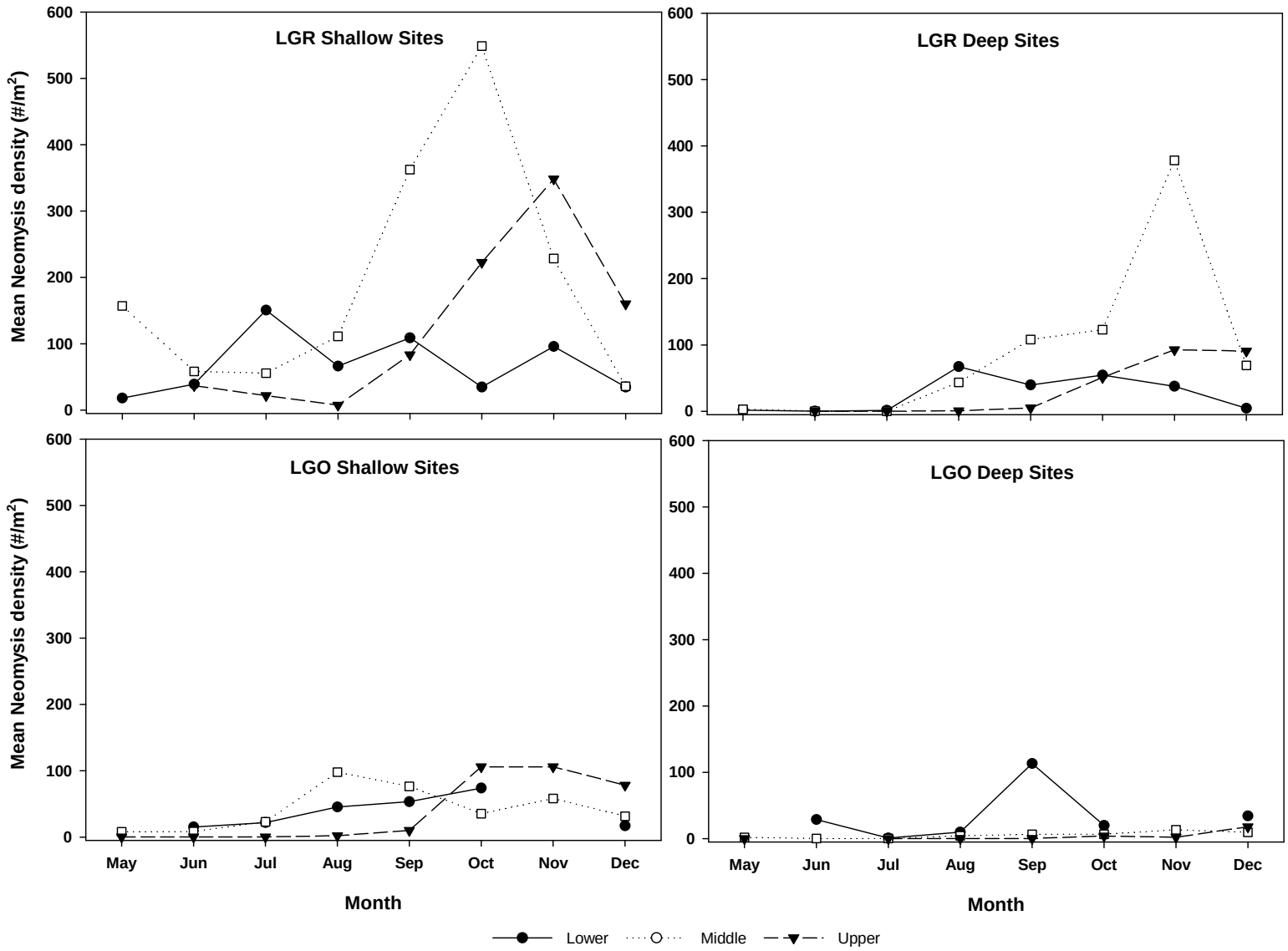

Figure 15. Monthly *Neomysis* densities at shallow and deep sampling sites in Lower Granite (LGR) and Little Goose (LGO) reservoirs, 2011. Densities are summarized for the lower (solid line), middle (dotted line), and upper (dashed line) reaches.

shallow and deep sites (Figure 15). As in Lower Granite Reservoir, *Neomysis* abundance was low in spring and early summer at shallow sites in Little Goose Reservoir and then began increasing in August and remained higher than spring abundances through December. At deep sites, *Neomysis* abundance was low (~13/m^2) in all reaches and months except in the lower reach in September (Figure 15).

Neomysis biomass showed trends similar to abundance in Lower Granite and Little Goose reservoirs both seasonally and by depth strata with some notable exceptions (Figure 16). At shallow sites in Lower Granite Reservoir, *Neomysis* biomass was highest in May, decreased through August, increased to a smaller peak in October, and decreased thereafter (Figure 16). By comparison, *Neomysis* abundance was relatively low in May and highest in October (Figure 15). This suggests that May samples contained larger, but fewer individuals, whereas October samples contained smaller, but more numerous individuals.

Corophium

In contrast to *Neomysis*, *Corophium* were not very abundant in beam trawl catches in either reservoir (Figure 17). At shallow sites in Lower Granite Reservoir, *Corophium* densities were consistently low in the upper reach as well as the other reaches, but some peaks were observed in the middle and lower reaches. At deep sites in Lower Granite Reservoir, *Corophium* were generally more abundant in the lower and middle reaches, but the highest abundance was observed in the upper reach in December (Figure 17). In Little Goose Reservoir, *Corophium* were also generally not abundant at shallow sites, but peaks in abundance were observed in September in both the lower and middle reaches (Figure 17). At deep sites in Little Goose Reservoir, high *Corophium* abundances were observed primarily in the lower reach in June and September.

Trends in *Corophium* biomass generally followed those of abundance in both reservoirs (Figure 18). An extremely high biomass was observed at shallow sites in the middle reach of Lower Granite Reservoir in September. Considerably higher *Corophium* biomasses were observed at deep sites, compared to shallow sites, in Lower Granite Reservoir in autumn, particularly in the lower reach (Figure 18). *Corophium* biomass was also higher at deep sites in Little Goose Reservoir compared to deep sites in Lower Granite Reservoir (Figure 18).

