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Electromyogram Telemetry, Nondestructive Physiological Biopsy, and Genetic Markers: Linking Recent Techniques with Behavioral Observations for the Study of Reproductive Success in Sockeye Salmon Mating Systems

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ABSTRACT There has been little investigation into the physiological and energetic factors affecting reproductive success in free-swimming, spawning fish. One of the major impediments has been the lack of methods for nonlethally studying individual-specific energetics and physiological condition, as well as the lack of utilization of technologies for associating specific behaviors with energetics, condition, and fertilization success. Here we review three approaches (electromyogram [EMG] telemetry, nondestructive physiological sampling, and microsatellite analysis) that have recently been used to examine individual specific differences in behavior, energetics, and physiology in spawning sockeye salmon *Oncorhynchus nerka*. We also review major findings from other studies that have used these approaches in the context of how they were, or could have been, used to assess reproductive success in sockeye. Electromyogram telemetry can provide good estimates of individual specific energy expenditures during spawning and help identify energetic costs of specific reproductive behaviors. Nondestructive physiological sampling can provide information into pre- and postspawning levels of energy, hormones, and metabolites, enabling assessments of physiological stress, reproductive preparedness, and osmoregulatory function. Microsatellite analysis is a genetic marker technique that enables an assessment of parentage. We discuss several of the strengths of these research approaches and the value of integrating them with studies of fish behavior as a means of significantly advancing our understanding of individual variation in reproductive success of spawning salmonids.

Sockeye salmon *Oncorhynchus nerka* cease foraging before entering freshwater (Brett 1995; Hinch et al. 2006), so they must complete migration, develop gonads and secondary sexual characters, and spawn using stored energy. Research examining energy use by sockeye salmon during upriver spawning migrations (Brett 1995; Hendry and Berg 1999; Crossin et al. 2004) has revealed that successful migrants use about 50% of reserve energy prior to spawning, though this varies among populations. If higher than usual temperatures or water flows are encountered, energy reserves could become further depleted, affecting migratory success (Rand and Hinch 1998; Hinch and Rand 2000; Rand et al. 2006) or energy partitioning to gonads

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(Patterson et al. 2004). Thus, the way in which energy is allocated during migration has serious implications for reproductive success (Roff 1992). Moreover, migrants that have reached spawning grounds, but have had their energy reserves severely diminished, may behave differently from those with more ample reserves—a factor that could affect spawning success. None of the implications of varying energy reserves and allocation schedules has been adequately studied in Pacific salmon or any other species in the wild.

In addition to becoming catabolic and directing energy towards gonad development and growth of secondary sexual characteristics, all adults returning from the sea experience other physiological changes. As sockeye prepare to enter freshwater, they adjust their osmoregulatory systems to cope with the dilute freshwater environment and continue to modify osmoregulation during the upriver migration (Shrimpton et al. 2005). For sockeye, specific changes include a 20% reduction in plasma chloride concentration and a 15% reduction in gill Na^+, K^+-ATPase activity (indices of osmoregulatory changes) from open ocean to freshwater entry, and further 10% and 65% reductions in these variables, respectively, following freshwater entry and arrival near spawning areas up to 45 d later (Hinch et al. 2006; Shrimpton et al. 2005). Cortisol levels (an index of stress) vary during the migration (Carruth et al. 2002) and are elevated two- to threefold when sockeye encounter high velocity currents and turbulent flows (Hinch et al. 2006). High river temperatures can also increase cortisol levels in migrating sockeye (Macdonald 2000). Elevated cortisol levels can significantly depress reproductive hormone expression (Hinch et al. 2006), so that a chronic experience of high levels of migratory stress could impair gonad development. Furthermore, because spawning occurs during the senescent phase of life in sockeye, other physiological processes (i.e., kidney and gill function, disease resistance) can become impaired as fish approach spawning grounds (Wagner et al. 2005). How this array of physiological and energetic changes influence spawning success is largely unknown.

