Evaluation of a New Coded Electromyogram Transmitter for Studying Swimming Behavior and Energetics in Fish

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Abstract.—A new coded electromyogram (CEMG) transmitter was recently introduced to the market to allow broader application and greater flexibility of configurations than the conventional noncoded version. CEMG transmitters were implanted into 20 steelhead *Oncorhynchus mykiss* and calibrated to swimming speed in a respirometer to determine the relationship between swimming speed and the output of the transmitters and also to determine how the output of a single transmitter varied when implanted in multiple fish. Linear regression models showed a strong positive relationship between the output from CEMG transmitters and swimming speed. However, grouping signals from multiple transmitters produced a less accurate relationship between CEMG output and swimming speed than using signals from individual transmitters. The results, therefore, do not suggest that the CEMG transmitters acted similarly in all fish. Calibration data from one transmitter were not readily transferable among multiple fish implanted with the same transmitter, suggesting that the same transmitter implanted in multiple fish also performed dissimilarly. These results indicate that experimental designs that require more precise estimates of muscular activity should use individual fish–CEMG transmitter calibrations. Variation in fish length, fish weight, location of transmitter implantation (distance from snout), and distance between the electrode tips did not account for the variation in models. The smaller size of the new CEMG transmitters will allow them to be used in a larger range of fish species and sizes. The fact that the transmitter has a coded transmission reduces the likelihood of interference from outside signals and allows multiple fish to be continuously logged on a single receiver. This could lead to reduced project costs because fewer receivers may be needed. However, one downfall of this new transmitter is that it has a smaller range of output, which may lead to lower accuracy in estimating swimming speeds.

Commercially available electromyogram (EMG) transmitters have been used since the early 1990s to examine the energetics and behavior of fish (see review by Cooke et al. 2004). The first-generation EMG transmitters used in fisheries research sampled electrical impulses from the muscles of fish and transferred this bioelectric information in the form of pulsed transmissions. This technology allowed no more than one transmitter to be monitored on a single radio frequency, which necessitated multiple receivers to ensure continuous reception of multiple tags. The first-generation transmitters were also quite large (18 g in air). A new, smaller, reengineered coded EMG (CEMG) transmitter (as light as 8 g; M. van den Tillart, Lotek Wireless, personal communication) was introduced to the market in 2003 (Lotek Wireless, Newmarket, Ontario) to allow broader application and greater flexibility of configurations. The new CEMG transmitter emits a coded pulse that allows multiple transmitters to be monitored on a single radio frequency, which reduces the number of receivers necessary for monitoring (thus reducing project costs) and also reduces the likelihood of interference from outside signals during the logging of transmissions.

Several aspects of the first-generation EMG transmitter have been studied. For example, soon after the introduction of the original EMG transmitter, several
research studies determined that transmitter output was strongly correlated with swimming speed (Booth et al. 1995; Økland et al. 1997). Calibrating EMG transmitters in individual fish has been shown to be more accurate than applying calibrations from one fish to uncalibrated fish (Geist et al. 2002) and positioning of electrodes (Beddow and McKinley 1999), and instruments for implanting electrodes (Bunt 1999) have been investigated.

Recent research studies have used the new CEMG transmitters (McFarlane et al. 2004; Murchie and Smokorowski 2004; Berejikian et al. 2007a, 2007b), yet performance of the CEMG transmitters in fish has not been formally evaluated. Calibration curves are used to relate the output of transmitters to swimming speeds for specific tag and fish pairings and can be used to evaluate the behavior and performance of EMG and CEMG transmitters. Since the new CEMG transmitter has been completely reengineered and is fundamentally a different transmitter, it is unclear whether its output will be well correlated with swimming speeds and similar among multiple fish, which would make it unnecessary to calibrate all fish individually. It is also unclear how the positioning of electrode tips will influence the effectiveness of the transmitters.