Caloric values

The mean monthly caloric content of prawns ranged from 4,724 Kcal in August to a peak of 4,898 Kcal in October and then declined to 4,700 Kcal by March, 2012 (Table 4). The mean monthly caloric content of *Neomysis* was variable and averaged 4,962 Kcal. Due to the difficulty in collecting sufficient quantities of *Corophium*, we could only estimate caloric content

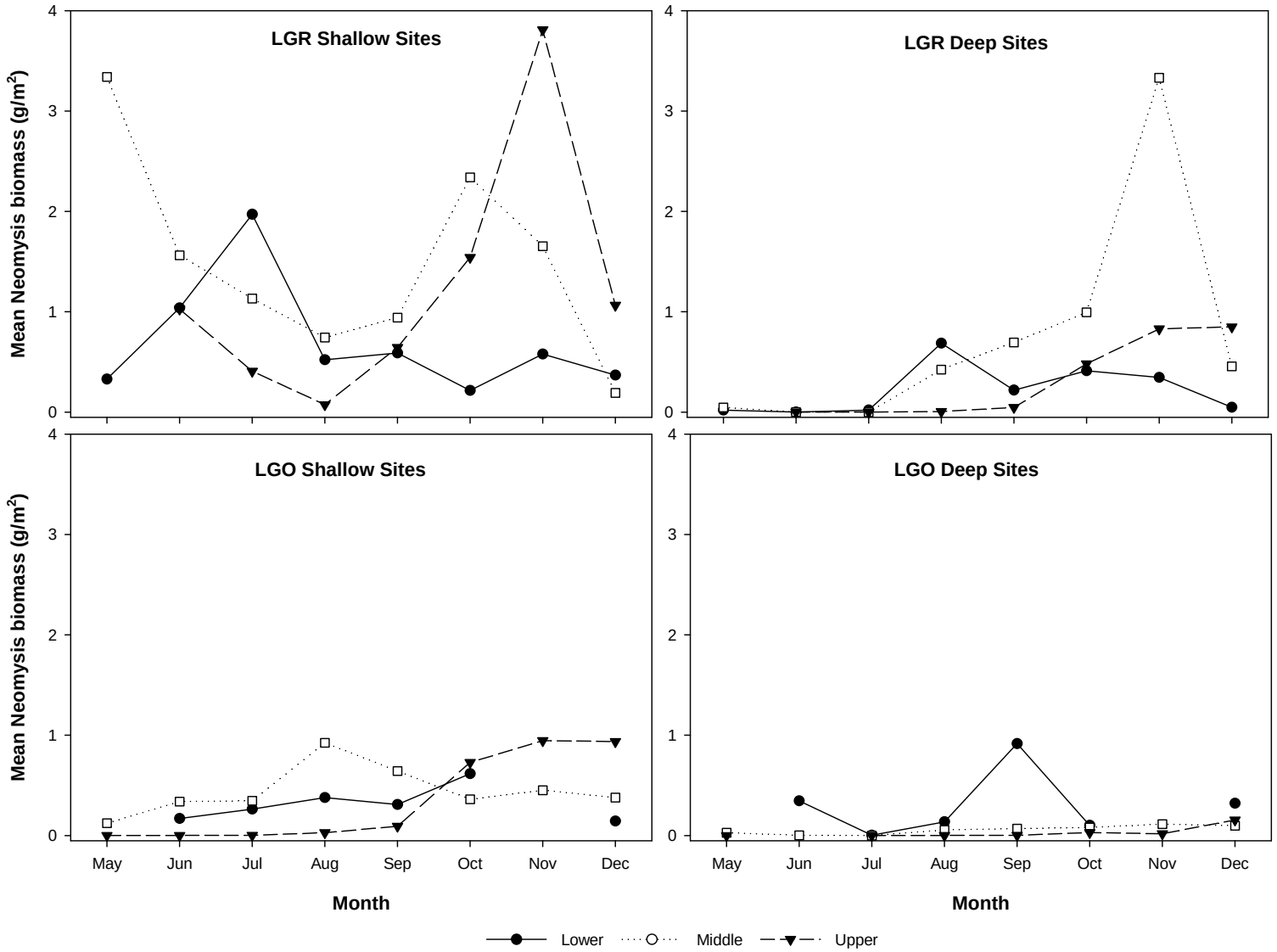

Figure 16. Monthly *Neomysis* biomass at shallow and deep sampling sites in Lower Granite (LGR) and Little Goose (LGO) reservoirs, 2011. Biomasses are summarized for the lower (solid line), middle (dotted line), and upper (dashed line) reaches.