The most prevalent approach to studying the associations between reproductive success and energy use, energy allocation and physiological condition in migrating and spawning adult fish is a ‘destructive’ sampling technique (Gilhousen 1980; Crossin et al. 2004). In these types of studies, changes in energy or physiology were determined by the differences observed between different groups of fish captured at distinct times during the migration. Implicit to this approach is the assumption that there is no differential mortality occurring during the migration or during spawning (i.e., between the sample times) owing to different initial energetic or physiological states. This assumption is clearly violated in years when the migratory conditions are particularly adverse; smaller sockeye with lower energy reserves are known to preferentially die during the migration (Rand et al. 2006). The main drawback with lethal sampling approaches is, therefore, that there is no way to link the condition of an individual to its reproductive success. A further complication is that several males often simultaneously spawn, or attempt to spawn, with the same female (Foote et al. 1997) and each female deposits her eggs in 3–5 batches with potentially different sires for each batch (Mehranvar et al. 2004). Thus, even if individuals are followed during migration or spawning, determining an individual’s contribution to egg fertilization, and hence spawning success, can be complex.

Recently, there has been increased emphasis on understanding the physiological and energetic mechanisms behind specific behaviors of wild animals (Cooke et al. 2000; Altmann and Altmann 2003; Wagner et al.

2004). But, given the difficulties outlined above, it is not surprising that few studies have rigorously investigated links between energy reserves, energy use, physiological condition, behavior, and reproductive success in free-swimming, spawning fish. We contend that one of the major impediments to studying these links has been the lack of appropriate technologies or methods for nonlethally tracking energetics and physiological condition of individual fish, as well as the lack of technologies for associating specific behaviors with energetics, condition, and fertilization success.

In this paper, we 1) briefly review three technologies (i.e., electromyogram telemetry, nondestructive physiological sampling, and microsatellite analysis) that have recently been used to examine individual specific differences in behavior, energetics and physiology in spawning sockeye salmon; 2) review some of the major findings from studies that have used these techniques to show their value in assessing reproductive success; and 3) discuss strengths of these research approaches and the value of combining them with studies of fish behavior to provide a suite of tools for significantly advancing our understanding of individual variability in reproductive success of spawning salmonids.

Energy Use and Electromyogram Telemetry

Electromyogram (EMG) radio telemetry is a technology that measures bioelectric voltage changes in the swimming musculature of fish and transmits this information to a receiver. By calibrating EMG signals with tail-beat frequency, swim speed, or oxygen consumption under controlled conditions, it is possible to quantify the metabolic costs of activity in wild fish (reviewed in Cooke et al. 2004). Some of the most extensive EMG studies have involved quantifying energetic costs of upriver migration in sockeye salmon (Hinch and Rand 1998; Rand and Hinch 1998; Standen et al. 2002). Electromyogram telemetry has also been used to study reproductive behavior in salmonids. Kaseloo et al. (1996) and Weatherley et al. (1996) used EMG telemetry to identify spawning episodes in lake trout *Salvelinus namaycush* in a laboratory spawning raceway and an experimental lake in Ontario, respectively. Økland et al. (2000) observed that many spawning behaviors in Atlantic salmon *Salmo salar* were associated with low EMG signals, which is indicative of greater muscle activity, as EMG signals are inversely proportional to muscle activity.

Healey et al. (2003) used EMG telemetry to calculate energetic costs of spawning in sockeye salmon. They made detailed behavioral observations on fish and simultaneously recorded EMG data. For each behavior, EMG signals were converted to energy cost (in calories per hour) by means of previously developed relationships between EMG signals, tail-beat frequency, and metabolism. Energy cost per unit time for each behavior was combined with measurements of the duration and frequency of the behavior to calculate the daily cost per behavior and the values for each behavior were summed to estimate energy expenditure. Healey et al. (2003) showed that estimates of total energy use during the spawning period (~7–10 d) based on EMG data (117.6 kcal for males, 155.7 kcal for females) were within the range of that estimated on the same population using destructive, proximate analysis approaches (59–157 kcal for males, 131–155 for females) where fish were sampled at spawning ground arrival and others were sampled postspawning when they were moribund (Macdonald et al. 2000; Crossin et al. 2004). Thus, EMG technology can provide realistic energy use estimates.

