Seasonal patterns of gonad size, liver size, and in vitro gonadal steroidogenic capacity in slimy sculpin (Cottus cognatus)

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ABSTRACT

The objective of this study was to characterize the reproductive seasonality of slimy sculpin (Cottus cognatus), a small-bodied, benthic spring spawning species. Sculpin are unique compared with other temperate fish species because they have a reproductive pattern where gonadal maturation occurs over winter in northern areas of its distribution. Previous studies have involved pre-spawning sampling (early spring in North America) and post-spawning sampling (early fall in North America). However, seasonal changes in gonadosomatic index, hepatosomatic index, condition factor, and in vitro gonadal production of estradiol (E2) and testosterone (T) in females and T and 11-ketotestosterone (11-KT) in males have not been characterized. Following a summer quiescent period, female sculpin showed an increase in gonadal hormone production during the fall, which was associated with increases in gonad and liver sizes, however males experienced a much shorter resting phase following spawning in May. Elevated production of both T and 11-KT appears to both signal the initiation of gonadal growth in September and contribute to gonadal maturation over the winter. This study is important because it is the first characterization of the seasonal reproductive pattern in sculpin and it describes the patterns of gonad development and seasonal changes in condition and liver size in this species as it prepares for spawning.

Key words | 11-ketotestosterone, estradiol, reproductive physiology, testosterone

INTRODUCTION

Sculpin (Cottus spp.) represent an important ecological component of northern temperate systems, but surprisingly little is known about their reproductive biology (devLaming et al. 1984). Freshwater sculpin are often locally abundant and occupy primarily swift water, boulder-gravel river habitats. These fish have been characterized as rock nesters (Natsumeda 2001), with males maintaining breeding space under rocks where they mate with females (Goto 1998). They are usually a spring spawning species in northern temperate waters, and are unique in that they do not start to accumulate gonadal tissue until the winter and early spring (Gray 2003). They are a complete and single spawning species, with fecundity ranging from 40 to 200 eggs depending on their size and age. Freshwater sculpin reproductive strategies do not appear to be similar to other temperate freshwater species, however, no comprehensive studies have been conducted.

Spring and early summer spawning species commonly use a reproductive strategy in which gonadal tissue develops in the fall with final maturation as water temperatures rise in the spring, as with white sucker (Catostomus commersoni) (Scott et al. 1984) or gonadal development occurs quickly in the spring, as with several species of dace (Galloway & Munkittrick 2006). Freshwater sculpin species such as the spoonhead sculpin (Cottus ricei) spawn immediately after ice-out in the
spring (Gibbons et al. 1998) and slimy sculpin spawn following the spring freshet (Gray 2003). This strategy requires that the bulk of gonadal tissue is generated over winter and under ice, when water temperatures are at or below freezing (Figure 1), and activity levels are usually limited. In this regard, sculpin also present an interesting model for energy storage, as they are actively synthesizing lipid-rich tissue (gonad) under ice while food sources are limited.

To ensure that a fish is in breeding condition at the appropriate season, physiological mechanisms must control the timing of gonad maturation (Wootton 1998). Previous studies have shown that female slimy sculpin gonad sizes are very small (<2–5% of body weight) at the beginning of the ice-cover period, and are then very large (30–40%) prior to spawning time following the spring freshet (Gray 2003). Gonadal steroid hormones are an important factor involved in these physiological processes, and timely and appropriate changes in gonadal steroidogenesis are necessary for successful reproduction (Ponthier et al. 1998). It is not known which hormones are responsible for initiating and sustaining the processes of sperm and oocyte maturation in sculpin, but testosterone and 11-ketotestosterone are known to be important in many species for males, and testosterone and 17β estradiol for females (Nagahama 1994). Hormonal assessment of reproduction is complicated by the small size of freshwater cottids (usually <5 g) and low blood volume precludes the use of blood samples for analysis of circulating sex steroids.