We conducted the present study to test three hypotheses about the performance of CEMG transmitters in adult steelhead Oncorhynchus mykiss: (1) that output from CEMG transmitters would be uncorrelated with swimming speed; (2) that for a group of fish implanted with unique CEMG transmitters, a common regression equation would describe the relationship between swimming speed and CEMG transmitter output with the same amount of variability as individual regression relationships for each fish; and (3) that for a group of fish implanted with the same CEMG transmitter, a common regression equation would describe the relationship between swimming speed and CEMG transmitter output with the same amount of variability as individual regression relationships for each fish in the group.

In addition, we conducted post hoc analyses to investigate whether fish length, fish weight, implantation location, and distance between electrodes further explained the observed variation in the regression parameters (slopes and intercepts) for individual fish. We also investigated how the distance between the gold electrode tips influenced the range of CEMG transmitter output between the fastest and slowest swimming speeds tested.

Methods

CEMG transmitters.—Electromyogram transmitters detect the voltage difference (potential) between electrodes in the muscles of fish. The voltage potential sensed at the gold electrode tips is transferred to the electronics by a pair of stainless steel electrode wires. The voltage is sampled thousands of times per second, rectified, and averaged on a per second basis. The transmitter then emits a coded signal that represents the average CEMG value over a user-defined time interval (2, 3, 4, or 5 s) set during manufacturing (3 s for this study). Theoretically, CEMG values range from 0 to 50, and larger values indicate higher muscle activity. The transmitters (CEMG-R11–25, Lotek Wireless) tested weighed 11.9 g in air (5.4 g in water) with electrode tips on and had a volume of 6 mL.

Fish holding.—The CEMG transmitters were tested in steelhead originating from the Skookumchuck River, Washington. These fish were reared from egg to smolt in freshwater at the University of Washington’s Big Beef Creek Research Station (Seabeck, Washington) from May 2001 through May 2002. The fish were transferred to the Northwest Fisheries Science Center, Manchester Research Station, Manchester, Washington, in May 2002 for rearing in a single tank (diameter, 4.6 m; depth, 1.0 m) supplied with seawater at 9–13.5°C. In November 2004, 20 fish were transferred to an identical tank and acclimated to freshwater over a 1-week period. These fish were transported to the Pacific Northwest National Laboratory (PNNL), Richland, Washington, wet laboratory on 9 December 2004. During the study period, fish were held outside the wet laboratory in 1,394-L circular tanks (diameter, 1.83 m; depth, 0.53 m). Fish were held and tested at 10.5 ± 1°C. Fish had a mean fork length of 505 mm (range, 440–588) and a mean weight of 1.3 kg (range, 0.8–2.0).

Transmitter implantation and calibration.—Beginning on 10 December 2004, fish were surgically implanted with CEMG transmitters by means of techniques similar to those of Hinch et al. (1996). Electrode tips were placed on the left side of the fish’s body. A single experienced surgeon (R. Brown) performed all surgeries as suggested by Cooke et al. (2004). Fish were not fed from 24 h before, or for 48 h after, surgical implantation and were allowed to rest at least 2 d between transmitter implantation and testing.

We calibrated the transmissions from CEMG transmitters against swimming speed using the methods of Geist et al. (2003) and Brown et al. (2006). Fish were anesthetized in 100 mg of MS-222 (tricaine methanesulfonate)/L of water. While the fish were quiescent, CEMG transmissions were collected to determine the resting CEMG rate. Transmissions were collected at this time because while fish are quiescent, implanted CEMG transmitters emit a nonzero value that is unrelated to the relationship between swimming speed and CEMG tag output (this allows tags to be
located if fish die during fieldwork). Transmissions from CEMG transmitters were recorded while the fish were swum at eight different speeds (30 through 170 cm/s in 20-cm/s increments) in a 2,200-L Brett-style respirometer. Each swimming speed was maintained until 30 CEMG readings were recorded while the fish was actively swimming in place. The order of speeds tested depended on the swimming behavior of the individual fish tested (i.e., velocity was increased until a fish swam actively and was changed until sufficient CEMG readings were recorded at each velocity). Fish were not tested at swimming speeds greater than 170 cm/s because the goal of the study was to examine the relationships between the outputs of CEMG transmitters while fish were swimming steadily and not while burst swimming.

