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[Article]

Spawning by Female Chinook Salmon Can Be Detected by Electromyogram Telemetry

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Abstract.—New methods to detect spawning of anadromous salmonids in their natural environment are needed to improve understanding of breeding behavior patterns, natural selection on reproductive traits (e.g., spawn timing), and interactions between artificially propagated and wild fish. We implanted maturing female Chinook salmon *Oncorhynchus tshawytscha* with coded electromyogram (CEMG) transmitters and continuously recorded spawning activity to develop an algorithm capable of accurately detecting spawning events from CEMG data. Marked increases in female digging frequencies immediately after spawning (cover digging) strongly correlated with CEMG values. The algorithm detected averages of 65% and 86% of the actual spawning events in 2003 and 2004, respectively. The algorithm accurately detected zero spawning events for the two female salmon that did not spawn. The presence of CEMG transmitters did not affect the digging frequency, number of nests constructed, or the reproductive life span of implanted fish. However, the CEMG tagging procedure or the presence of tags significantly increased egg retention. Pedigree analyses of DNA confirmed that females implanted with CEMG transmitters exhibited significantly lower individual reproductive success relative to that of nontagged females (73% and 66% reduction in two separate experiments). Subsequent research in adult steelhead *O. mykiss* has indicated that alternative implantation techniques hold promise for reducing the effects of the tags on reproductive success. We suggest that remote monitoring of salmonid spawning behavior is now possible with CEMG technology and should be tested in natural habitats.

New methods to detect spawning of anadromous salmonids in their natural environment are needed to improve understanding of breeding behavior, natural selection on reproductive traits, and interactions between artificially propagated and wild fish. Currently, salmonid spawning events can only be detected visually, which is often hindered by limited access to spawning areas, high turbidity, deep water, or high velocities in streams. Moreover, anadromous salmonids spawn at least as frequently at night as during the day (Berejikian et al. 2005; McMichael et al. 2005; Rubin et al. 2005), and the act of each spawning event lasts just several seconds (Briggs 1953; Burgner 1991; Berejikian et al. 1997). Throughout a spawning period of several days, females construct multiple nests (collectively called a "redd"). Once the female has excavated a suitable nest depression, the male and female align side by side, their backs arched and mouths wide open as they deposit their gametes (spawn). Females participate in fewer than a dozen

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spawning events during their reproductive life span (Berejikian et al. 2000; de Gaudemar et al. 2000). Consequently, a female Pacific salmon *Oncorhynchus* spp. may spend a total of only about 1–2 min engaged in the act of egg deposition (i.e., spawning) during its life span.

Until recently, estimates of salmon reproductive fitness required behavioral assessments that included direct observation of spawning events (e.g., Fleming and Gross 1994). With the recent development and increasing popularity of DNA-based pedigree analyses, the reproductive fitness of Pacific salmon can be measured more directly (Wilson and Ferguson 2002). However, quantifying natural selection on reproductive traits requires partitioning and evaluating each of the proximate causes of reproductive fitness, which include survival to spawning, reproductive behavior, gamete quality, and offspring survival. Estimates of natural selection on spawning frequency and timing of individual fish would be improved if data from DNA pedigree analyses were combined with estimates of spawning frequency and timing. For example, adult salmon may experience selective mortality after reaching the spawning grounds, resulting in partial or complete failure to spawn (Quinn and Kinnison 1999). Spawn timing affects emergence timing and may consequently affect offspring growth and survival (Einum and Fleming 2000). Observing or remotely detecting actual spawning events in natural streams would replace less precise surrogate estimates of spawn timing, such as migration timing (Seamons et al. 2004).

Pacific salmon exhibit stereotypical nest digging and courtship behaviors involving muscle activity that differs from that occurring during basal swimming. For example, females' digging motions performed to excavate nests and cover deposited eggs involve strong, rapid trunk muscle undulations (more than 10 rapid body flexures may be involved in a single dig). The frequency of female digging activity is fairly constant during the hour immediately preceding spawning (about 0.3 digs/min) but increases dramatically during the first 5 min after spawning (about 6.0 digs/min) as the female covers eggs in the nest pocket to prevent them from being washed away or preyed upon (Berejikian et al. 1997, 2000). Near the time of spawning, variability in digging frequencies among females is relatively low, and nest-covering digs occur with high predictability, even when spawning fish densities and consequent aggressive interactions are high (Berejikian et al. 1997).

The recent development of electromyogram (EMG) transmitters that read electrical pulses across muscle tissue and transmit the data to fixed or mobile radio receivers provides the opportunity to detect changes in muscle activity associated with swimming and behavioral activities, such as spawning. The EMG technology has been applied to work on fish energetics, migration, fishway passage, aquaculture, and reproductive ecology (reviewed by Cooke et al. 2004). Work on largemouth bass *Micropterus salmoides* indicates that egg deposition and parental care activities are associated with elevated EMG values (Cooke et al. 2001). Some studies on salmonids have suggested that patterns in EMG output are useful in detecting reproductive activity (Kaseloo et al. 1996; Weatherly et al. 1996). Combining behavioral observation and EMG monitoring has also improved understanding of the energetic costs associated with various reproductive behaviors in sockeye salmon *O. nerka* (Healey et al. 2003).

This study was conducted to develop quantified relationships between values transmitted from EMG tags and female digging behavior. We hypothesized that distinct and common patterns in the EMG values would occur just before, during, and just after spawning and would provide a sufficiently distinct signal to develop an algorithm capable of accurately detecting spawning events when applied to all females in the population. Two major assumptions when using EMG transmitters are that the transmitters (1) do not interfere with normal reproductive behavior and (2) do not reduce the reproductive success of tagged fish (Cooke et al. 2001). Salmon implanted with EMG transmitters have been observed to spawn (Healey et al. 2003) but may suffer water-hardening of eggs, which precludes fertilization (Cooke et al. 2004). Therefore, we tested for transmitter effects on reproductive behavior and adult-to-fry reproductive success by comparing EMG-tagged and nontagged Chinook salmon *O. tshawytscha* with a combination of 24-h behavioral observations and DNA microsatellite pedigree analyses.