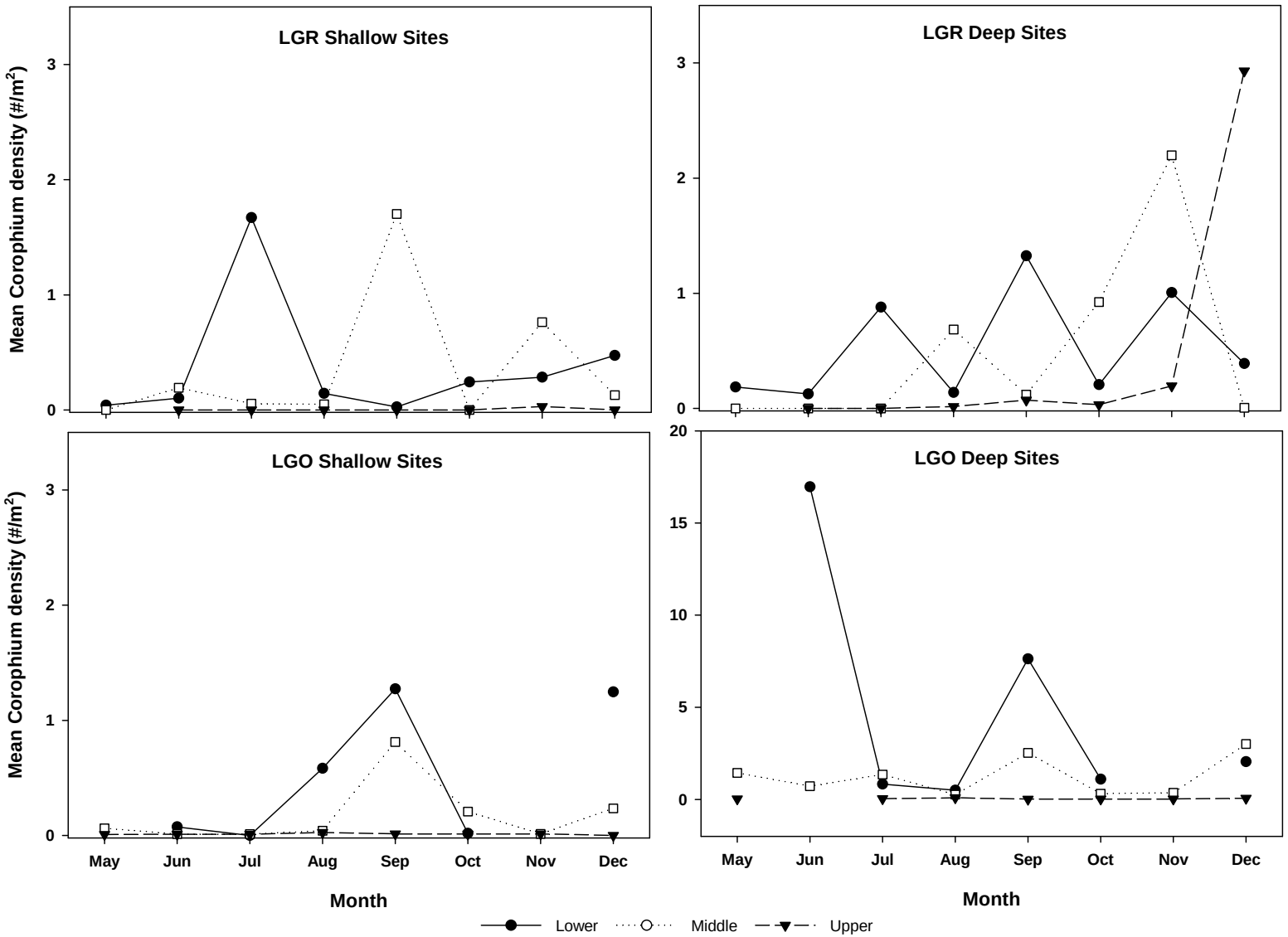

Figure 17. Monthly *Corophium* densities at shallow and deep sampling sites in Lower Granite (LGR) and Little Goose (LGO) reservoirs, 2011. Densities are summarized for the lower (solid line), middle (dotted line), and upper (dashed line) reaches.

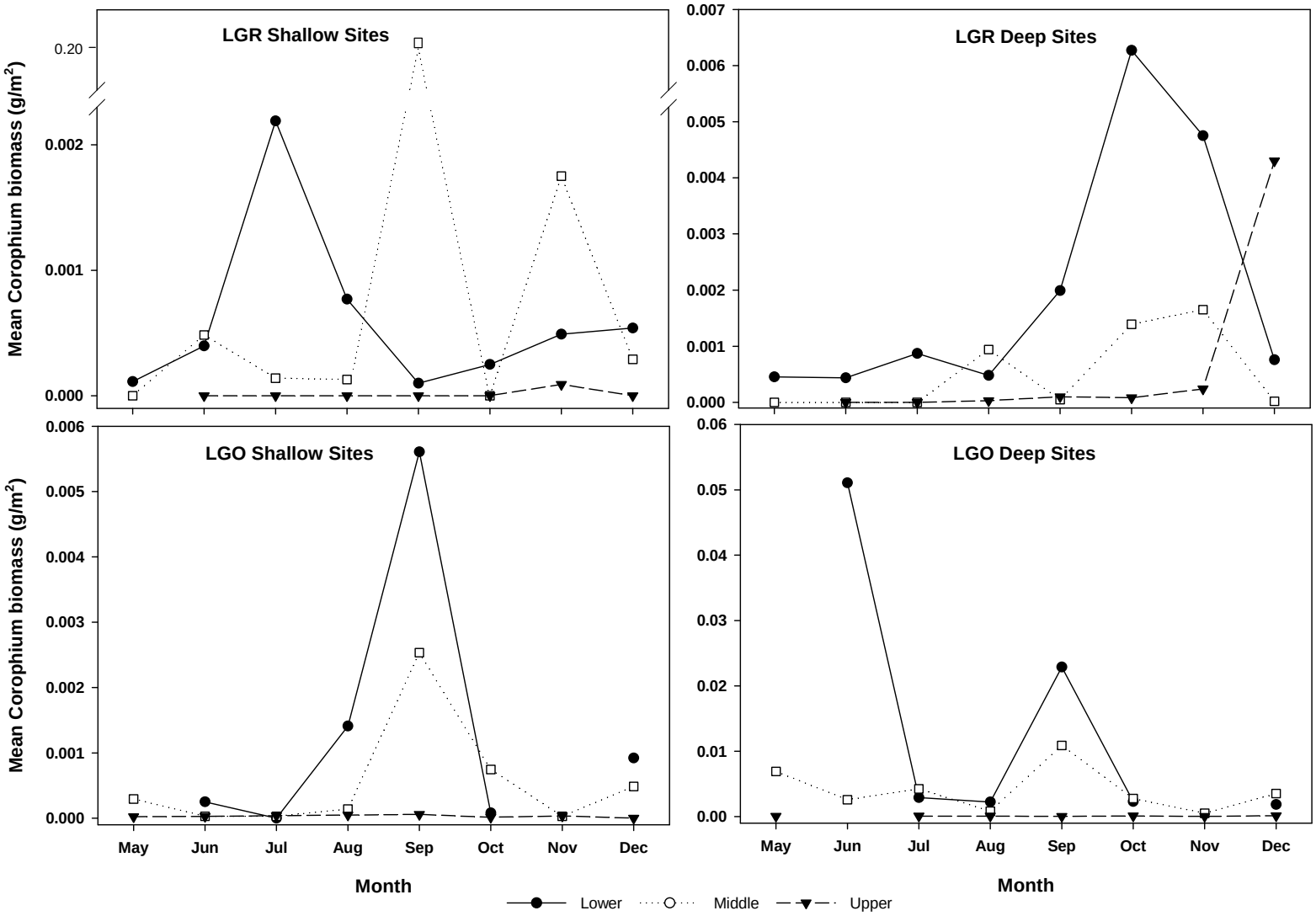

Figure 18. Monthly *Corophium* biomass at shallow and deep sampling sites in Lower Granite (LGR) and Little Goose (LGO) reservoirs, 2011. Biomasses are summarized for the lower (solid line), middle (dotted line), and upper (dashed line) reaches.

Table 4. Mean monthly caloric content of prawns, *Neomysis*, and *Corophium* collected in Lower Granite and Little Goose reservoirs in 2011 and 2012. *N* is the number of samples analyzed.

Year	Month	*N*	Caloric content (Kcal)	SD
		Prawns		
2011	August	3	4,724	66.3
2011	September	3	4,834	61.3
2011	October	2	4,898	124.5
2011	November	3	4,816	2.8
2012	January	7	4,782	31.5
2012	February	3	4,747	17.3
2012	March	2	4,700	19.1
2012	April	4	4,749	73.8
2012	May	31	4,788	72.0
Grand mean			**4,782**	
		Neomysis		
2011	August	4	5,046	25.1
2011	September	1	4,889	
2011	October	9	5,071	84.2
2012	February	4	4,980	82.9
2012	April	10	4,956	40.1
2012	May	23	4,830	195.4
Grand mean			**4,962**	
		Corophium		
2011	May	1	4,147	
2011	July	1	4,384	
2011	September	1	6,246	
Grand mean			**4,926**	

for three samples. The caloric content of *Corophium* increased from 4,147 Kcal in May to 6,246 Kcal in September (Table 4).

DISCUSSION

In Lower Granite and Little Goose reservoirs, prawns were more abundant in shallow water in the spring and early summer and then shifted to deeper water in summer and autumn, but prawns were present in both habitats throughout the year. Juvenile and adult prawns were present at both shallow and deep sites from May through June, but the distinction between the two size classes was not as clear after May. There tended to be larger prawns in deeper compared to shallow water in all months. Length-frequency plots suggest that most young-of-year prawns move to deep water in autumn, but many remain in shallow water. The almost near absence of adult prawns in shallow water from September through December indicates that they must prefer deeper waters during this time of year.