One of the other unique aspects of Healey et al. (2003) was their assessment of energy use associated with the display of specific behaviors and the revelation that across all spawning classes (e.g., dominant males, subdominant males, nesting females, guarding females), "holding behavior", where fish maintain position with minimal swimming, was by far the most energetically costly activity (Figure 1). This occurred because fish on the spawning grounds spent almost all their time in holding behavior. Other courtship behaviors, such as aggression and nest preparation, that could be costly when displayed, did not occur frequently enough to account for large total energy expenditures. This implies that a premium is put on energy conservation during spawning, and raises the question of whether those individuals that begin spawning with higher energy reserves have higher reproductive success, or whether the fish are capable of trading off energy used to maintain spawning ground longevity (e.g., holding position), with that used for specific active behaviors to gain access to mates or defend a spawning redd. This question can only be addressed by technologies that allow researchers to measure energy reserves and energy use of individuals throughout their spawning life.

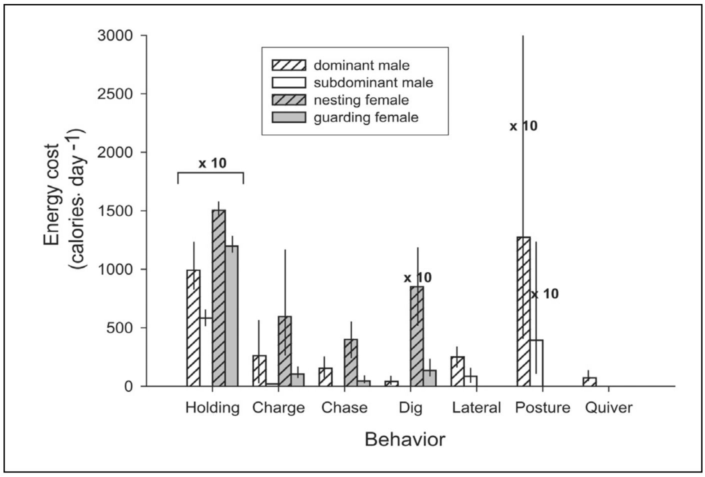

Figure 1. The geometric mean calories/d cost of behaviors performed by Early Stuart sockeye salmon, a Fraser River stock. Note that for holding, female digging, and male posture displays, bars shows one-tenth the actual cost. Vertical lines show 95% confidence intervals. Results are based on observations from 31 males and 18 females. Adapted from Healey et al. (2003).

The above studies were carried out using the old non-coded EMG tags, which are no longer available. New coded EMG tags that are now on the market emit coded pulses of averaged EMG signals over a time interval set by the manufacturer. These new tags may be problematic for studies designed to look at the energy use of specific, short-duration behaviors due to the averaging of signals. In addition, these transmitters have a small working range of signals and there is substantial variability in the output between transmitters and between fish (unpublished data; R.S. Brown, personal communication).

Electromyogram telemetry approaches have to be calibrated and it is likely that these calibrations will need further attention to environmental-, stock- and species-specific differences in metabolism. With the advent of reliable field-based, swim tunnel respirometry (Farrell et al. 2003), field calibrations are now possible. The importance of these considerations is that the relationship between metabolic rate and EMG signals will vary among species. Differences in metabolic rate and swimming performance certainly exist for seawater and freshwater-acclimated adult sockeye (Wagner et al. 2006) and differences in metabolic capacity exist between different stocks of adult sockeye from the same watershed (Lee et al. 2003a, 2003b).

Nondestructive Physiological Examinations

Individual-specific energy levels can be assessed on spawning grounds in a variety of manners, but all approaches have limitations.

Muscle biopsy, whereby a small piece of muscle tissue (~1 g) is removed from an individual, can be used to assess instantaneous energy state in adult salmonids (Crawford et al. 1977). Tissue energy content is estimated using bomb calorimetry, proximate analyses, or by drying and weighing the sample as lipid content can be estimated from known relationships between moisture and lipids (Higgs et al. 1979; Crossin and Hinch 2005). Gill biopsies have also been taken, removing the tips from a few gill filaments, with success. Biopsy techniques are constrained by the limited amount of tissue that can be removed and this is especially true for gill tissue.