Methods

Study population and location.—Maturing fall Chinook salmon were obtained in September 2003 and 2004 from the University of Washington's Big Beef Creek Research Station located at the Big Beef Creek estuary in Hood Canal (Kitsap County, Washington). Adults were transported to the National Oceanic and Atmospheric Administration–Fisheries Manchester Research Station (MRS), Manchester, Washington, on 24 September 2003 (8 females and 8 males) and on 13 September 2004 (22 females and 10 males).

The study was conducted in a 40-m-long × 6-m-wide spawning channel located at the MRS. In 2003, the channel (described in Berejikian et al. 2000) was

divided lengthwise into two sections, and fish had access to 50% of the channel (120 m^2 of available spawning area; flow = 3,842 L/min). In 2004, the channel was configured to create a meandering flow pattern (described in Berejikian et al. 2003), and fish had access to the entire channel (240 m^2 of available spawning area; flow = 4,800 L/min).

Transmitter implantation.—In 2003, coded EMG (CEMG) radio transmitters (Lotek Wireless, Inc., Newmarket, Ontario) were implanted into four of the eight females and four of the eight males. In 2004, 14 of the 22 females and none of the 10 males received CEMG transmitters. The CEMG transmitters detect the voltage difference (potential) between electrodes in the muscles of the fish and converts it into a coded signal, which represents the average CEMG value (range = 0–50) over a user-defined time interval set during manufacturing. Higher values indicate higher muscle activity.

In 2003, all eight females had ovulated and all males were spermiating, indicating reproductive maturity at the time of tagging. In 2004, we attempted to include an equal mix of ovulated and nonovulated females in the study by collecting adults 10 d earlier in the calendar year than in 2003. Eight of the 14 tagged (57%) and 4 of the 8 nontagged (50%) females had not ovulated at the time of implantation and stocking into the channel in 2004.

The CEMG transmitters were implanted following methods similar to those of Hinch et al. (1996) and Geist et al. (2003). The procedure was the same in both years and was done by a single experienced surgeon (R.S.B.). The fish were anesthetized with tricaine methanesulfonate (MS-222; 80–100 mg/L), weighed, measured, and placed on a tagging cradle. A flow of oxygenated water containing a 40-mg/L dose of anesthesia irrigated the gills throughout the procedure. A 2–4-cm incision was made in the belly, anterior to the pelvic girdle. Two polyvinyl-coated steel-wire electrodes with solid gold tips (7 mm long, 1 mm in diameter) were secured in the red musculature just under the surface of the skin beneath the lateral line. The electrodes were inserted on the left side of the fish, and the antenna extended posteriorly through the body wall. The tag itself was placed in the body cavity. The incision was closed with three or four 2–0 silk sutures, and triple antibiotic ointment was applied to the surface of the closed incision to prevent infection. Each fish was also fitted with a 3-cm-diameter, individually numbered Petersen disc tag in the dorsal musculature for visual identification. In 2004, the suture line was dried with forced warm air and cyanoacrylate adhesive was applied to minimize suture failure and leaking of body fluids.

In 2003, each of the eight transmitters (5 cm long, 1.5 cm in diameter, 10 g in air) emitted a pulse every 3.2 s on a different radio frequency to microprocessor-controlled receivers (Lotek Wireless, Inc.; Model SRX 400). In 2004, 12 of the 14 tags (6.1 cm long, 1.1 cm in diameter, 11.9 g in air) were uniquely coded, and groups of three coded transmitters operated on each of four unique frequencies (i.e., three codes per frequency). The transmitters emitted a pulse every 3.0 s, and the three tags within a frequency were activated at approximately 1.0-s intervals to stagger the transmission of signals and avoid simultaneous transmission by multiple tags on the same frequency. Two transmitters used in 2004 transmitted a pulse every 4.0 s, and each transmitted on its own frequency as in 2003. A small fin tissue sample was taken from every adult and preserved in 100% nondenatured alcohol to supply DNA needed for the pedigree analyses (below).

Recording of CEMG.—In 2003, three receivers were used to record CEMG values from implanted female salmon. Two receivers were each set to continuously record CEMG values from a separate, single corresponding CEMG transmitter with the goal of obtaining continuous CEMG data associated with prespawning, spawning, and postspawning activity for replicate spawning events for each female. Hereafter, we refer to this as "focal sampling." The third receiver was configured to "scan" for transmissions from all four CEMG-tagged females. In this configuration, values from an individual female were transmitted to the receiver every 3.2 s for a total of 48 s. The data were then automatically downloaded (taking ~16 s) to the receiver's internal memory, and the receiver automatically switched to record data from the next female. The cycle was repeated continuously from the time the females were introduced to the stream channel until the death of the last female.

In 2004, each of four receivers was dedicated to monitoring one of four separate frequencies. Three transmitters emitted individually coded signals at each of the four frequencies (three tags per frequency; n = 12 females). Two additional receivers recorded data from the two tags that transmitted on unique frequencies. Data were collected continuously throughout the duration of the study. Minor daily interruptions in EMG data collection occurred during the time data from the receivers were being downloaded (30–45 min/d).

Spawning behavior.—The methods for monitoring spawning behavior of Chinook salmon were the same in 2003 and 2004. Four floodlights positioned horizontally 4.5 m above the channel provided enough light (mean ± SD = 4.8 ± 0.9 lx) for nighttime video recording. Four cameras (Watec Company Ltd.,

Taiwan; model 902HS; 00015-lx sensitivity at F-stop 1.4; 26-mm lens) were positioned approximately 3.8 m above the stream channel so that each camera captured images from 25% of the channel. Video images were recorded on time-lapse recorders (Gyyr, Inc., Anaheim, California; Model TLC 2124-GY) at approximately 5 frames/s between 1700 and 0730 hours. The video recorders and CEMG receivers were synchronized daily. All nocturnal spawning events captured on videotapes were observed and documented when the videotapes were reviewed between 0800 and 1200 hours the next day.