The distribution of prawns in Lower Granite and Little Goose reservoirs may be controlled in part by water velocities. Prawns in both reservoirs were either absent or present in low abundance in most samples collected in the upper reaches regardless of the time of year, but were more abundant in lower reservoir reaches. Velocities are highest in the upper reaches of reservoirs (Tiffan et al. 2009), and it is plausible that velocity limits the upstream expansion of their range to these areas. Furthermore, 2011 was a high flow year and prawns may have been displaced from the upper portions of the reservoirs. Our sampling in 2012—a more typical water year—will provide data for comparison to further examine the longitudinal distributions of prawns in these reservoirs.

There is little information on the biology of the Siberian prawn that is published in English so it is difficult to provide a context for our results. To provide perspective for our fecundity data, we examined data from other related prawn and shrimp species. Corey and Reid (1991) summarized data on the fecundity of ten species of the family Palaemonidae. Mean egg numbers ranged from 93 to over 11,000, but fecundities were typically less than 1,000. By comparison, the prawns we examined contained a mean of 171 eggs/female. Female prawns carry their eggs on their abdomen until they hatch. We first observed ovigerous females in July and did find eggs into December, but these did not appear to be viable. Prawn reproduction probably occurs mainly from midsummer to early autumn in lower Snake River reservoirs. Given the patterns in our length-frequency data, we suspect that prawns produced in the summer and autumn mature into reproductive adults by the following summer, but additional data is need to confirm this.

Prawns are omnivorous (Bell and Coull 1978) and are probably opportunistic foragers. The very limited diet information that we collected from adult prawns suggests that they prey on benthos, including *Corophium* and *Neomysis*. *Corophium* may be at greater risk of prawn predation because they are slower moving than *Neomysis*, which have a high escape capacity (Irvine et al. 1990). However, given the high densities of *Neomysis* we observed, they are probably also important in prawn diets, and prawns are probably not food limited in Lower Granite or Little Goose reservoirs. It is interesting that the location of some of the highest prawn densities we observed (lower reach of Little Goose Reservoir) was the same for the highest *Corophium* densities we observed. Additional analyses of prawn diets will be incorporated in future reports.

Neomysis densities were higher in Lower Granite than in Little Goose Reservoir. Mean densities were generally <100/m^2 in both reservoirs but maximum seasonal densities in Lower Granite Reservoir ranged from 378/m^2 at deep sites to 548/m^2 at shallow sites. These densities were higher than reported for studies in oligotrophic systems but lower than densities reported for eutrophic systems (Table 5). It is somewhat difficult to compare our results to other studies because of differences in sampling methods. *Neomysis* are commonly collected in vertical net tows so aerial densities represent that from the entire water column, or the depth of the tow. These tows are typically made at night because *Neomysis* are distributed throughout the water column at night (Cooper et al. 1992; Haskell and Stanford 2006). In contrast, we sampled during the day by towing a trawl along the bottom when *Neomysis* are concentrated there. We believe that most *Neomysis* were benthically, rather than pelagically, oriented during our daytime sampling and thus our density estimates should be somewhat comparable to other studies, at least at a coarse scale.

Neomysis densities were generally greater in shallow water than in deep water, which is consistent with findings of Murtaugh (1981b). One reason for this is that food may more abundant in shallower areas. In Lower Granite Reservoir, the shallow sampling areas in both the upper and middle reaches were located behind islands away from the main river channel. These may be important zooplankton production areas because velocities are lower there, and zooplankton may be transported out of these areas at lower rates. The lower velocities in shallow habitats may also explain the higher *Neomysis* densities found there because Neomysis would also be less likely to be transported out those habitats compared to deeper main-channel habitats where the velocities would be higher.

We summarized data collected on *Corophium* because we routinely collected them during our sampling and they are seasonally important in the diet of rearing subyearling fall Chinook salmon (Tiffan et al. 2012). However, our beam trawl probably did not effectively sample them because they are tube dwellers and the trawl was most effective at collecting epibenthic invertebrates. In our collections, *Corophium* density was typically about 1/m^2. By comparison, Seybold and Bennett (2010) reported *Corophium* densities in excess of 1,500/m^2 in

Table 5. A comparison of density (maximum seasonal estimate) and biomass (corresponding with maximum density) of *Neomysis* in freshwater and estuarine systems. Much of this table was reproduced from Cooper et al. (1992). The results from our study are shown in bold and split out by shallow and deep sites. Lower Granite Reservoir is abbreviated as LGR

Lake or estuary	Density (#/m^2)	Biomass (g/m^2)	Lake trophic status	Source
Kasumigaura	11,000	6.0	Ultra-eutrophic	Toda et al. (1981) Toda et al. (1982)
Sacramento-San Joaquin estuary	7,000	4.5	NA	Siegfried et al. (1979)
Fraser River estuary (side channel)	1,000	0.6	NA	Johnston and Northcote (1989)
Ferring	882	---	Ultra-eutrophic	Søndergaard et al. (2000)
LGR shallow	**548**	**2.3**	**Oligotrophic**	**This study**
LGR deep	**378**	**3.3**		
Washington	406	0.3	Eutrophic	Murtaugh (1981b) Edmondson and Lehman (1981)
Muriel	117	0.07	Ultra-oligotrophic	Nidle et al. (1984) Cooper (1988)
Kennedy (Clayoquot arm)	71	0.05	Ultra-oligotrophic	Nidle et al. (1984) Nidle and Shortreed (1985, 1987)
Fraser River estuary (main channel)	10	0.001	NA	Northcote et al. (1976)

lower Snake River reservoirs collected with a Peterson dredge. We collected benthic samples with a dredge in 2012 to better understand the abundance of *Corophium* and other benthic invertebrates in the soft substrates of Lower Granite and Little Goose reservoirs.

The proliferation of both prawns and *Neomysis* in Lower Granite and Little Goose reservoirs has added a new dimension to the food web and their effects are not fully understood at this time. It is well known that *Neomysis* prey on zooplankton and can selectively depredate larger *Daphnia* and reduce their abundance (Murtaugh 1981b; Haskell and Stanford 2006). This is important because subyearling fall Chinook salmon also feed on zooplankton when they rear in reservoir habitats (Curet 1993; Rondorf et al. 1990; Tiffan et al. 2012). However, once subyearlings grow large enough, they begin to prey on *Neomysis*, and Tiffan et al. (2012) showed that *Neomysis* can compose up to 98% of subyearling diets in late spring in Lower Granite Reservoir. *Neomysis* are also consumed by larger reservoir-type fall Chinook salmon in autumn, but to a lesser extent (Tiffan and St. John 2011). It is likely that only juvenile prawns in shallow habitats would be potentially used by larger subyearling smolts and reservoir-type juveniles in late summer and autumn, but we have not documented their consumption by juvenile salmon. However, by this time juvenile fall Chinook salmon typically use pelagic habitats and their use of benthic prey like prawns may be minimal but no data exist to either reject or confirm this notion.