A protocol has been developed to measure the in vitro steroid biosynthetic capability of gonadal tissue as a surrogate for levels circulating in blood, and circulating levels have been shown to correlate with in vitro production levels (McMaster et al. 1995). The technique has been used for a variety of small-bodied species, including Johnny darter (Etheostoma nigrum; McMaster et al. 2002), pearl dace (Margariscus margarita; Tetreault et al. 2003), longnose dace (Rhinichthys cataractae; McMaster et al. 2004), bluntnose minnow (Pimephales notatus), and common shiner (Luxilus cornutus; McMaster unpublished data), as well as white sucker (McMaster et al. 1995), longnose sucker (Catostomus catostomus; McMaster et al. 2004).
yellow perch (*Perca flavescens*) and brown bullhead (*Amerius nebulosus*; *McMaster et al. 2002*). Hormones produced by the gonads interact to regulate the growth and structural integrity of the reproductive organs, the production of gametes, the patterns of sexual behavior, the phenotypic differences between the sexes, and the continuation of the species (*Tetreault et al. 2003a; McMaster et al. 2004, 2005*).

Sculpin have been used recently for freshwater environmental monitoring related to agriculture (*Gray et al. 2002; Gray 2003; Gray & Munkittrick 2005*), pulp and paper and sewage effluents (*Gibbons et al. 1998; Galloway et al. 2003*) as well as oil sands operations (*Tetreault et al. 2003a, 2003b*). However, impact studies can be difficult to interpret without life history information, which may provide an ecological or seasonal basis for the changes that may be documented (*Munkittrick et al. 2000*).

In central Ontario, studies determining the most appropriate period to sample greenside darter for monitoring determined that fish response endpoints have the potential to be quite variable in the period just prior to spring spawning and that late fall is best suited for biological monitoring (*Tetreault 2012*). Additionally, collections of rainbow darter in the spring prior to spawning suggest that rapid changes in final gonadal maturation may contribute significant within-site variability in reproductive parameters, which may impair the ability to detect site differences. Investigation of the most appropriate period to sample two other sentinel fish species in Alberta, fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*), for the purpose of biological monitoring of municipal wastewater, demonstrated seasonal site differences with respect to fish condition, gonadal development and histopathology as well as significant alterations in steroid production depending on the season (spring, summer, fall) (*Tetreault et al. 2012*).

Limited information about seasonal cycles can also make the data difficult to interpret in the context of other species and previous studies. To date, no study has addressed how spring spawning slimy sculpin function over the winter and the rate at which recrudescence occurs under ice cover. This study presents the seasonal characterization of gonadal size, liver size, condition, and *in vitro* gonadal hormone production in slimy sculpin, and describes its reproductive cycle based on these observed patterns.

**MATERIALS AND METHODS**

Slimy sculpin were collected from the upper Kennebecasis River (45°49′37″N, 65°13′9″W), southern New Brunswick (Canada). This section of the river is primarily ground-water-fed and remains ice free, allowing for continuous monthly collections year round (May 2003–May 2004). Water temperatures were collected using HOBO temperature recorders, and daily maximum temperatures were reported in this study (Figure 1). Slimy sculpin were collected by sampling faster runs and riffles (approx. 1.1–1.5 m/s) approximately 0.5–0.75 m deep with boulder/cobble substrates, between the hours of 10:00 and 13:00. Sculpin were collected with dipnets (2 × 1.2 m, 6-mm mesh size) and a backpack electrofishing unit (Smith-Root type VII). Collections targeted a minimum of 20 adult males and 20 adult females per sampling period. Holding time in the cooler did not exceed 4 h, based on results of previous experiments on the effects of holding time on steroid production (*Tetreault 2002*). Each adult fish was rendered unconscious by concussion, followed by spinal severance, and measured for total length (±1 mm), body weight (±0.01 g), gonad weight (±0.001 g), and liver weight (±0.001 g). Only mature sculpin were sampled during this study. During periods of low gonadal development, a minimum length of 50 mm was used for sampling to ensure fish were mature. During periods where gonadal growth was discernable externally, samples included some smaller, but obviously sexually mature fish. A gonadosomatic index (GSI) was calculated according to the following formula: gonad weight/body weight – gonad weight × 100. A hepatosomatic index was calculated using the formula liver weight/body weight – liver weight × 100. Condition factor was calculated as \[ k = \frac{\text{weight}/(\text{length})^3}{100}. \]