During daylight, three remote underwater cameras were used to record the behavior of females before, during, and after individual spawning events. Active construction of a nest by a female while being attended by a courting male indicated that spawning was imminent. An underwater camera was positioned near nesting females and the video was recorded in real time (JVC Company of America, Wayne, New Jersey; Model HR S-7300-U). We were able to document the time, location, and female participant for every spawning event that occurred during the study with a combination of direct observation, daytime underwater video, and nighttime overhead video.

For each female, we reviewed the day- and nighttime video recordings and entered the nest construction digs and nest-covering digs alongside the actual time (to the nearest second) that the behavior occurred. Digs were recorded for 60 min before and 30 min after each documented spawning event. Each individual dig was entered into the spreadsheet alongside the corresponding CEMG value.

Within 12 h of death, females were removed from the stream channel and were necropsied. The number of eggs retained was estimated by weighing three aliquots of 25 eggs to determine the weight per egg and dividing the weight of the entire retained mass of ovulated eggs by the average egg weight.

Tag effects on individual reproductive success.—Emergent fry were captured in downstream traps and removed by seining and electroshocking. Subsamples of 999 fry in 2003 and 1,056 fry in 2004 were analyzed to determine their parentage. Genomic DNA was isolated from the adult and fry fin tissue samples (adult samples were collected at the time of tagging) with Wizard genomic DNA purification kits (Promega Corporation, Madison, Wisconsin) following the manufacturer's instructions. The isolated genomic DNA was used in polymerase chain reactions (PCRs) to amplify seven microsatellite loci—*Ogo4* (Olsen et al. 1998), *Oki23mmbl* (A. Spidle, U.S. Geological Survey; GenBank accession number AF272822), *Ots2M* (Greig et al. 2003), *Ots3* (Banks et al. 1999), *Ots104* (Nelson and Beacham 1999), *Ots519* (Naish and Park 2002), and *Ssa408* (Cairney et al. 2000). The PCRs were carried out in 10-μL reactions containing 10 mM of tris-HCl (pH = 9.0), 1.75 mM of $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, 0.20–0.50 μM of fluorescently labeled forward and reverse primers, 0.6–0.9 U of *Taq* polymerase, 0.09 mg of bovine serum albumin, and about 100 ng of genomic DNA. Thermal cycler conditions consisted of an initial denaturation step of 95°C for 2 min, followed by 33 cycles of 94°C for 40 s, annealing temperature for 40 s, 72°C for 40 s, and a final step of 54°C for 40 min. Annealing temperature of 47°C was used for *Ots3* and *Ots104*, and 54°C was used for all other loci.

The resulting PCR products were subjected to a fragment analysis with a 310 or 3100 capillary electrophoresis system (Applied Biosystems, Foster City, California). To determine every individual's genotype for each locus, results of the electrophoretic runs were analyzed with GeneScan and Genotyper software (Applied Biosystems).

Genotypes of the fry were compared with those of the adults by use of CERVUS (Marshall et al. 1998) to assign each fry to a unique parental pair. We initially used an exclusionary approach, but if exclusion failed to assign a fry to a unique parental pair, a likelihood analysis was used.

Spawning detection algorithm.—We first evaluated the characteristics of the CEMG data generated around the time of spawning to determine the accuracy with which known spawning events could be detected unambiguously from CEMG data. The period surrounding spawning showed an uncharacteristically high frequency of high CEMG values intermixed with longer periods of rest (i.e., consistently low values). We then developed an algorithm and applied it to all females in both years. The criteria for identifying a "detected" spawning event from the CEMG data were arrived at iteratively; that is, the algorithm described below was modified to both maximize the number of true detections and minimize "false detections" (where spawning had not actually occurred) and "missed detections" (where spawning had occurred, but the algorithm did not detect it). All CEMG values for a single female were first converted to a standard normal variable with a mean of 0 and a variance of 1. A moving SD of 100 consecutive standard normal values was calculated (noted as Y_j). The following variables were calculated:

$$Z_h = |(Y_j - Y_{j+1})| \text{ for } j = 1, \ldots N - 1$$

and

$$Z_{\hat{h}}; = (Z_h + Z_{h+1} + Z_{h+2} \ldots Z_{h+99}) \cdot 100^{-1}$$
$$\text{for } h = 1, \ldots N - 99.$$

The following decision rules were arrived at iteratively:

$$\text{If } Z_{\hat{h}} > Y_j \text{ and } Z_{\hat{h}} > 1.2, \text{then } X_i = 1; \text{and}$$

$$\text{if } Z_{\hat{h}} \leq Y_j \text{ or } Z_{\hat{h}} \leq 1.2, \text{then } X_i = 0.$$

A moving average of X_i was calculated as

$$X_{\hat{i}} = (X_i + X_{i+1} + X_{i+2}, \ldots X_{i+999}) \cdot 1,000^{-1}$$
$$\text{for } i = 1, \ldots N - 999.$$

A spawning event was considered "detected" when $X_{\hat{i}}$ was greater than 1.0. Because $X_{\hat{i}}$ is a moving average of 1,000 values, it will exceed 0 for a minimum of 1,000 consecutive rows of data (hereafter, a cluster) whenever $X_{\hat{i}}$ is greater than 1.0. One-thousand rows represented (1) a minimum of 58 min for the four tags used in 2003, producing a mean "effective" burst rate of 3.4 s (see Results for effective burst rate calculations), (2) 67 min for the two tags used in 2004, producing a mean effective burst rate of 4.0 s, and (3) 145 min for the 12 tags used in 2004, producing a mean effective burst rate of 8.6 s. We took the greatest value of $X_{\hat{i}}$ within a cluster as the actual time of the detected spawning.