Muscle biopsy has been used to assess prespawning energy levels in sockeye (Hendry et al. 2001; Mehranvar et al. 2004) but it has one large disadvantage. Most energy reserves are stored in the musculature along the body. In upriver migrating salmon, energy reserves are preferentially utilized first near tail and then towards the head (Burgetz et al. 1998; Crossin and Hinch 2005). Thus, the accuracy of this biopsy technique is strongly dependant on two factors: the location along the body where the sample is taken and the extent of energy depletion during migration. In years when migration conditions are extreme, during which energy reserves have been severely depleted, the accuracy of the determinations may be limited by the biopsy size, particularly if samples are derived from posterior body locales. Approaches that assess energy content of entire fish bodies nondestructively could generate not only more accurate estimates of energy reserves, but also eliminate the need to remove a plug of tissue which could lead to infection or disease and thereby affect spawning success.

Several techniques are available to nondestructively assess energy levels in fish (reviewed in Crossin and Hinch 2005). Microwave transmission is one of the easiest approaches for field use and is widely used in the finfish aquaculture industry for assessing the lipid content of market fish. This technique has been used with wild adult sockeye salmon (e.g., Cooke et al. 2005, 2006; Young et al. 2006) and wild Atlantic salmon (*Salmo salar*; Hendry and Beall 2004). The hand-held device that we use emits a low-powered wave (frequency, 2 GHz ± 2,000 MHz; power, 2 mW) that interacts with water in the somatic tissues and returns a signal of different frequency that is detected by sensors in the probe. The changes in frequency are proportional to the water content of the tissue so that the probe measures tissue water content. As there is a strong inverse relationship between the water and lipid content in fish tissues (e.g., Higgs et al. 1979), outputs from the probe can be calibrated to tissue lipid levels from laboratory measures of tissue lipid in sacrificed animals. Once calibrated, the probe can be used to estimate lipid content of live fish tissue. We have developed sockeye-specific calibrations to convert meter readings to estimates of gross somatic energy for several populations from river entry to spawning (Crossin and Hinch 2005). The microware technology can resolve the expected differences in energy content along the length of the fish (Crossin and Hinch 2005); therefore it is critical that an exact protocol be used (Cooke et al. 2005; Crossin and Hinch 2005).

The microwave transmission technology to estimate energy content in spawning sockeye is not without its difficulties. We have had issues with developing reliable calibrations to estimate energy content in spawning sockeye, probably related to the low lipid content of spawning sockeye and the sensitivity of the meter. Water replaces fat and protein as they are metabolized, so at extremely low energy density, fish tissues have high concentrations of water. Sensors in the microwave probe can become saturated when water concentrations are too high, creating high error in readings of some salmon on the spawning grounds (Crossin and Hinch 2005). Regardless of population, sockeye from the Fraser River, British Columbia tend to reach spawning grounds with about 5–6 MJ/kg of energy remaining in their somatic tissues (Crossin et al. 2004). Unfortunately, these values are near the sensitivity of the meter and energy densities at or below this level create a problem. Therefore, although the meter is an extremely valuable tool during the adult migration, its utility becomes limited on the spawning grounds. In fact, in a pilot study we could not detect any differences in microwave transmission readings of an individual's body energy density measured at the start and end of its spawning life (unpublished data), although our work and that of others using destructive bomb calorimetry has shown energy depletion on the spawning grounds (Crossin et al. 2004). Hendry and Beall (2004), by contrast, successfully used the same microwave energy meter to measure energy changes throughout the spawning period in Atlantic salmon. They found that energy declined over time and varied with behavioral class. The differences in successful use of the energy meter between Hendry and Beall's (2004) study and our work on spawning groundfish may result from two factors. First, there may be differences in how Atlantic and sockeye salmon utilize energy reserves, considering that Atlantic salmon are iteroparous so they require energy for their return seaward migration. Thus, spawning Atlantic salmon in Hendry and Beall's (2004) study may not have reached tissue water levels that saturated the meter. Second, there may be differences in the energy meter settings used in the two studies, which could result in differential sensitivity of the meter to water saturation. Therefore, caution must be used when attempting to use this technology on spawning salmon.