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APPENDICES

Appendix 1. Gene ontology functional categories enriched in the lists of genes differentially expressed between Snake River fall Chinook salmon at different stages of development and migration timing. GO terms that make up the clusters are shown along with their corresponding p-values from the overrepresentation analysis.

Cluster description	GO term	*P*
	SNK parr vs. SNK early-migrating smolts—all DE genes	
DNA repair	GO:0006259 DNA metabolic process	3.8E-10
	GO:0006974 response to DNA damage stimulus	1.5E-05
	GO:0006281 DNA repair	3.2E-05
	GO:0033554 cellular response to stress	2.0E-04
Cell cycle/ Mitosis/ Spindle organization	GO:0007051 spindle organization	3.1E-08
	GO:0022403 cell cycle phase	3.2E-08
	GO:0000279 M phase	1.3E-07
	GO:0051301 cell division	3.5E-07
	GO:0000280 nuclear division	5.5E-07
	GO:0007067 mitosis	5.5E-07
	GO:0007049 cell cycle	5.7E-07
	GO:0000087 M phase of mitotic cell cycle	6.9E-07
	GO:0007017 microtubule-based process	1.1E-06
	GO:0048285 organelle fission	1.2E-06
	GO:0000278 mitotic cell cycle	1.5E-06
	GO:0022402 cell cycle process	4.2E-06
	GO:0000226 microtubule cytoskeleton organization	5.9E-06
	GO:0007052 mitotic spindle organization	1.3E-04
	GO:0051297 centrosome organization	6.2E-04
	GO:0031023 microtubule organizing center organization	1.0E-03
	GO:0051225 spindle assembly	7.5E-03
	GO:0007010 cytoskeleton organization	1.1E-02
Steroid metabolism	GO:0016126 sterol biosynthetic process	8.7E-08
	GO:0008202 steroid metabolic process	1.3E-07
	GO:0006694 steroid biosynthetic process	4.6E-07
	GO:0016125 sterol metabolic process	2.8E-06
	GO:0008203 cholesterol metabolic process	2.0E-04
	GO:0006695 cholesterol biosynthetic process	2.6E-04
	GO:0008610 lipid biosynthetic process	3.3E-04
	GO:0016126 sterol biosynthetic process	8.7E-08
Chromosome organization/ DNA packaging	GO:0034728 nucleosome organization	1.0E-03
	GO:0006334 nucleosome assembly	2.4E-03
	GO:0031497 chromatin assembly	4.0E-03
	GO:0051276 chromosome organization	5.1E-03
	GO:0065004 protein-DNA complex assembly	6.2E-03

	GO:0006323 DNA packaging	8.1E-03
	GO:0006333 chromatin assembly or disassembly	3.7E-02
Tetrapyrrole, cofactor, and pigment metabolism	GO:0033013 tetrapyrrole metabolic process	1.6E-03
	GO:0006778 porphyrin metabolic process	1.6E-03
	GO:0051186 cofactor metabolic process	7.4E-03
	GO:0042440 pigment metabolic process	4.5E-02
Iron homeostasis	GO:0055072 iron ion homeostasis	8.1E-04
	GO:0048878 chemical homeostasis	2.1E-02
	GO:0006879 cellular iron ion homeostasis	2.6E-02
	GO:0055066 di-, tri-valent inorganic cation homeostasis	2.9E-02
Heme binding/ Oxygen binding and transport	GO:0005506 iron ion binding	1.7E-03
	GO:0020037 heme binding	6.8E-03
	GO:0046906 tetrapyrrole binding	1.2E-02
	GO:0019825 oxygen binding	1.3E-02
	GO:0015671 oxygen transport	3.6E-02
	GO:0005344 oxygen transporter activity	3.6E-02
Immune response	GO:0050778 positive regulation of immune response	2.3E-03
	GO:0002253 activation of immune response	3.6E-03
	GO:0002252 immune effector process	1.4E-02
	GO:0006956 complement activation	2.2E-02
	GO:0006959 humoral immune response	2.4E-02
	GO:0002541 activation of plasma proteins involved in acute inflammatory response	2.4E-02
	GO:0048584 positive regulation of response to stimulus	3.9E-02
Isoprenoid, vitamin, and hormone metabolism	GO:0006720 isoprenoid metabolic process	4.6E-03
	GO:0006766 vitamin metabolic process	9.0E-03
	GO:0006721 terpenoid metabolic process	1.5E-02
	GO:0034754 cellular hormone metabolic process	3.4E-02
	GO:0019748 secondary metabolic process	3.4E-02
Fatty acid metabolism	GO:0006631 fatty acid metabolic process	9.9E-03
	GO:0006633 fatty acid biosynthetic process	1.7E-02
	GO:0016053 organic acid biosynthetic process	2.0E-02
	GO:0046394 carboxylic acid biosynthetic process	2.0E-02
Exodeoxyribonuclease activity	GO:0004529 exodeoxyribonuclease activity	1.4E-02
	GO:0016895 exodeoxyribonuclease activity, producing 5'-phosphomonoesters	1.4E-02
	GO:0004536 deoxyribonuclease activity	2.6E-02
DNA binding/ Mismatch repair	GO:0032135 DNA insertion or deletion binding	8.6E-03
	GO:0032405 MutLalpha complex binding	8.6E-03
	GO:0003690 double-stranded DNA binding	1.3E-02
	GO:0032404 mismatch repair complex binding	1.4E-02

	GO:0030983 mismatched DNA binding	2.8E-02
	GO:0051052 regulation of DNA metabolic process	3.7E-02
	GO:0006298 mismatch repair	4.5E-02
Nucleotide biosynthesis	GO:0044271 nitrogen compound biosynthetic process	2.9E-03
	GO:0034654 nucleobase, nucleoside, nucleotide and nucleic acid biosynthetic process	4.7E-02
	GO:0034404 nucleobase, nucleoside and nucleotide biosynthetic process	4.7E-02
DNA damage response	GO:0042770 DNA damage response, signal transduction	1.1E-02
	GO:0000077 DNA damage checkpoint	2.7E-02
	GO:0031570 DNA integrity checkpoint	3.7E-02
Male sexual development	GO:0008584 male gonad development	1.1E-02
	GO:0046546 development of primary male sexual characteristics	3.0E-02
	GO:0046661 male sex differentiation	4.5E-02
	GO:0008406 gonad development	4.6E-02
Adaptive immune response	GO:0002252 immune effector process	1.4E-02
	GO:0002449 lymphocyte mediated immunity	3.7E-02
	GO:0002250 adaptive immune response	4.6E-02
	GO:0002460 adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	4.6E-02
ATP/ Nucleoside binding	GO:0005524 ATP binding	2.8E-02
	GO:0032559 adenyl ribonucleotide binding	3.2E-02
	GO:0001882 nucleoside binding	3.3E-02
	GO:0000166 nucleotide binding	4.2E-02
	GO:0030554 adenyl nucleotide binding	4.8E-02
	GO:0032555 purine ribonucleotide binding	4.9E-02
	GO:0032553 ribonucleotide binding	4.9E-02
SNK parr vs. SNK early-migrating smolts—DE genes correlated with ATPase		
Steroid metabolism	GO:0008202 steroid metabolic process	4.9E-06
	GO:0016126 sterol biosynthetic process	8.9E-06
	GO:0006694 steroid biosynthetic process	1.8E-05
	GO:0016125 sterol metabolic process	6.0E-05
	GO:0008203 cholesterol metabolic process	1.3E-03
	GO:0008610 lipid biosynthetic process	3.4E-03
	GO:0006695 cholesterol biosynthetic process	5.1E-03
DNA repair	GO:0006259 DNA metabolic process	1.3E-07
	GO:0006281 DNA repair	4.8E-04
	GO:0006974 response to DNA damage stimulus	1.5E-03
	GO:0033554 cellular response to stress	1.8E-02