Each detection was categorized as either accurate or false. An accurate detection was one that occurred within 1 h of an actual spawning event determined by visual observation. A false detection was one that occurred either more than 1 h from the nearest observed spawning event or between two accurately detected spawning events. Total detections were the sum of the false and accurate detections calculated for each fish individually.

Statistical analyses.—The effects of CEMG tagging (tagged or not) on longevity (time from introduction to the spawning channel until death), number of spawning events, egg retention, and individual adult-to-fry reproductive success were analyzed by two-sample *t*-tests within each year. Simple linear regression analyses were performed to determine the relationship between egg retention and reproductive success.

Results

Spawning Frequency

In 2003, we observed (directly and from video recordings) all four CEMG-tagged females and three of four nontagged females spawning at least once. One nontagged female died prematurely from an apparent wound on the belly that was evident at the time of stocking; this fish was not included in the analyses. In 2004, 12 of the 14 CEMG-tagged females and 6 of the 8 nontagged females spawned at least once. One of the two nontagged females that did not spawn was removed from the spawning channel after all of the males had perished. The female had not ovulated at the time she was removed, so she did not have the opportunity to reproduce and was not included in the statistical analyses.

Tag and Receiver Performance

The signals from transmitters that were constantly monitored by a receiver in 2003 were logged every 3.2 s. The receivers took approximately 0.25 s to download the data to memory. Thus, CEMG signals were recorded every 3.45 s, and each data point represented the average muscle activity for 92.7% of a given 3.45-s time interval.

In 2004, the receivers for the two tags configured to transmit on unique frequencies (as in 2003) took an average of 0.21 s to download the data to memory. Thus, CEMG signals were recorded every 4.21 s, and each data point represented the average muscle activity for 95.0% of a given 4.21-s time interval. The remaining 12 tags used in 2004 were configured such that three unique codes were placed on each of the four radio frequencies. The tags transmitted a pulse every 3.00 s. However, the average time between consecutive recorded data points across all 12 females was 8.56 ± 0.65 s (mean ± SD). Data loss was caused by two factors. First, a lack of proper decoding among tags on the same frequency that periodically transmitted at the same time caused approximately 15% of the pulses to return an error code and no CEMG value. Second, after a receiver logged data from the three tags, it took an additional 3.00 s for the receiver to download the data to memory and resulted in an additional loss of about 50% of the pulses. Thus, approximately 65% of the data transmitted by the tags were not stored into memory and were unavailable for analysis.

There was a clear relationship between digging frequency and CEMG values during the 90 min surrounding spawning in both years (Figure 1). In 2003, females surgically implanted with CEMG tags spawned a combined 26 times during the study. The CEMG receivers were set to record CEMG values during 15 of the 26 spawning events. The CEMG data were not collected during the other 11 spawning events because only two recorders were dedicated to focal sampling and, therefore, only two (or later three) fish could be recorded at once. The algorithm detected 9 of a possible 15 spawning events (60%; range = 20–100%) by the four females, and no spawning events were falsely detected (Table 1; Figure 2). The consistently low and declining values from one female suggest poor transmitter function or improper implan-