Gonadal tissues were placed in medium 199 (M199; containing Hank’s salts without bicarbonate; GIBCO, Burlington, ON, Canada), which was supplemented with 25 mM Hepes, 4.0 mM sodium bicarbonate, 0.01% streptomycin sulfate, and 0.1% bovine serum albumin (pH 7.4) at 4 °C until preparation for culture; holding time for gonadal
tissue never exceeded 6 h. Small sections (18–25 mg) of the gonadal tissue were placed into M199 in 20-mL sample tubes on ice. In vitro incubation of the gonadal tissue was conducted in 24-well tissue culture plates (Falcon 3047; Fisher Scientific, Toronto, ON, Canada). Tissues were subjected to two treatments: basal (M199 alone) or stimulated ( forskolin + M199) (Sigma F6886). Forskolin is a diterpene activator of the adenylate cyclase pathway which mimics gonadotropin action. This compound bypasses the gonadotropin receptor which increases cyclic adenosine monophosphate (AMP) production and subsequent gonadal steroid production (McMaster et al. 1995). The level of forskolin-stimulated steroid production provides information regarding the integrity and maximal capacity of the tissue to produce steroid hormones. Tissues were incubated for 18 h at 16°C, after which the media from each of the wells was removed, placed in cryovials, and stored at −80°C until the time of analysis.

Hormones were analyzed at the National Water Research Institute (Burlington, ON). Concentrations of testosterone (T) (both sexes), 17β-estradiol (E2) (females), and 11-ketotestosterone (11-KT) (males) released into the media during the incubation period were quantified by radioimmunoassay (RIA) as described by McMaster et al. (1992). Media were assayed in duplicate at a volume of 100–200 μL for each hormone and values were converted to correct for size of sub-sample of tissue analyzed, and expressed in pg/mg of gonadal tissue. For T, E2, and 11-KT, inter-assay variabilities were <10% and intra-assay variability for each steroid was approximately 5%. As described in McMaster et al. (1992), intra-assay variability was determined using a pooled sample run five times within one RIA and those values were within 5% of one another. Inter-assay variability was determined from that same pooled sample run in duplicate on each RIA spin and compared between RIA spins. The variability in those values was less than 10%. T and E2 antibodies were purchased from Medicorp (Prod#07-189016, #07-138016 Montreal, QC, Canada) and radiolabeled T, E2, and 11-KT from Amersham Pharmacia Biotech ( 3H-T Prod# TRK 402; 3H-E2 Prod# TRH 322; 3H-11-KT Prod# TRQ 8945, Baie D’Urfe, QC, Canada). Unlabeled T and E2 were purchased from Sigma–Aldrich; KT antibody was received from Dr Glen Van Der Kraak (University of Guelph, Guelph, ON, Canada) and purchased from Helix Biotech (0.5024 mg/mL; Vancouver, BC, Canada).

The gonad histology of slimy sculpin was examined to monitor progression in gonadal development at this site. Oocytes were categorized as previtellogenic, endogenous vitellogenic, or vitellogenic according to their stage of maturation. The relative abundance of each oocyte stage was used to monitor this development. Gonadal tissue was stained with hematoxylin and eosin and fixed onto slides prior to examination with a Zeiss compound light microscope. To ensure an unbiased representation of each slide, standard stage coordinates were used to select 20 sections for examination. An image of the field-of-view (FOV) at each section was recorded using Eclipse software. The oocyte stage tally was pooled across images for each fish, and the proportion of oocytes at each stage of maturation was calculated by dividing by the total number of cells counted in a fish. Data were pooled across images within a fish. The sum area covered by a oocyte stage was divided by the sum of the total area measured to calculate the relative abundance for a fish. The average proportion was then taken across all fish collected within a sampling period and then the 2 months examined (March and May) were compared.

Due to the seasonal aspect of this study, samples in a given month were only compared to the previous and following month’s samples. For example, May was compared to April and June, but not to other months. Estimates of condition (weight vs. length), gonad size (gonad weight vs. body weight), and liver size (liver weight vs. body weight) were evaluated using analysis of covariance (ANCOVA) among adjacent months (SYSTAT v 9.0). Body weight was the covariate used in these statistical tests. Tukey’s post hoc test was then used when \( p < 0.05 \).