Cell cycle/ Mitosis/ Centrosome and spindle organization	GO:0051297 centrosome organization	5.0E-05
	GO:0031023 microtubule organizing center organization	8.4E-05
	GO:0007017 microtubule-based process	2.5E-04
	GO:0000226 microtubule cytoskeleton organization	7.9E-04
	GO:0007051 spindle organization	9.1E-04
	GO:0051225 spindle assembly	1.6E-03
	GO:0022403 cell cycle phase	1.7E-03
	GO:0007049 cell cycle	4.5E-03
	GO:0000279 M phase	5.0E-03
	GO:0000280 nuclear division	5.2E-03
	GO:0007067 mitosis	5.2E-03
	GO:0000087 M phase of mitotic cell cycle	5.6E-03
	GO:0048285 organelle fission	7.0E-03
	GO:0000278 mitotic cell cycle	1.2E-02
	GO:0022402 cell cycle process	1.3E-02
	GO:0007010 cytoskeleton organization	4.3E-02
Tetrapyrrole, heterocycle, and pigment metabolism	GO:0033013 tetrapyrrole metabolic process	1.6E-03
	GO:0006778 porphyrin metabolic process	1.6E-03
	GO:0018130 heterocycle biosynthetic process	2.7E-03
	GO:0042440 pigment metabolic process	7.6E-03
	GO:0046148 pigment biosynthetic process	3.5E-02
	GO:0051186 cofactor metabolic process	3.8E-02
Heme binding/ Oxygen binding and transport	GO:0019825 oxygen binding	2.1E-03
	GO:0020037 heme binding	2.2E-03
	GO:0046906 tetrapyrrole binding	3.6E-03
	GO:0015671 oxygen transport	1.3E-02
	GO:0005344 oxygen transporter activity	1.4E-02
	GO:0015669 gas transport	2.9E-02
Negative regulation of transcription	GO:0045892 negative regulation of transcription, DNA-dependent	7.1E-03
	GO:0051253 negative regulation of RNA metabolic process	8.3E-03
	GO:0010629 negative regulation of gene expression	1.1E-02
	GO:0016481 negative regulation of transcription	1.5E-02
	GO:0000122 negative regulation of transcription from RNA polymerase II promoter	2.7E-02
	GO:0045934 negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	3.2E-02
	GO:0051172 negative regulation of nitrogen compound metabolic process	3.5E-02
SNK early-migrating smolts vs. SNK late-migrating smolts—all DE genes		
Cell cycle / Mitosis	GO:0000279 M phase	7.8E-05

	GO:0000280 nuclear division	3.5E-04
	GO:0007067 mitosis	3.5E-04
	GO:0000087 M phase of mitotic cell cycle	3.8E-04
	GO:0048285 organelle fission	4.6E-04
	GO:0022403 cell cycle phase	5.1E-04
	GO:0000278 mitotic cell cycle	5.3E-04
	GO:0022402 cell cycle process	4.8E-03
	GO:0051301 cell division	6.3E-03
	GO:0007017 microtubule-based process	8.7E-03
	GO:0007049 cell cycle	1.1E-02
	GO:0003777 microtubule motor activity	4.0E-02
Mitotic spindle organization	GO:0007052 mitotic spindle organization	7.3E-03
	GO:0007017 microtubule-based process	8.7E-03
	GO:0007051 spindle organization	4.0E-02
DNA packaging / Chromatin assembly	GO:0006323 DNA packaging	8.8E-03
	GO:0006334 nucleosome assembly	2.2E-02
	GO:0031497 chromatin assembly	2.8E-02
	GO:0065004 protein-DNA complex assembly	3.3E-02
	GO:0034728 nucleosome organization	3.3E-02

Appendix 2. Gene ontology functional categories enriched in the lists of genes differentially expressed between Clearwater and Snake River natural juvenile fall Chinook salmon. GO terms that make up the clusters are shown along with their corresponding p-values from the overrepresentation analysis.

Cluster description	GO term	*P*
	CLW vs. SNK parr—all DE genes	
Immune response	GO:0006952 defense response	2.2E-04
	GO:0006956 complement activation	2.4E-04
	GO:0002253 activation of immune response	2.7E-04
	GO:0002541 activation of plasma proteins involved in acute inflammatory response	2.8E-04
	GO:0002526 acute inflammatory response	4.8E-04
	GO:0006959 humoral immune response	1.3E-03
	GO:0009611 response to wounding	1.4E-03
	GO:0050778 positive regulation of immune response	1.5E-03
	GO:0051605 protein maturation by peptide bond cleavage	2.0E-03
	GO:0002252 immune effector process	2.2E-03
	GO:0006954 inflammatory response	2.8E-03
	GO:0016485 protein processing	5.9E-03
	GO:0051604 protein maturation	8.9E-03
	GO:0048584 positive regulation of response to stimulus	1.0E-02
	GO:0002684 positive regulation of immune system process	1.3E-02
	GO:0006955 immune response	2.0E-02
	GO:0006958 complement activation, classical pathway	2.5E-02
	GO:0002455 humoral immune response mediated by circulating immunoglobulin	2.7E-02
Vitamin B_6 binding	GO:0019842 vitamin binding	1.7E-03
	GO:0070279 vitamin B6 binding	7.4E-03
	GO:0030170 pyridoxal phosphate binding	7.4E-03
Negative regulation of apoptosis	GO:0006916 anti-apoptosis	9.5E-03
	GO:0043066 negative regulation of apoptosis	2.4E-02
	GO:0043069 negative regulation of programmed cell death	2.5E-02
	GO:0060548 negative regulation of cell death	2.6E-02
	CLW vs. SNK early-migrating smolts—all DE genes	
Cell cycle/ Mitosis/ Spindle organization	GO:0022403 cell cycle phase	6.7E-11
	GO:0007049 cell cycle	7.8E-11
	GO:0000279 M phase	1.7E-10
	GO:0022402 cell cycle process	3.4E-09
	GO:0007017 microtubule-based process	3.4E-09