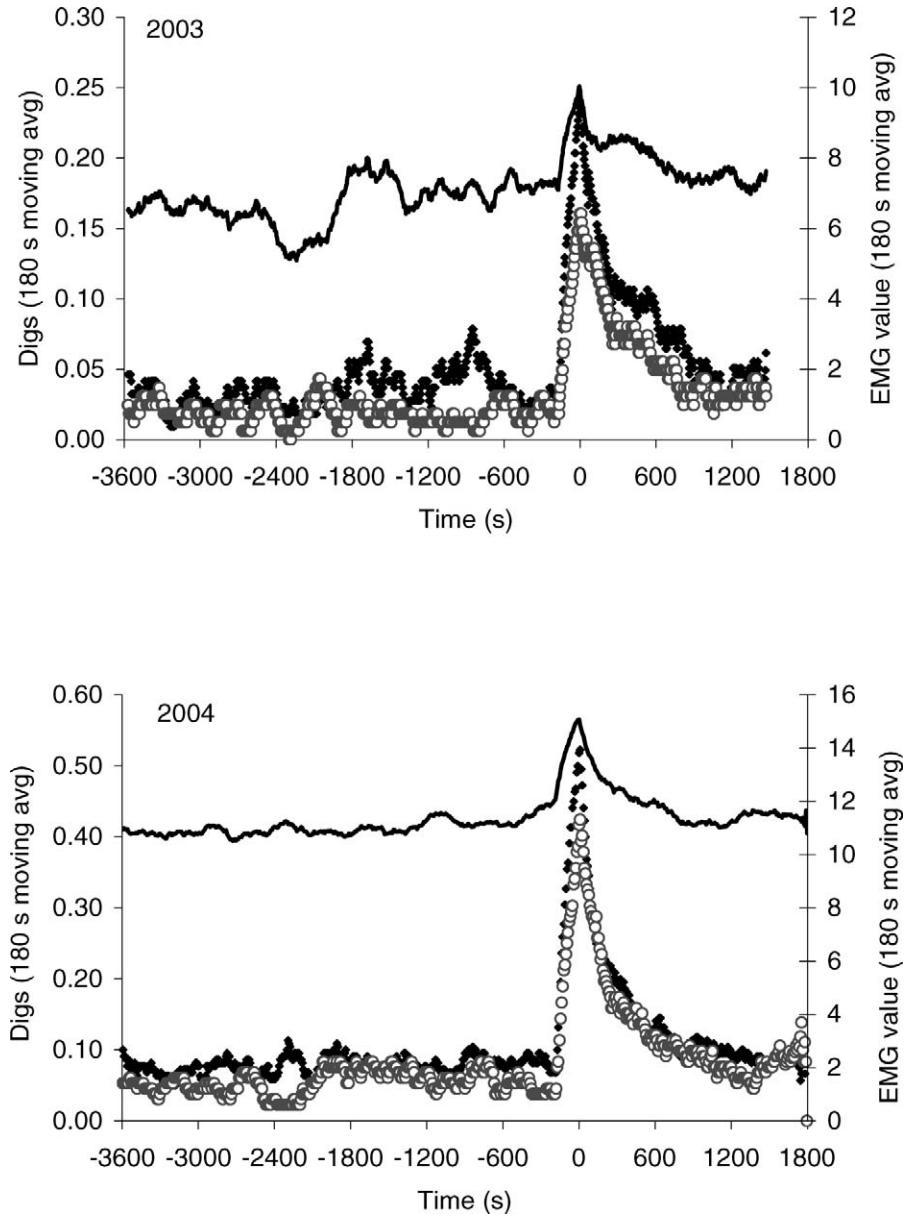

FIGURE 1.—Moving averages of female Chinook salmon digging frequency during spawning, as determined by observing coded electromyogram (CEMG)-tagged (black diamonds) and nontagged (open circles) individuals at the Manchester Research Station, Washington, in 2003 and 2004. The 180-s moving averages of CEMG values are shown by the solid line in both graphs. In 2003, digs were binned into 3.45-s intervals, which was the average pulse frequency of CEMG tags in that year. A moving average of 52 consecutive data points (representing approximately 180 s) was calculated for each fish. The data were then averaged for each 3.45-s interval across the four tagged and three nontagged females that spawned. In 2004, digs were binned into 8.56-s intervals, which was the average CEMG pulse interval across all spawning events for all females in 2004. A moving average of 21 data points (representing 180 s) was calculated for each fish. The data were then averaged across 10 tagged spawning females and 6 nontagged spawning females. To standardize, we present data from the second spawning event for each female.

TABLE 1.—Female Chinook salmon spawning events that were recorded by video or direct observation and those detected by a coded electromyogram (CEMG) algorithm Manchester Research Station. The CEMG transmitter frequency ranged from 151.000 to 151.890 MHz. Maturity status at the time of tagging was ovulated (OV), not ovulated–belly soft (NS) or not ovulated–belly firm (NF). The number of observed spawning events in 2003 during which CEMG data were recorded is shown in parentheses. The CEMG data were continuously recorded for all spawning events and for all females in 2004.

Year	Disc tag	CEMG Frequency (code)	Maturity	Observed events	Mean time interval (*s*)[a]	Accurate detections (*n*)	Accurate detections (%)	False detections (*n*)	Percent total detections
2003									
	03A	640 (9)	OV	7 (3)	3.4	3	100	0	100
	03B	560 (7)	OV	6 (5)	3.4	1	20	0	20
	03C	600 (8)	OV	6 (3)	3.4	2	67	0	67
	03D	520 (6)	NF	7 (4)	3.4	3	75	0	75
2004									
	04A	480 (8)	OV	3	8.4	0	0	0	0
	04B	400 (3)	NS	9	8.0	7	77	0	77
	04C	440 (5)	OV	5	8.9	4	80	0	80
	04D	440 (6)	NS	3	8.4	3	100	1	125
	04E	440 (4)	NF	0	—	0	—	0	—
	04F	400 (1)	NS	6	8.1	2	33	0	33
	04G	400 (2)	NF	3	7.4	3	100	4	233
	04H	890 (12)	NF	7	4.2	3	43	2	71
	04I	480 (9)	OV	6	9.3	3	50	1	67
	04J	520 (12)	NS	5	9.3	3	60	1	80
	04K	520 (10)	NS	0	—	0	—	0	—
	04L	480 (7)	NS	5	8.9	2	40	1	60
	04M	520 (11)	OV	5	8.7	2	40	1	60
	04N	000 (11)	OV	2	4.2	2	100	1	150

[a] The mean time interval is the average duration between consecutive CEMG pulses recorded by a receiver throughout the course of the study.

tation (Figure 2, fish number 03C). Missed detections from this one female accounted for four of the six overall missed detections. The detection rate for the remaining three females ranged between 67% and 100%.

In 2004, there were 59 spawning events, and the algorithm detected 34 (58%). Application of the algorithm led to detection of 12 spawning events that were not observed (i.e., false detections; Table 1). The algorithm detected at least one spawning event in 11 out of 12 females that were observed spawning (Table 1; Figure 2) and correctly detected zero spawning events in the 2 females that did not spawn at all throughout the course of the study (Table 1; Figure 2).

We added the false detections to the accurate detections and calculated a total detection rate for each female to simulate a scenario in which the algorithm would be applied in the same manner to different data sets without knowledge of the observed spawning events. By this approach, the total detection rate of spawning events in 2003 averaged 65% (95% confidence interval [CI] = 32.7–98.2%; Table 1). The total detection rate in 2004 averaged 86.3% (95% CI = 52.4–120.2%; Table 1). The correct nondetections for the two females that did not spawn perhaps suggest a greater accuracy than is reflected by the calculated mean, but there is no direct way to include these in the calculation.

Tagging Effects on Behavior and Reproductive Success

Electromyogram-tagged females spawned significantly more often than nontagged females in 2003 and spawned about equally as often as nontagged females in 2004 (Table 2). The CEMG-tagged females retained more eggs than nontagged females in both years (Table 2). Thus, problems with the high egg retention in CEMG-tagged females were caused by the tagging procedure or the tag itself, rather than by a failure to construct nests and spawn. There were no significant differences in longevity (time from release until death) between tagged and nontagged females (Table 2). The CEMG-tagged females spawned significantly, but only slightly, earlier than nontagged females in 2003, and no difference in time of first spawning was evident in 2004 (Table 2). Female egg retention accounted for a significant portion of the variability in offspring production ($r^2 = 0.45$; $P < 0.01$). Nontagged females produced more fry than did tagged females; the difference was marginally nonsignificant in 2003 and significant in 2004 (Table 2).