A video camera recorded the behavior of the swimming fish while another recorded signals logged on the radio receiver (SRX 400, Lotek Wireless). Video recordings from both cameras were recorded simultaneously by means of a multiplexer. Video recordings were reviewed after testing to exclude CEMG readings that occurred when fish were not swimming steadily. Between 0% and 50% of signals were excluded. Signals were mostly excluded at the higher swimming speeds where unsteady swimming was more common. Data taken while fish were swimming unsteadily were excluded because the goal of the study was to examine the relationships between the outputs of CEMG transmitters during nonburst swimming. However, an assessment of the use of CEMG transmitters to examine burst swimming would be useful (see Geist et al. 2003 for a discussion of EMG signals).  

Twelve fish were implanted with unique CEMG transmitters to test the second hypothesis that output from CEMG transmitters would not be correlated with swimming speed. However, an assessment of the use of CEMG transmitters to examine burst swimming would be useful (see Geist et al. 2003 for a discussion of EMG signals). Several variables were examined to determine whether they influenced the relationship between swimming speed and the output of CEMG transmitters. Simple linear regression analyses relating the four independent variables (fish weight, fish length, location of electrode placement, and the distance between electrode tips) and two dependent variables (the regression intercepts and slopes) were generated for the 12 fish implanted with different CEMG transmitters and the three groups of 3 fish implanted with the same CEMG transmitter. In addition, the relationship between the range of CEMG output and distance between electrode tips was investigated with simple linear regression. The range of CEMG output for an individual fish was calculated as the difference between the mean CEMG value when the fish was swimming at 170 cm/s minus the mean CEMG value when the fish was swimming at 30 cm/s.  

Results  

All linear regression models for the 12 steelhead tested showed a strong positive relationship between output from CEMG transmitters and swimming speed. (Table 1; Figures 1, 2). Relationships between swimming speed and CEMG transmitter output were
significant for each of the 12 fish \((P < 0.001)\); therefore, the null hypothesis that CEMG output is uncorrelated with swimming speed was rejected.

The two regression models used to test whether individual CEMG transmitters performed similarly when implanted in a group of 12 different fish (hypothesis 2) are shown in Table 2. The value of the calculated \(F\)-statistic for the reduced model was greater than the critical \(F\)-value, indicating that the reduced model was significantly different and less accurate than the full model \((\alpha = 0.05)\). The full model containing a separate intercept and a separate slope for each fish describes the relationship between swimming speed and CEMG output with the least amount of variability. The reduced model explained only 33% of the variation \((R^2)\) compared with 96% in the full model (see Table 2). Grouping signals from multiple transmitters produced a less accurate relationship between CEMG output and swimming speed than using signals from individual transmitters. Thus, the results of this analysis do not suggest that the CEMG transmitters functioned similarly in all fish.

The two regression models used to test whether the same CEMG transmitter performed similarly when implanted in three different fish (hypothesis 3) are shown in Table 3. The reduced model was not the preferred model for any of the three groups of fish tested. Calibration data from one transmitter were not readily transferable among multiple fish implanted with the same transmitter. Thus, there is no evidence to suggest that the same CEMG transmitter will perform similarly when it is implanted in different fish.

The variation in the slopes and intercepts of the regression equations (Table 1) could not be further explained by fish length, fish weight, location of transmitter implantation (distance from snout), or distance between the gold electrode tips using linear regression. None of these variables accounted for a significant portion of the variability in the slopes and intercepts of the regression equations.