Gonadal tissue weight determined how many replicates were possible for in vitro studies, and average values for in vitro hormone production were obtained from three replicates when possible. Data are shown for eight to 10 fish per sample, to illustrate the variability as well as the polarization of some individuals. A single mean was calculated for each month, and we attempted to characterize months when the fish appeared to be ‘shut down’ or ‘dormant’ with regard to gonadal hormone production. Hormone production data were analyzed using nonparametric Kruskal–Wallis (NCSS© 2004).
RESULTS

Average sizes for female fish ranged from 1.7 to 3.3 g, and 2.2 to 5.0 g for males (Table 1). Male gonad sizes increased rapidly throughout September, reaching their maximum size (2.29 ± 0.07%) in early November (Figure 2(a)). Testis size decreased significantly after November (p = 0.016), and then continued to gradually decrease until spawning (1.80 ± 0.08%), although the decreases were not statistically significant between adjacent months (p = 0.14, 0.8, 0.69, 0.76). Male gonad sizes were at their minimum during July (0.21 ± 0.04%), however both T and 11-KT showed significant increases in gonadal in vitro productive capacity during July, compared to June (p = 0.028 and p = 0.032, respectively) (Figures 2(b) and 2(c)). Based on the patterns of gonad size, the reproductive cycle for male sculpin was divided into four ‘seasons’: post-spawning (June), recrudescence (July–October), maturation (late October–April), and spawning (May). Similarly in females, the reproductive cycle was defined as post-spawning (June–August), recrudescence (September–February), pre-spawning (March–April), and spawning (May) (Figure 3).

<table>
<thead>
<tr>
<th>Females</th>
<th></th>
<th>Males</th>
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<tr>
<td></td>
<td>Length (mm)</td>
<td>Body wt (g)</td>
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<tr>
<td>May</td>
<td>61.29 ± 1.45 (21)</td>
<td>2.65 ± 0.24 (21)</td>
</tr>
<tr>
<td>June</td>
<td>62.18 ± 1.36 (22)</td>
<td>2.47 ± 0.19 (22)</td>
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<tr>
<td>July</td>
<td>58.19 ± 1.44 (21)a</td>
<td>1.94 ± 0.24 (21)a</td>
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<tr>
<td>August</td>
<td>67.33 ± 4.31 (6)a</td>
<td>3.25 ± 0.64 (6)a</td>
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<tr>
<td>September</td>
<td>60.77 ± 0.98 (22)a</td>
<td>2.18 ± 0.09 (22)a</td>
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<tr>
<td>October</td>
<td>61.00 ± 1.35 (22)</td>
<td>2.26 ± 0.17 (22)</td>
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<tr>
<td>November</td>
<td>58.22 ± 1.09 (23)</td>
<td>1.70 ± 0.10 (23)a</td>
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<tr>
<td>January</td>
<td>57.90 ± 2.05 (20)</td>
<td>1.87 ± 0.22 (20)</td>
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<td>February</td>
<td>54.68 ± 1.51 (25)</td>
<td>1.76 ± 0.17 (25)</td>
</tr>
<tr>
<td>March</td>
<td>57.93 ± 1.70 (30)</td>
<td>2.12 ± 0.21 (30)</td>
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<tr>
<td>April</td>
<td>50.93 ± 1.42 (15)a</td>
<td>2.54 ± 0.22 (15)</td>
</tr>
<tr>
<td>May</td>
<td>46.13 ± 1.02 (23)a</td>
<td>1.99 ± 0.15 (23)a</td>
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<tr>
<td></td>
<td>53.35 ± 0.91 (55)a</td>
<td>1.95 ± 0.12 (55)</td>
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*Indicates significant change from preceding month (p < 0.05). December sampling was not possible due to high water events, resulting in an early January collection, leading to two collections in February 3 weeks apart.