Discussion

We developed an analytical method for optimizing the detection of spawning events in Chinook salmon with CEMG signals. The method relied on the consistently greater frequency of higher CEMG values associated with females' cover digging immediately after spawning. High CEMG values were observed at

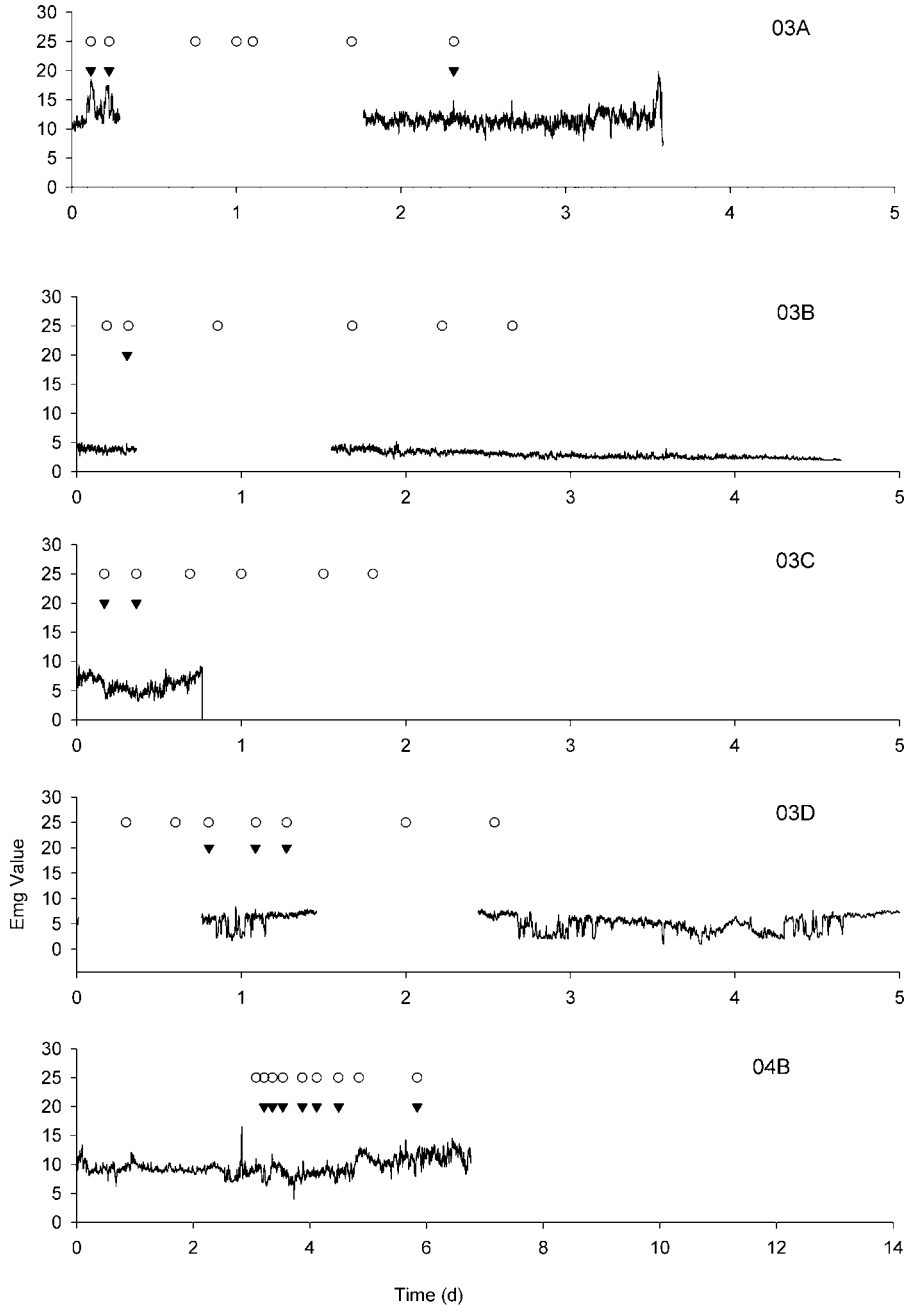

FIGURE 2.—Moving averages of coded electromyogram (CEMG) values for spawning female Chinook salmon monitored at the Manchester Research Station, Washington, in 2003 and 2004. Gaps in the CEMG values indicate periods for which no CEMG data were collected. Each circle denotes a spawning event observed either directly or from video recordings, and each triangle denotes a spawning event detected by analysis of CEMG data. The first four graphs show all data from all four females monitored in 2003 (03A–D), the second four graphs show a representative subsample of females monitored in 2004 that successfully spawned (04B, 04C, 04F, 04G), and the last two graphs show the two females monitored in 2004 that did not spawn (04E and 04K).