The outputs of CEMG transmitters while fish were at rest (i.e., motionless) did not always fall in line with the linear relationship between swimming speed and CEMG output (Figure 3). While fish were at rest, signals from the 12 CEMG transmitters had a mean of 6.1 (SD, 3.2; range of means, 2.0–11.1). For several CEMG transmitters, the inclusion of the resting outputs fit the linear relationship seen between CEMG transmitter output and swimming speed (Figure 3). However, several other CEMG transmitters did not have resting outputs that fit the linear relationship (Figure 3). When resting rate CEMG values were added to regressions between transmitter output and swimming speed, the mean \(R^2\) dropped from 0.96 to 0.93 (simple linear regression: SD, 0.07; range, 0.68–0.99).

Transmitters had a relatively small working range of output at the swimming speeds tested. Transmitters had a mean rate of 10.3 (SD, 4.5; range of means, 4.2–15.9) while fish were swimming at 30 cm/s. Transmitters had a mean rate of 19.6 (SD, 4.5; range of means, 12.3–26.9) while fish were swimming at 170 cm/s. There was a mean range of 9.3 (SD, 1.7; range, 6.7–11.5) between the transmitter output at the lowest (30 cm/s) and the highest (170 cm/s) speeds that fish swam. There was a mean range of 13.5 (SD, 1.6; range of means, 10.2–16.8) between the rate while resting and the rate at 170 cm/s. Distance between the electrode tips was significantly and positively correlated to the range of output between the lowest and highest swimming speeds \((R^2 = 0.45, P < 0.01; \text{Figure 4})\).

**Discussion**

The first hypothesis was rejected because output from CEMG transmitters correlated well with swimming speed. The second hypothesis was rejected because the regression relationships between swim-

<table>
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<th>Slope</th>
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<th>(R^2)</th>
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<td>0.651 (0.0116)</td>
<td>0.96 (0.03)</td>
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</table>
ming speed and CEMG output varied greatly among transmitters. The variation among fish and transmitters precluded generation of an accurate generalized equation relating swimming speed to CEMG output for a group of fish implanted with different transmitters. This has practical implications for the design of experiments using CEMG transmitters. The results indicate that applying individual calibrations of each CEMG transmitter output against swimming speed would provide more accurate results than applying calibration curves from one tagged fish to other tagged fish. Furthermore, the variation in the regression relationship persisted when a single transmitter was implanted in several fish, suggesting that even when a single tag is implanted in multiple fish the results will not be as accurate as when each implanted fish is calibrated. The third hypothesis was rejected because a fully reduced model with a common intercept and slope was not the best model for any of the three groups of fish implanted with the same CEMG transmitter.

The resting rate output of the CEMG transmitter did not appear to fall in line with the linear regression equation between transmitter output and swimming speed. Geist et al. (2002) found the same lack of fit between observed resting output and the intercept of the regression equation in juvenile white sturgeon *Acipenser transmontanus* in a similar experiment with the original EMG transmitter.

Using generalized regression equations for a group of fish implanted with CEMG transmitters is likely to increase the error in estimating the swimming speeds of fish. In some experiments, the increased estimation error may not be critical. For example if researchers are interested only in examining relative changes in swimming speed or identifying spawning events or other punctuated periods of high muscular activity such as burst swimming, accurate estimates of swimming speed may not be needed. However, if researchers are using swimming speed estimates to examine energy use in fish, they probably will want more accurate estimates of swimming speed obtained through the individual calibration process. Even when a fish implanted with a CEMG transmitter is calibrated in the laboratory and then examined in the field, considerable error in the estimates of swimming speed can occur. For example, the CEMG output of the fish in Figure 1B shows that a CEMG output of 18 could be seen at swimming speeds ranging from 70 to 150 cm/s. Therefore, researchers should strive to obtain sufficient sample sizes to deal adequately with the potential for larger experimental error. Our results indicate that inaccuracy may be reduced if the range of CEMG transmitter output between the slowest and fastest swimming speeds were larger than we observed. Additional gains in this range may be made possible by design changes in the manufacture of CEMG transmitters.