In *vitro* steroid biosynthetic capacity in male testis tissue was highly variable between individuals, within a collection. Highest individual values for T were seen during August, immediately preceding the large increase in gonad size during September and early October. Similarly, males showed increased synthetic capacity for 11-KT in August, but maximum levels were not observed until November. This is followed by a significant decrease in testis weight (p = 0.016) and presumably spermatocyte maturation. However, individual males continued to show low steroid productivity, and there was no relationship between T production and relative gonad size ($r^2 = 0.082$, data not shown), or between T and 11-KT ($r^2 = 0.40$, data not shown). In contrast to the male reproductive cycle, female gonad sizes increased gradually during the winter in preparation for the next spawn (May) (Figure 3(a)). During the winter, at water temperatures below 1 °C, the ovaries increased in size from less than 1% in October to more than 12% when water temperatures began to rise in April. Rapid growth occurred in this tissue between early April and May, increasing significantly from around 12–30% (p < 0.001).
Figure 2 | Monthly changes (mean monthly values) in male slimy sculpin: (a) gonadosomatic index; (b) gonadal in vitro steroidogenic capacity to produce 11-ketotestosterone; (c) gonadal in vitro steroidogenic capacity to produce testosterone. Solid lines represent seasonal changes in the monthly mean. Dashed lines define ‘shut off’ as defined by the maximum production value in the months of minimum steroidogenic capacity. *(GSI)/‡(11-ketotestosterone)/†(testosterone) indicates significant (p < 0.05) change from the preceding month using Mann-Whitney nonparametric probabilities. For GSI, values are presented as mean ± SEM (error bars, N = 20 per collection time point).
Figure 3 | Monthly changes (mean monthly values) in female slimy sculpin: (a) gonadosomatic index (gonad weight/body weight × gonad weight × 100); (b) gonadal forskolin stimulated in vitro steroidogenic capacity to produce testosterone; (c) gonadal in vitro steroidogenic capacity to produce 17β-estradiol. Solid lines represent seasonal changes in the monthly mean. Dashed lines define ‘shut off’ as defined by the maximum production value in the months of minimum steroidogenic capacity. *(GSI)/‡(estradiol)/†(testosterone) indicates significant \( p < 0.05 \) change from the preceding month using Mann–Whitney nonparametric probabilities. For GSI, values are presented as mean ± SEM (error bars, \( N = 20 \) per collection time point).
The gonadal histology of slimy sculpin was examined during February and May to follow natural gonadal development in this species. Gonadal development appeared normal other than the timing of the maturation during mid-winter ice cover. Female gonads showed a gradual and marginal increase in size from August until November, when gonadal development accelerates. Histological evaluation demonstrates the progression in female ovarian development from February to May with higher percentages of the follicles at the previtellogenic stage in February ($p < 0.013$) relative to May (Figure 4). Even though gonadal size increases from $<3\%$ in October to $>12\%$ in March, there was no significant increase in gonadal size between March and April. However, as water temperatures increase in May, ovarian size more than doubles in size (Figure 3(a)).

In May, more of the follicles have progressed through the endogenous vitellogenic stage into the vitellogenic stage with a greater proportion of the follicles in the vitellogenic stage ($p < 0.03$) in preparation for spawning (Figure 4).

For females, both $E_2$ and T production capacity were diminished during the collections following the spawning period (Figures 3(b) and 3(c)). T capacity remained low until early February as recrudescence ends ($p < 0.001$). Following this initial increase, T production remained elevated until spawning. In September, $E_2$ production increased as recrudescence began and stayed elevated in most fish until late February. $E_2$ production dropped off in the months preceding spawning, with a marked reduction in May ($p = 0.023$). As $E_2$ capacity declined, T production began to increase starting in early February ($p = 0.003$).