There is a great deal of individual variability in behavior (Healey et al. 2003) and spawning success that cannot be explained solely by differences in initial energy state, or in energy-use strategies (Mehranvar et al. 2004). Understanding differences in physiological status among individual spawners may help explain some of this variability. Until recently it was not known how physiologically stressful spawning was for anadromous salmon, nor how variable it could be between sexes or individuals. Blood from destructively sampled sockeye collected at several points along their migration and on spawning grounds (Figure 2, Hinch et al. 2006) revealed that plasma cortisol concentrations in females were higher on the spawning grounds than at any other point along the migration (four to eight times higher) except for passage through the Hell's Gate canyon, during which cortisol concentrations were 30% higher than on the spawning grounds. Hell's Gate is the most difficult point of passage for sockeye in the Fraser River, BC (Hinch and Rand 1998; Hinch and Bratty 2000). Cortisol concentrations in males showed a pattern similar to females, though the magnitude of the differences among sample locations was less. Females had cortisol concentrations nearly twice those of males and had twice the individual variability (mean ± 1 SE; 410 ± 130 ng/mL, 220 ± 80 ng/mL; females and males, respectively). Aside from the Hell's Gate canyon, the highest variability in cortisol concentrations among females was found at the spawning grounds. As individuals had to be sacrificed upon arrival at the spawning grounds to obtain these measurements, it was not possible to relate individual variability in cortisol to behavioral class or spawning success. This problem can now be circumvented with our new blood biopsy technique for adult salmon.

Cooke et al. (2005) have recently developed a nondestructive technique for assessing the physiological state of migrating adult salmon by rapidly drawing 3 mL of blood

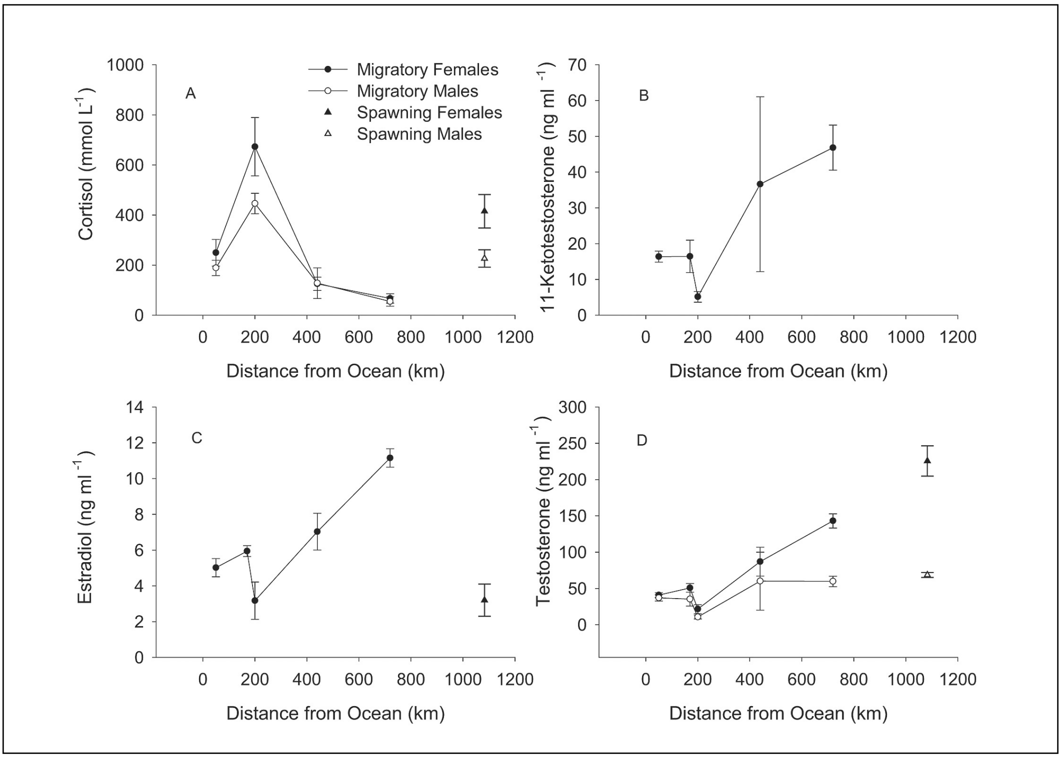

Figure 2. Mean (±SE; n = 10) plasma concentrations of selected hormones (A-cortisol, B-ketotestosterone, C-estradiol, D-testosterone) from Early Stuart sockeye salmon destructively sampled during upstream spawning migration and at spawning grounds in 2001 (0 rkm = mouth of Fraser River). At rkm 200 fish negotiate a very difficult hydraulic barrier (Hell's Gate) where plasma cortisol levels are significantly elevated; this stress is also associated with depression of all three sex hormones. Adapted from Hinch et al. (2006).