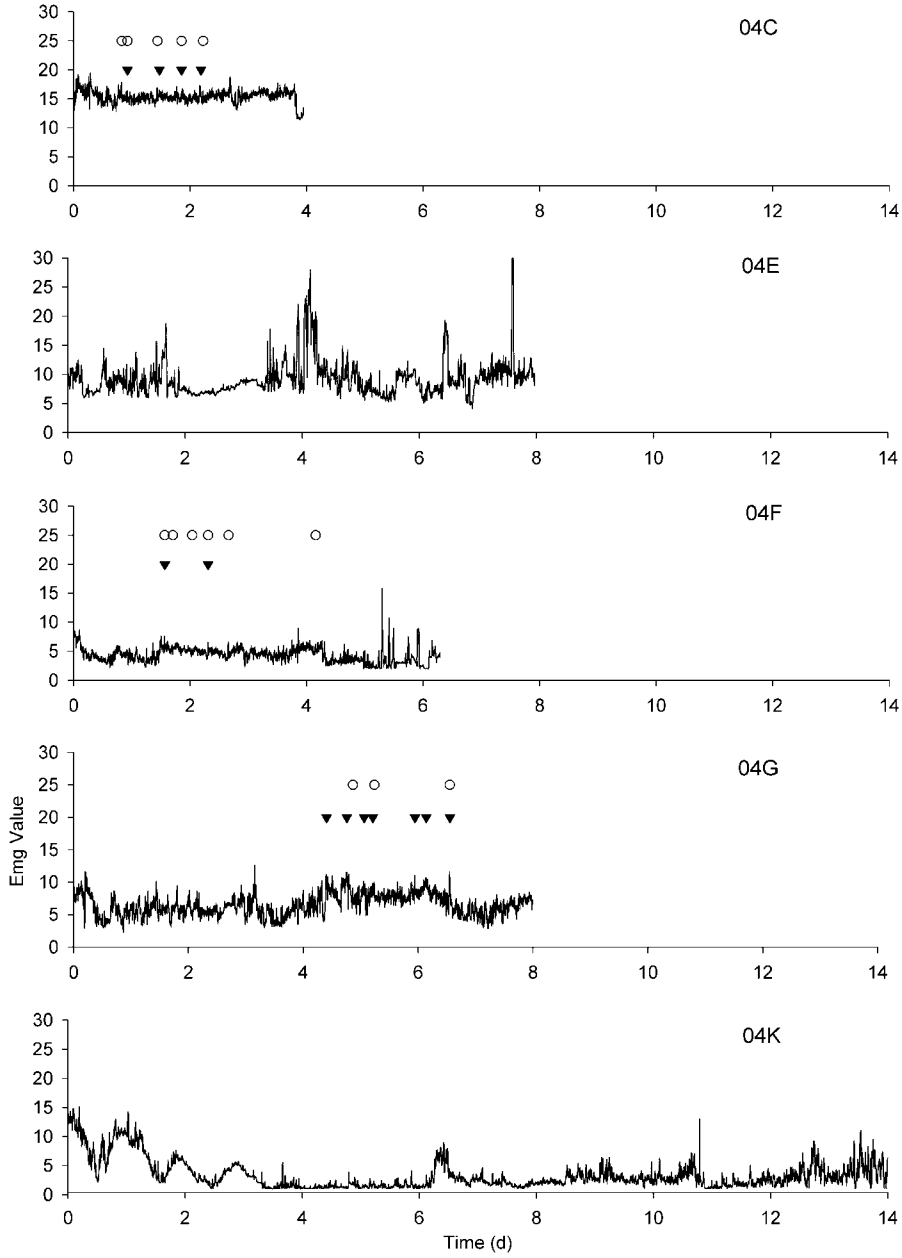

FIGURE 2.—Continued.

various other times during the reproductive life span, but they did not substantially obscure the pattern associated with digging behavior surrounding the spawning event. The accuracy of the detection method, which was applied to all fish in both years in exactly the same manner, was similar between years and among tag types, which had different effective burst rates (3.4, 4.0, and 8.6 s). However, no false detections occurred in 2003, whereas both tag types produced false detections in 2004, suggesting that either the faster burst rate in 2003 or the conditions under which the fish were spawning (i.e., lower density in 2003 than in 2004) accounted for the greater number of false detections in 2004.

This is the first study to match patterns in CEMG activity with a large number of distinct spawning

TABLE 2.—The mean, SD, test statistic (t), and significance (P; significant results in bold) of reproductive performance measures for coded electromyogram (CEMG) tagged and nontagged female Chinook salmon at the Manchester Research Station, Washington, in 2003 and 2004. Reproductive success is the number of fry produced by each female. Egg retention is the number of eggs retained in the body cavity at death. The sample sizes (n) for latency to first spawning are less than for other variables because some fish did not spawn.

	Tagged			Nontagged				
	Mean	SD	n	Mean	SD	n	t	P
2003								
Reproductive success	75.00	72.93	4	216.33	88.27	3	2.330	0.067
Egg retention	3,010	896	4	921	790	3	−3.198	**0.024**
Spawning events	6.55	0.57	4	3.33	0.57	3	−7.181	**0.001**
Latency to first spawning (d)	0.35	0.10	4	0.63	0.13	3	3.317	**0.021**
Longevity (d)	4.57	1.41	4	4.88	0.58	3	0.352	0.740
2004								
Reproductive success	25.43	26.60	14	92.00	62.34	7	3.476	**0.030**
Egg retention	3,326	1,253	14	1,311	1,240	7	−3.485	**0.002**
Spawning events	4.21	2.55	14	3.86	2.48	7	−0.305	0.763
Latency to first spawning (d)	1.05	1.24	12	2.53	3.60	6	1.496	0.154
Longevity (d)	5.91	3.60	14	7.62	4.26	7	0.970	0.344

events by numerous females that could each be verified visually. Prior studies had suggested that CEMG telemetry would be useful in monitoring spawning behavior of salmonids. Weatherly et al. (1996) tracked two male and one female lake trout *Salvelinus namaycush*. Those authors speculated that high muscle activity in one of the males during the height of spawning activity in a natural lake meant that the male had participated in spawning. Kaseloo et al. (1996) studied a single pair of lake trout in a fabricated tank and found a clear connection between increased muscle activity and a single identified spawning event. The authors noted that the complex interactions involving larger numbers of fish spawning in the wild may also result in spikes in CEMG values similar to those observed during spawning. Healey et al. (2003) found that, aside from holding position in the current, digging during the spawning period is the most energetically expensive behavior (based on elevated CEMG values); however, they also found that aggressive charges and chases represent a significant portion of energy expenditure during reproduction. In our study, aggression associated with contests for nesting territories and nest guarding after spawning also produced elevated CEMG values, often equal to or greater than the cover digs we quantified (data not presented), but these high CEMG values did not produce a repeatable pattern. Nevertheless, the stereotypical behavior pattern that occurs before and after spawning in Chinook salmon (see also Berejikian et al. 2000) allowed us to develop an algorithm to detect the spawning events with reasonably high accuracy and in the presence of potentially confounding signals from other behaviors.