	GO:0007051 spindle organization	4.3E-08
	GO:0007067 mitosis	4.5E-08
	GO:0000280 nuclear division	4.5E-08
	GO:0000087 M phase of mitotic cell cycle	5.9E-08
	GO:0048285 organelle fission	1.1E-07
	GO:0000278 mitotic cell cycle	2.6E-07
	GO:0000226 microtubule cytoskeleton organization	2.9E-07
	GO:0051301 cell division	2.9E-05
	GO:0007010 cytoskeleton organization	6.9E-05
	GO:0007052 mitotic spindle organization	1.5E-04
DNA repair	GO:0006259 DNA metabolic process	9.4E-10
	GO:0006281 DNA repair	1.6E-04
	GO:0006974 response to DNA damage stimulus	5.6E-04
	GO:0033554 cellular response to stress	1.4E-02
Steroid and	GO:0006694 steroid biosynthetic process	4.8E-06
isoprenoid	GO:0008202 steroid metabolic process	5.0E-06
metabolism	GO:0016126 sterol biosynthetic process	2.1E-05
	GO:0016125 sterol metabolic process	1.3E-04
	GO:0008203 cholesterol metabolic process	2.5E-04
	GO:0006695 cholesterol biosynthetic process	3.0E-04
	GO:0006720 isoprenoid metabolic process	8.5E-04
	GO:0008299 isoprenoid biosynthetic process	1.3E-03
	GO:0008610 lipid biosynthetic process	3.4E-03
DNA packaging/	GO:0034728 nucleosome organization	2.1E-05
Chromosome	GO:0006334 nucleosome assembly	3.9E-05
organization	GO:0006323 DNA packaging	6.9E-05
	GO:0031497 chromatin assembly	8.7E-05
	GO:0065004 protein-DNA complex assembly	1.7E-04
	GO:0051276 chromosome organization	2.4E-04
	GO:0006333 chromatin assembly or disassembly	2.7E-03
	GO:0034621 cellular macromolecular complex subunit organization	4.1E-03
	GO:0034622 cellular macromolecular complex assembly	7.7E-03
	GO:0006325 chromatin organization	4.8E-02
DNA unwinding	GO:0006261 DNA-dependent DNA replication	3.6E-03
for replication	GO:0006268 DNA unwinding during replication	6.2E-03
	GO:0032508 DNA duplex unwinding	1.3E-02
	GO:0032392 DNA geometric change	1.3E-02
Centrosome	GO:0051297 centrosome organization	6.0E-03
organization	GO:0031023 microtubule organizing center organization	8.8E-03
	GO:0007098 centrosome cycle	4.8E-02

DNA mismatch repair	GO:0032135 DNA insertion or deletion binding	9.0E-03
	GO:0032405 MutLalpha complex binding	9.0E-03
	GO:0032404 mismatch repair complex binding	1.5E-02
	GO:0030983 mismatched DNA binding	2.9E-02
	GO:0051052 regulation of DNA metabolic process	4.2E-02
	GO:0006298 mismatch repair	4.8E-02
Vitamin metabolism / pyridine nucleotide biosynthesis	GO:0006766 vitamin metabolic process	1.0E-02
	GO:0019363 pyridine nucleotide biosynthetic process	2.9E-02
	GO:0009110 vitamin biosynthetic process	3.3E-02
	GO:0019748 secondary metabolic process	3.9E-02
CLW vs. SNK early-migrating smolts—DE genes correlated with ATPase		
DNA repair	GO:0006259 DNA metabolic process	1.3E-08
	GO:0006281 DNA repair	3.1E-04
	GO:0006974 response to DNA damage stimulus	8.0E-04
	GO:0033554 cellular response to stress	8.4E-03
Steroid metabolism	GO:0008202 steroid metabolic process	5.7E-07
	GO:0016125 sterol metabolic process	1.4E-05
	GO:0006694 steroid biosynthetic process	4.2E-05
	GO:0016126 sterol biosynthetic process	5.1E-05
	GO:0008203 cholesterol metabolic process	5.4E-05
	GO:0006695 cholesterol biosynthetic process	2.9E-03
	GO:0008610 lipid biosynthetic process	7.8E-03
Cell cycle/ Mitosis/ Spindle and centrosome organization	GO:0022403 cell cycle phase	6.1E-05
	GO:0022402 cell cycle process	6.8E-04
	GO:0007017 microtubule-based process	9.2E-04
	GO:0007049 cell cycle	9.6E-04
	GO:0000279 M phase	1.0E-03
	GO:0000226 microtubule cytoskeleton organization	1.1E-03
	GO:0007059 chromosome segregation	1.8E-03
	GO:0007051 spindle organization	3.4E-03
	GO:0051297 centrosome organization	4.8E-03
	GO:0000280 nuclear division	5.0E-03
	GO:0007067 mitosis	5.0E-03
	GO:0000087 M phase of mitotic cell cycle	5.5E-03
	GO:0031023 microtubule organizing center organization	6.4E-03
	GO:0048285 organelle fission	6.7E-03
	GO:0000278 mitotic cell cycle	7.3E-03
	GO:0007010 cytoskeleton organization	3.2E-02
	GO:0051301 cell division	4.9E-02

Negative regulation of transcription/ Steroid hormone receptor activity	GO:0003707 steroid hormone receptor activity	1.2E-03
	GO:0004879 ligand-dependent nuclear receptor activity	2.7E-03
	GO:0010629 negative regulation of gene expression	1.7E-02
	GO:0045892 negative regulation of transcription, DNA-dependent	1.8E-02
	GO:0051253 negative regulation of RNA metabolic process	2.0E-02
	GO:0016481 negative regulation of transcription	2.6E-02
	GO:0010605 negative regulation of macromolecule metabolic process	3.6E-02
	GO:0031327 negative regulation of cellular biosynthetic process	4.1E-02
	GO:0010558 negative regulation of macromolecule biosynthetic process	4.1E-02
	GO:0009890 negative regulation of biosynthetic process	4.9E-02
	GO:0045934 negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	5.0E-02
	CLW vs. SNK late-migrating smolts—all DE genes	
Cell cycle/ Mitosis/ Spindle organization	GO:0000279 M phase	1.5E-10
	GO:0022403 cell cycle phase	7.3E-10
	GO:0000280 nuclear division	1.3E-09
	GO:0007067 mitosis	1.3E-09
	GO:0000087 M phase of mitotic cell cycle	1.8E-09
	GO:0007049 cell cycle	3.5E-09
	GO:0048285 organelle fission	4.4E-09
	GO:0022402 cell cycle process	4.5E-08
	GO:0007017 microtubule-based process	1.1E-07
	GO:0000278 mitotic cell cycle	2.4E-07
	GO:0051301 cell division	1.0E-06
	GO:0007051 spindle organization	5.0E-06
	GO:0007059 chromosome segregation	6.4E-06
	GO:0000226 microtubule cytoskeleton organization	8.8E-06
	GO:0007010 cytoskeleton organization	2.6E-03
Steroid and isoprenoid metabolism	GO:0008202 steroid metabolic process	2.3E-09
	GO:0016126 sterol biosynthetic process	4.5E-08
	GO:0016125 sterol metabolic process	1.5E-07
	GO:0006694 steroid biosynthetic process	3.0E-06
	GO:0008203 cholesterol metabolic process	4.9E-06
	GO:0006695 cholesterol biosynthetic process	2.9E-05
	GO:0008299 isoprenoid biosynthetic process	8.9E-04
	GO:0006720 isoprenoid metabolic process	1.9E-03
	GO:0008610 lipid biosynthetic process	8.8E-03
DNA repair	GO:0006259 DNA metabolic process	2.6E-09