from the caudal peduncle and removing a small piece (0.3 g) of gill tissue from unanesthetized fish. Based on field trials and postrelease telemetry for up to 2 months, this biopsy approach did not affect immediate survival (24 h in a net pen), subsequent migration rates or survival to spawning grounds of sampled and released subjects. From the biopsy material, we have been able to determine concentrations of metabolites, hormone titers, ionic status, and degree of physiological stress, reproductive preparedness, and osmoregulatory function. Unlike destructive approaches, this technique permits the same individuals to be sampled at the start and completion of spawning. When this technique is coupled with telemetry, it becomes a very powerful tool for relating physiological state with individual behavior (Cooke et al. 2005).

In addition, we have conducted a pilot study using this biopsy technique on sockeye spawning at Weaver Creek Spawning Channel (Fraser River, BC) and have found that the tissue sampling did not alter spawning behavior or length of spawning life, despite the fish being so close to their programmed death. For these studies, blood was biopsied twice, at the outset and when the fish became moribund. Preliminary results suggest some remarkable changes in physiological condition over the spawning period (average duration 5 d, range among fish 2–9 d). Plasma testosterone fell by 84% in females and 50% in males and plasma lactate increased by 8–10 times in both sexes (Figure 3). The reduction in sex hormone concentration over the spawning period was similar to the increase which occurred during the migration (see Figure 2). The differences in absolute levels of testosterone between Figures 2 and 3 may be a stock effect as the data in Figure 2 are for early Stuart sockeye whereas those in Figure 3 are for Weaver Creek sockeye. Lactate is a stress hormone and byproduct of anaerobiosis, so its large increase could reflect the stress of reproduction and glycogen depletion from the muscle during reproduction related activities. High levels of plasma lactate can impair swimming and even cause direct mortality resulting from acidosis (Wood et al. 1983). Thus, the dramatic increase in lactate in spawning fish could affect spawning by altering behavior and spawning ground longevity. It is also worth noting that within the sexes, individuals began spawning with nearly identical levels of lactate but finished with much more variable levels, which may be explained when we couple these results with behavioral observations made on each individual.

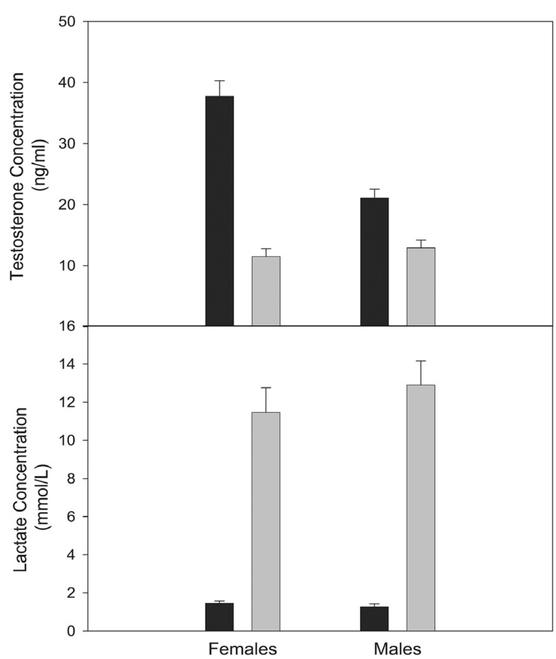

Figure 3. Mean (±SE) plasma testosterone and lactate concentrations nondestructively sampled from females (*n* = 15) and males (*n* = 11) upon arrival onto the Weaver Creek spawning channel (dark bars) and sampled again following spawning (light bars). Fish were externally tagged with Petersen discs and visually located daily. Postspawning sampling occurred when fish started to lose swimming ability and appeared moribund. The mean longevity for these fish was 5 d (range 2–9 d). Within each sex, differences between arrival and moribund testosterone and lactate were significant at *P* < 0.001. This Fraser River stock spawns 150 km upriver from the ocean. Source: Hruska (Unpublished data).