Other approaches or tag types may be further developed to remotely detect spawning in salmonids, although a direct comparison of alternative methods was beyond the scope of this study. Tags sensitive to acceleration have been used to identify spawning-related activity, such as female digging and male quivering, from swimming-related activity in Atlantic salmon *Salmo salar* (Økland et al. 1996). Tags recording the bioelectrical voltage changes (EMGs) from axial red musculature accurately recorded the intensity of swimming- and breeding-related behaviors in the same species (Økland et al. 2000). Økland et al. (2000) noted that the EMG approach offered a more precise measure of activity than the activity tags used in their prior study (Økland et al. 1996). Brown et al. (in press) evaluated the new CEMG transmitter and noted the advantages of its smaller size, reduced likelihood of interference from outside signals during the logging of transmissions, and ability to simultaneously and continuously monitor multiple tags on a single receiver, as we did in 2004. Moreover, the new CEMG tags are now the standard, and older versions (i.e., EMG) may be difficult to obtain. However, they also noted that the new transmitter has a smaller range of output, which may lead to lower accuracy in estimating the swimming speed of fish. The smaller output range may compromise spawning detection as well.

The use of CEMG technology may prove useful in field detection of spawning for other anadromous salmonids. Predictable postspawning increases in digging frequency have been documented for other species (chum salmon *O. keta*: Schroder 1981; coho salmon *O. kisutch*: Berejikian et al. 1997; Atlantic salmon: de Gaudemar et al. 2000; sockeye salmon: Healey et al. 2003). Female digging in Atlantic salmon produced a repeatable CEMG signal (pulse interval)

that was significantly greater than the resting CEMG pulse interval (Økland et al. 2000) and similar to what we observed in Chinook salmon. Criteria for detection may need to be developed for each species independently. However, the methods developed in our study should allow for this technique of spawning detection to be applied under field conditions, provided that close behavioral monitoring is in place to verify the results of initial studies. In particular, the results suggest that documenting presence or absence of spawning and determining spawn timing would be quite accurate. Further laboratory development and, perhaps, alternative data analysis techniques should be developed to reduce the error rate in the number of spawning events detected per individual female. In addition, other types of tags, such as those that record activity (Økland et al. 1996), pressure changes in the body cavity or other changes associated with spawning should be further explored.

The algorithm used to detect spawning events was developed essentially by exploratory data analyses. There may be other approaches that would actually improve the detection accuracy over the method we developed. Spawning detection success did not appear to be affected by the average CEMG values recorded for a particular fish throughout its reproductive life span, so the method seems to be fairly robust with respect to variation among fish or tags. However, some variability caused by tag placement or the tags themselves should be expected. For example, the algorithm failed to detect four out of five spawning events for female 03D (2003 experiment). That female's CEMG values were consistently low, diminished over time, and showed very little variability. In a different study with steelhead *O. mykiss*, Brown et al. (in press) found a positive correlation ($R^2 = 0.45$) between the interelectrode distance and the range of EMG transmitter values of individual fish swimming at different speeds (i.e., 30 cm/s and the rate at 170 cm/s). Thus, consistent electrode placement may be important when using CEMG data for spawning detection.

This is the first study to quantify the effect of implanted CEMG transmitters on reproductive behavior or reproductive success in salmonids (adults to fry) in a manner that included nontagged controls. Atlantic salmon did not appear to be impaired by the presence of CEMG tags in an artificial spawning arena (Økland et al. 2000). Studies conducted at times other than during reproduction have indicated that migratory behavior was similar between sockeye salmon surgically implanted with CEMG tags and those only gastrically implanted with radio transmitters (Hinch et al. 1996). In addition, Beddow and McKinley (1998) found that maximum sustained swimming speed was unaffected by CEMG tag presence in Atlantic salmon.

Female Chinook salmon implanted with CEMG tags retained the majority of their eggs, which significantly reduced their reproductive success compared with nontagged females in both years. Postmortem dissections did not reveal any apparent blockage caused by the tag itself. We suspect that the high egg retention was caused by the loss of ovarian fluid during the surgery or through the incision afterwards. Ovarian fluid was observed draining from the incision during surgery, and the postmortem inspections revealed that none of the incisions had healed, thereby allowing ovarian fluid to escape the body cavity and water to intrude. The role of ovarian fluid in facilitating egg deposition is unclear, but ovarian fluid may serve as a lubricant or to maintain hydrostatic pressure in the body cavity. Cooke et al. (2004) indicated that at least one other study (unpublished) on maturing sockeye salmon noted water-hardened eggs in CEMG-tagged females.

The utility of CEMG telemetry to quantify energy use or spawning activity depends on the ability to tag reproductively maturing salmon without compromising their reproductive success, particularly when used on members of populations listed as threatened or endangered under the U.S. Endangered Species Act. In a follow-up to the present study (Berejikian et al., in press), we demonstrated that placement of CEMG tags between the skin and muscle tissue below the lateral line (as in Healey et al. 2003), rather than in the body cavity, significantly reduced egg retention in naturally spawning steelhead. Fish tagged internally, as in this study, retained 46% of their estimated fecundity, compared with 10% in subdermally tagged females and 2% in nontagged females. The new subdermal tag implantation technique should allow researchers to use CEMG tags on maturing fish with minimal effects on egg deposition.

Perhaps the greatest challenge to the use of CEMG telemetry for monitoring reproductive behavior in natural systems is the limited range for detecting signals emitted from the tags. The range changes with water depth, antenna configuration, topography, and other factors and, at present, is not likely to exceed a 0.5–1.0-km radius. Thus, implementation of the methods described here may be currently limited to populations that naturally spawn in discrete areas or in field studies that use weirs to limit the movements of fish. However, improvements in the range of transmission or more extensive or sophisticated receiver configurations (e.g., Gerlier and Roche 1998; Boggs et al. 2004) would broaden the utility of this approach to larger breeding areas.

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