**Figure 4**  Frequency of the various stages of oocyte development for slimy sculpin collected during March (open bars) and May (shaded bars). Inset pictures are representative sections from fish collected in March (left) and May (right). The letters designate the type of follicles: (a) previtellogenic, (b) endogenous vitellogenic, (c) vitellogenic. Size marker represents 300 microns. Values are presented as mean ± SEM (error bars).
There was no significant decrease in condition factor following spawning for male sculpin \( (p = 0.170) \) (Table 1). However, during recrudescence, there were changes in condition \( (p < 0.0001) \). In November, when both gonad and liver showed significant increases, condition decreased \( (p < 0.0001) \). Additionally, condition increased more than 10% in the early February collection \( (p < 0.0001) \), from 0.90 to 1.06 in developing males. Following spawning, liver size in male sculpin was constant, with no detectable change until September \( (p = 0.004) \) (Figure 5(a)). This coincided with a period of rapid testis growth. While testis size reached its maximum in October (Figure 2(a)), liver size recovered and began to increase in November \( (p < 0.001) \), continuing through until March (Figure 5(a)).

Unlike males, females exhibited a significant decrease in condition following the spawn \( (p = 0.022; \text{Table 1}) \). Initially during recrudescence, there was no change in condition, but condition decreased in late fall \( (p < 0.0001) \) corresponding with increases in both gonad \( (p = 0.002; \text{Figure 3(a)}) \) and liver size \( (p = 0.031; \text{Figure 5(b)}) \). Beginning in February, there was a slight but significant increase in condition \( (p < 0.0001) \).

Liver size was dynamic in females, initially increasing after spawn \( (p = 0.016) \) and then decreasing until after the onset of recrudescence. Beginning in October, liver size increased significantly each month, reaching maximum size in early March. In females, liver size continues to increase during the winter period of gonadal development. From September to March relative liver size increased by 400%. During the final phases of gonadal growth, male and female liver sizes dropped rapidly \( (p = 0.004 \text{ and } p = 0.009, \text{respectively}) \).

**DISCUSSION**

Slimy sculpin have a gonadal maturation pattern unlike most other freshwater northern temperate fishes, where the females undergo significant ovarian development during the winter, ice-covered months when water temperatures are \(< 1 \degree C\). Other single-spawning freshwater temperate species in eastern Canada include white sucker, northern pike \( (Esox lucius) \), and yellow perch. Yellow perch show an increase from about 10% in October to 12% in January and 17% in February, leading to a spawning GSI around 20% \( (\text{Henderson et al. 2000}) \). Female white sucker gonadal growth start in late August and reach >70% of spawning levels by mid-October (unpublished data), while pike show a GSI of 8% by November, and a gradual increase over winter \( (\text{Lenhardt 1992}) \). Multiple spawning species, such as goldfish \( (Carassius auratus L.) \) increase gonadal growth from September to October, but remain unchanged between November and April \( (\text{Munkittrick & Leatherland 1984}) \). European species that spawn in spring/early summer also show little female gonadal development over the winter including roach \( (Rutilus rutilus) \) as a single spawner, and the bleak \( (Alburnus alburnus) \) and white bream \( (Blicca bjoerkna) \) \( (\text{Rinchard & Kestemont 1996; Rinchard et al. 1997}) \) or perch \( (Perca fluviatilis) \) \( (\text{Blanchard et al. 2005}) \) or chub \( (Leuciscus pyrenaicus) \) \( (\text{Encina & Granado-Lorencio 1997}) \).

Based on hormonal changes and changes in organ sizes, the reproductive cycles of females were divided into four seasons, an extended post-spawning season from June to August, a period of gonadal recrudescence starting slowly.
in September and leveling off in April, a rapid pre-spawning period leading up to a synchronized spawning event. In comparison, after a post-spawning period in July, males showed a period of rapid gonadal growth starting in August and reaching maximum size during the October collection, followed by a period of slow gonadal maturation between November and April, and a prolonged guarding and spawning period from April to July. These stages do not coincide with other freshwater temperate, spring spawning species as most development is done prior to ice up.

For post-spawning females, both T and E2 production appears to be shut off in August, followed by increased E2 productive capacity in September, remaining high and peaking in early February, followed by a marginal decline at spawning time. In contrast, T remains low until February, peaking in April and remaining high until spawning. This pattern is not typical of temperate, spring spawning species such as the rainbow trout where E2 rises in June, peaks in November, and declines in December. Testosterone begins to rise in July, peaks in November, and declines steadily into April (Scott et al. 1980a).