Mating Success and Microsatellite Analysis

Studies of salmonid reproductive behavior have traditionally assumed that dominant males (assessed by aggressive behavior and consort position near the receptive female) are the ones that fertilize the largest number of eggs (i.e., Schroder 1982; Quinn and Foote 1994). However, it is well known that nondominant males sneak in on spawning events to fertilize eggs (Schroder 1982; Foote 1990; Foote et al. 1997). In studies on mammals, birds, and lizards, genetic markers have revealed that behavioral measures are not always an accurate measure of mating success (Moller and Birkhead 1993; Hughes 1998; Coltman et al. 1999; Lebas 2001). Though genetic mating success has been examined in Atlantic salmon (Garant et al. 2001; Taggart et al. 2001), the relationship between dominance and genetic reproductive success is poorly understood in most salmonid mating systems.

Microsatellite markers are becoming widely used in studies of animal and plant population structure (Ciofi et al. 2002; Aldrich et al. 1998), mating strategies (Semple et al. 2001) and parentage (Worthington-Wilmer et al. 1999; Carling et al. 2003). These tandem repeats of very short nucleotide sequences are highly polymorphic, codominant, selectively neutral, and are easy to amplify and score. Using this genetic tool, sockeye mating systems have recently been examined to determine whether behavioral measures can be used to predict reproductive success and how variable individuals are in reproductive success.

Groups of wild male and female sockeye salmon were introduced into spawning arenas in the Weaver Creek Spawning Channel located in coastal British Columbia. The behavior of the fish was monitored until spawning was complete and genetic reproductive success was determined from the microsatellite fingerprints of adults and offspring (Mehranvar et al. 2004). Males and females spawned with up to four different partners (average for males, 1.85; average for females, 2.5). These results are consistent with earlier behavioral studies on reproductive strategies in sockeye that report high levels of competition for access to multiple mates (Hanson and Smith 1967), and that have observed some males adopting sneaker tactics for gaining fertilizations (Schroder 1982; Gross 1985; Foote 1990). Mehranvar et al. (2004) found that some males attained dominance and had primary access to one or more females, whereas others clearly used sneaker tactics to fertilize eggs. The high number of mates acquired by individual females was in part due to the joint effect of dominant and sneaker male tactics during single spawning events and to a switch in dominant partners between spawning events.

Mehranvar et al. (2004) also found that behavioral measures of reproductive success in males (i.e., social dominance, time as consort, number of female partners) were strongly correlated with genetic reproductive success (i.e., proportion of offspring sired and number of females mated with), but explained only 33–40% of the variance in reproductive success (Mehranvar et al. 2004). A few subordinate males fathered a large proportion of offspring, probably due to sneaker tactics. For example, one small male, who was ranked very low on the two behavioral dominance indices, was genetically quite successful, acquiring matings with 80% of the females and fertilizing 18% of the eggs. Thus, although behavioral indices can allow researchers to make general predictions about male reproductive success, these predictions may be biased towards large, dominant males. Only longevity on the spawning grounds was correlated with indices of female reproductive success. Spawning longevity may have positive fitness consequences, as the longer a female defends her nest the lower the probability it will be dug up by a later spawning female (Quinn and Foote 1994). This correlation may, therefore, be reflective of male mate choice, as factors associated with longevity may be used by males to assess female quality.