The placement of the electrode tips along the fish’s body and fish size did not explain a significant amount of the variance in the regression models. However, the range in output of CEMG transmitters between lowest and highest swimming speeds varied with the placement of the electrode tips. It is unlikely that placing the electrode tips an exact distance apart during each implantation would improve the application of CEMG equations across fish. This is because even when tips were placed at exactly the same distance apart, there
was still a great deal of variation in transmitter output among fish (Figure 3). However, if researchers do want to increase the range of CEMG transmitter output between the lowest and highest swimming speeds, they may want to experiment with placing electrode tips farther apart. In addition, researchers should keep in mind that locations of electrodes may not remain stable over long periods of time, which may influence the accuracy of the application of field results. Research should be conducted to determine how well electrodes stay in place over time, and if movement is seen, what factors may be involved in this movement and how the movement influences the output of CEMG transmitters.

The pulse interval of transmitters may also influence the accuracy of CEMG transmitters, which are available with a pulse transmitted every 2, 3, 4, or 5 s. The transmitters used in this study had a 3-s pulse interval. A larger pulse interval (e.g., 5 s) would probably decrease researchers’ ability to identify short-duration swimming behavior. A smaller pulse interval, however, would increase the likelihood that signals will overlap during reception if more than one transmitter per receiver is used (see below).

Additional advances could be made that would allow an activity transmitter to be used much more widely. In recent work, the authors were able to log three different transmitters on the same frequency. However, there was a problem with signals overlapping and thus not being readable. Some (−13%; authors’ unpublished data) of the signals were not usable owing to a signal overlap that occurred while scanning three transmitters on one frequency. Therefore, CEMG transmitters that have less variance in the timing of their signal outputs would be useful. In addition, shortening the length of the signal transmission would allow users to monitor more transmitters on the same frequency.

This transmitter would also be more widely useful if size and frequency emission ranges were wider.
Although the new transmitter is much smaller than the original, it is still much too large for use in many juvenile fish. A smaller transmitter could be used in a larger size range of fish. In addition, we are not currently aware of any commercially available EMG transmitter that can be easily used in deep or marine waters. An EMG transmitter transmits only at 148–152 MHz and not at ultrasonic frequencies (20–420 kHz). Thus, the signal from the transmitter is attenuated and cannot transmit through more than about 10–12 m of freshwater. Some authors (Geist et al. 2005) have placed arrays of antennas on the bottom of a river to be able to conduct EMG studies on bottom-dwelling fish (white sturgeon) in deep water. However, in brackish or marine waters, transmitters working at the currently available frequency range would have such a limited range that they probably would not be useful. Thus, we suggest that an EMG transmitter with output in the acoustic range would be useful.

In conclusion, the CEMG transmitter output correlated well with swimming speed. However, our research indicates that applying individual calibrations of CEMG output against swimming speed would provide more accurate results than applying calibration curves from one tagged fish to other tagged fish. The new CEMG transmitter has several advantages over its predecessor. Its smaller size will allow it to be used in a larger range of fish species and sizes. The fact that it has a coded transmission reduces the likelihood of interference from outside signals during the logging of transmissions and allows multiple fish to be continuously logged on a single receiver. This could lead to

![Image](image1.png)

**FIGURE 3.**—An example of the relationship between mean CEMG transmitter output and swimming speed for three transmitters surgically implanted in three different adult steelhead. Each transmitter (A, B, and C) was implanted in three separate steelhead. Resting rate values are shown but are not included in the regression relationships exhibited by the lines on each graph.

![Image](image2.png)

**FIGURE 4.**—Relationships between the distance between the tips of CEMG transmitter electrodes and the range of CEMG transmitter output for adult steelhead surgically implanted with transmitters. The range of CEMG transmitter output is the difference between the mean rate at 30 cm/s and the mean rate at 170 cm/s.

<table>
<thead>
<tr>
<th>Transmitter</th>
<th>Model</th>
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reduced project costs because fewer receivers may be needed. However, one downfall of this new transmitter is that it has a smaller range of output. This may lead to lower accuracy in estimating the swimming speed of fish.

Acknowledgments

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References