	GO:0006281 DNA repair	5.8E-04
	GO:0006974 response to DNA damage stimulus	9.8E-04
	GO:0033554 cellular response to stress	4.6E-03
DNA packaging/	GO:0006323 DNA packaging	5.8E-07
Chromatin	GO:0006334 nucleosome assembly	1.4E-05
assembly	GO:0065004 protein-DNA complex assembly	1.4E-05
	GO:0034728 nucleosome organization	1.4E-05
	GO:0031497 chromatin assembly	3.9E-05
	GO:0034621 cellular macromolecular complex subunit organization	1.8E-03
	GO:0006333 chromatin assembly or disassembly	3.0E-03
	GO:0034622 cellular macromolecular complex assembly	4.0E-03
Centrosome	GO:0000226 microtubule cytoskeleton organization	8.8E-06
organization	GO:0051297 centrosome organization	8.9E-04
	GO:0031023 microtubule organizing center organization	1.6E-03
	GO:0007098 centrosome cycle	1.7E-02
Mitotic	GO:0007059 chromosome segregation	6.4E-06
chromosome	GO:0000070 mitotic sister chromatid segregation	3.4E-03
condensation and	GO:0000819 sister chromatid segregation	3.4E-03
segregation	GO:0030261 chromosome condensation	1.3E-02
	GO:0007076 mitotic chromosome condensation	2.2E-02
Microtubule motor	GO:0003777 microtubule motor activity	4.6E-04
activity	GO:0007018 microtubule-based movement	2.0E-03
	GO:0003774 motor activity	3.4E-02
DNA mismatch	GO:0032405 MutLalpha complex binding	1.1E-03
repair binding	GO:0032404 mismatch repair complex binding	2.7E-03
	GO:0032135 DNA insertion or deletion binding	2.5E-02
DNA unwinding	GO:0006261 DNA-dependent DNA replication	2.4E-03
for replication	GO:0006268 DNA unwinding during replication	2.6E-03
	GO:0032508 DNA duplex unwinding	7.5E-03
	GO:0032392 DNA geometric change	7.5E-03
Exodeoxyribonuclease	GO:0016895 exodeoxyribonuclease activity, producing 5'-	
activity	phosphomonoesters	2.7E-03
	GO:0004529 exodeoxyribonuclease activity	2.7E-03
	GO:0008853 exodeoxyribonuclease III activity	1.3E-02
	GO:0004536 deoxyribonuclease activity	2.4E-02
NAD or NADH	GO:0048037 cofactor binding	8.9E-03
binding	GO:0051287 NAD or NADH binding	1.2E-02
	GO:0050662 coenzyme binding	1.4E-02
Coenzyme and	GO:0006575 cellular amino acid derivative metabolic process	1.8E-03
glutathione	GO:0006749 glutathione metabolic process	1.1E-02

metabolism	GO:0051186 cofactor metabolic process	3.2E-02
	GO:0006790 sulfur metabolic process	3.4E-02
	GO:0006732 coenzyme metabolic process	3.8E-02
	GO:0006518 peptide metabolic process	4.1E-02
ATP/ Nucleoside binding	GO:0005524 ATP binding	3.8E-02
	GO:0030554 adenyl nucleotide binding	3.8E-02
	GO:0032559 adenyl ribonucleotide binding	4.6E-02
	GO:0001882 nucleoside binding	4.7E-02
CLW vs. SNK late-migrating smolts—DE genes correlated with ATPase		
DNA replication and repair	GO:0006260 DNA replication	1.8E-11
	GO:0006259 DNA metabolic process	1.0E-07
	GO:0006281 DNA repair	1.5E-03
	GO:0006974 response to DNA damage stimulus	9.5E-03
Steroid metabolism	GO:0016126 sterol biosynthetic process	3.2E-06
	GO:0006694 steroid biosynthetic process	5.0E-06
	GO:0008202 steroid metabolic process	5.3E-06
	GO:0016125 sterol metabolic process	1.4E-04
	GO:0006695 cholesterol biosynthetic process	3.1E-03
	GO:0008203 cholesterol metabolic process	3.4E-03
	GO:0008610 lipid biosynthetic process	9.2E-03
Protein polymerization/ Microtubule-based movement	GO:0007017 microtubule-based process	5.1E-05
	GO:0051258 protein polymerization	1.8E-03
	GO:0034622 cellular macromolecular complex assembly	8.9E-03
	GO:0007018 microtubule-based movement	1.9E-02
Cell cycle/ Mitosis/ Centrosome and spindle organization	GO:0007017 microtubule-based process	5.1E-05
	GO:0000226 microtubule cytoskeleton organization	2.4E-04
	GO:0022403 cell cycle phase	2.9E-04
	GO:0051297 centrosome organization	3.8E-04
	GO:0031023 microtubule organizing center organization	5.8E-04
	GO:0007049 cell cycle	1.3E-03
	GO:0022402 cell cycle process	2.5E-03
	GO:0007051 spindle organization	3.7E-03
	GO:0000279 M phase	4.2E-03
	GO:0000280 nuclear division	5.9E-03
	GO:0007067 mitosis	5.9E-03
	GO:0000087 M phase of mitotic cell cycle	6.4E-03
	GO:0048285 organelle fission	7.7E-03
	GO:0000278 mitotic cell cycle	8.8E-03
	GO:0007098 centrosome cycle	1.2E-02

	GO:0007059 chromosome segregation	1.3E-02
	GO:0007010 cytoskeleton organization	1.5E-02
	GO:0051301 cell division	2.1E-02
Chromatin/	GO:0034728 nucleosome organization	2.6E-03
Nucleosome	GO:0034621 cellular macromolecular complex subunit organization	6.1E-03
assembly	GO:0034622 cellular macromolecular complex assembly	8.9E-03
	GO:0006334 nucleosome assembly	1.1E-02
	GO:0031497 chromatin assembly	1.5E-02
	GO:0065004 protein-DNA complex assembly	2.0E-02