Linking Technologies and Future Challenges

We briefly reviewed three techniques (EMG telemetry, physiological bioassay, microsatellite markers) that have recently been applied to the study of sockeye salmon mating systems. Each has generated novel insights into factors potentially affecting spawning success—insights that were well beyond what has been learned from basic behavioral observations. However these approaches have largely been used in isolation. We contend that only through the integration of these or related approaches, can we gain a more complete understanding of the causes and consequences of individual variability in mating

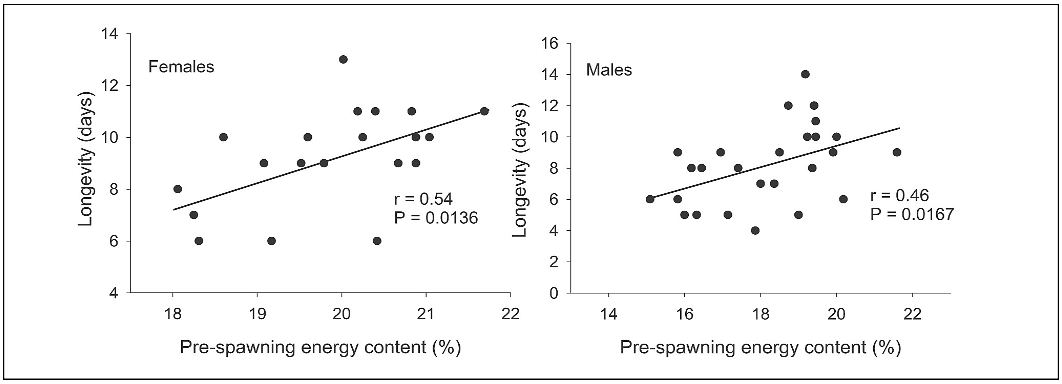

Figure 4. Relationship between prespawning energy content, as determined by muscle plug biopsy, and longevity (days) in female (*n* = 20) and male (*n* = 27) sockeye salmon upon entry into the Weaver Creek Spawning Channel. Pearson correlation, associated P-value and regression line of best fit are indicated. Adapted from Mehranvar (2002).

and reproductive success in any salmonid.

Where integration has been attempted, the results have been revealing. For example, by combining behavioral observation with EMG telemetry, Healey et al. (2003) were able to show that routine activities consumed the greatest amount of energy during spawning and that only two specific behaviors (i.e., digging in females and posture displays in males) consumed any significant amount of energy. Mehranvar et al. (2004) combined muscle biopsy (for energy content) with behavioral observation and genetic analysis of reproductive success. Although spawners did not select mates with a particular energy content, energy levels were positively correlated with longevity on the spawning grounds (Figure 4; Mehranvar 2002). Longevity is an important trait for reproductive success. The longer that males survive, the more opportunities they have to spawn (Morbey and Abrams 2004) and for females it ensures that as many eggs as possible get deposited and that redds are guarded as long as possible to prevent other females from disturbing them (Quinn and Foote 1994; McPhee and Quinn 1998; Hendry et al. 2004). Thus, initial energy state appears to be important, though indirectly, to reproductive success in sockeye. Energy reserves did not determine male status or reproductive success but microsatellite analysis confirmed that dominant males sired the most offspring. Subordinate males sired more offspring than expected—a result that could not have been determined by either technique in isolation.

The challenges for future studies on salmonid mating systems will be to quantify the specific roles of initial energetic state and physiology with respect to spawning success and fitness. As we have shown, energy state can be assessed with muscle biopsy and/or microwave transmission. Blood and gill samples can be used to measure several aspects of physiological condition (e.g., level of reproductive readiness, stress, etc.) at different times in the same individuals. If continuous information on energy use or the cost of specific spawning activities is desired, EMG telemetry can be an effective tool. It may also be possible to use blood samples to assess energy use from changes to circulating levels of nonesterified free fatty acids (e.g., Ballantyne et al. 1996). Tissue or blood samples can also be used for physiological genomics research (e.g., Rise et al. 2004). Recent developments in gene array technology, particularly for salmonids, have provided opportunities to assess a much broader suite of physiological processes than is possible with standard assays by revealing the up-regulation or down-regulation of thousands of genes. We also contend that, whenever feasible, it is important that genetic marker approaches be incorporated into future examinations of salmonid mating systems. As an example, genetic fingerprinting could have been used to overcome limitations encountered by Healey et al. (2003) in trying to relate energetics of different behavioral classes to spawning success. Few spawning events were actually observed because of night time spawning and the quickness of fertilization events. Genetic fingerprinting could have been used to overcome these issues.

Acknowledgments

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