Recrudescence in females is initiated in September and corresponds to a significant increase in E2. At the time of pre-spawning, E2 production has dropped significantly, while T remains elevated. This switch in the steroid pathway may be related to other hormones involved in maturation and ovulation. In white sucker, during gonadal maturation, E2 production is lessened while 17α-20β-dihydroxy 4-pregnen-3-one is up-regulated (Van der Kraak et al. 1992). This hormone has been shown to be the maturation inducing hormone in many fish species (Nagahama 1994). The large increase in ovarian size in April is not accompanied by increased hormone productive capacity (on a per mg tissue basis), but is accompanied by a rapid decline in liver size (energy storage).

The male period of gonadal growth in August to October is preceded by a significant increase in both 11-KT and T production in July, and a peak in KT production capacity in August, immediately preceding the large investment in gonadal weight. However, during the period of rapid gonad growth (Sept–Oct), both T and 11-KT production are near post-spawning levels. In November, elevated production of both T and 11-KT appear to trigger the initiation of testis maturation, as testis size decreases. Both 11-KT and T production stabilize about 2 months prior to spawning, perhaps indicating that sperm maturation is complete, and males may be ready to spawn prior to females. In a number of fish species, T dominates during early spermatogenesis and it is thought that T may control spermatogenesis and that 11-KT may play more of a role in spermiation, expression of secondary sexual characteristics, and sexual behavior (Scott et al. 1980b).

The fish collected in this study exhibited sexual-size dimorphism – the males were larger than the females. In this species, males are responsible for several of the following behaviors in preparation for spawning: movement to and choice of a breeding site, preparation of a spawning site, defense of a spawning site, courtship, and parental care. In some species, 11-KT is associated with the expression of secondary sex characteristics, e.g., parental behaviors (Liley et al. 1986; Kindler et al. 1989). Given the pattern of 11-KT observed, perhaps it also played a role in the reproductive behavior in the slimy sculpin. Additionally, these data suggest that T and 11-KT may work in conjunction throughout the observed season; however, additional research is needed to investigate the interplay between these hormones, as well as the seasonal expression of testicular enzymes.

Male gonad growth appears to be fueled by liver stores, as the gonad growth period is accompanied by a decrease in liver size, and another decline in liver size occurs in April and May, as the males have been shown to be guarding their nests during that time (Keeler & Cunjak 2007). In a previous comparative study of stable isotopes (Jardine et al. 2005), male slimy sculpin sampled in April exhibited 15N enrichment, suggesting they may be in poor nutritional condition prior to spawning in May. Female sculpin in this study did not show significant 15N enrichment, suggesting that their prey consumption rates remained constant throughout the winter. Jardine et al. (2005) suggested this may be a consequence of cessation or reduction in feeding during the establishment of territories and nest guarding by males. Given this metabolically challenging environment, it is likely female sculpin have developed a divergent approach to reproductive and energetic investment. Dahle et al. (2003) also demonstrated sex differences in energy utilization from the liver in the Atlantic cod (Gadus morhua L).

Stimulation tests, such as the in vitro assay used in this study, are designed to take advantage of known endogenous
control mechanisms to assess steroidogenic capacity of the tissue to produce hormone (Griffin 2000). Variability between fish in vitro steroidogenic capacities in a given month generates questions about reproductive asynchrony and how microhabitat differences may influence physiological endpoints. During phases of the reproductive cycle when there are critical changes in gonadal development, the variability in steroidogenesis is high. This indicates there is not synchrony between fish, or the fish utilize differences in microhabitat to give themselves benefits in terms of performance. This is a worthy hypothesis for future study in the slimy sculpin.

This study presents the reproductive cycle of both male and female slimy sculpin which is characterized by distinct seasonal variations in energy storage, represented by liver size and condition factor, and energy expenditure represented by gonad size. Data on organ sizes, condition, and hormone production can indicate when males and females are shifting reproductive stages from pre- and to post-spawning, to recrudescence, and then to final maturation and will assist in interpreting population data collected in environmental studies. Sculpin are unique, compared with other temperate fish species, gonadal steroid production is more variable, and their reproductive pattern reveals that gonadal maturation can take place in water temperatures below 1 °C in central to northern Canadian streams.

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