**Supplementary**

**Supplementary table 1**.Scoring system of histological images related to cell stage and their corresponding characteristics, nuclear diameter, and maturity stage.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Cell stage** | **Description** | **Maturity stage** |
| **Spermacytogenesis** | **Clonal expansion** | **Spermatogonia A un-differentiated and differentiated** | Large nuclear diameter ~10 um, some heterochromatin. It can be foamy apperance of sertoli cells and presence of tubuli. Spermatogonia A stain light purple with AB/PAS. | **Stage I** |
| **Spermatogonia B** | Nuclear diameter ~8 um, high amount of heterochromatin, and/or presence of mucus and ducts. Mucus stain dark blue with AB/PAS. | **Stage II** |
| **Maturation** | **Spermatocytes I & II** | Nuclear diameter ~ 4 um | **Stage III** |
| **Spermiogenese** | **Differentiation** | **Spermatides** | Nuclear diameter ~ 2 um, densly packed nucleis, stain dark purple with AB/PAS | **Stage IV** |
| **Spermatozoa** | Nuclear diameter > 2 um, small densly packed nucleis with tail, stain dark blue with AB/PAS | **Stage V** |

**Supplementary table 2.** Thermal growth coefficient (TGC), Specific growth rate (SGR), Specific feeding rate (SFR), Feed conversion ratio (FCR) and g feed/g fish of Atlantic salmon exposed to continuous (LL), short-to-long (SL) and decreasing daylength (DL) for 18 weeks in brackish water. After which all groups were exposed to seawater and similar decreasing daylength for 11 weeks (19-29). Data presented as mean and SEM. Distinct letters denote statistically significant difference between groups. P-value (P < 0.05) in Bold indicates significant effect of photoperiodic regime.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Week** | **Parameter** | **DL** | **SEM** | **LL** | **SEM** | **SL** | **SEM** | **P-value** |
| **1-4** | **TGC** | 3.84 | 0.09 | 3.80 | 0.09 | 3.86 | 0.07 | *0.901* |
| **SGR** | 2.63 | 0.02 | 2.68 | 0.07 | 2.73 | 0.04 | *0.408* |
| **SFR** | 1.55b | 0.05 | 1.57b | 0.03 | 1.71a | 0.03 | ***0.045*** |
| **FCR** | 0.61 | 0.02 | 0.61 | 0.01 | 0.66 | 0.02 | *0.188* |
| **Feed/g fish** | 59.31b | 0.62 | 61.55b | 1.89 | 67.90a | 1.04 | ***0.008*** |
| **5-8** | **TGC** | 4.40 | 0.13 | 4.71 | 0.10 | 4.86 | 0.17 | *0*.121 |
| **SGR** | 2.48 | 0.09 | 2.58 | 0.04 | 2.67 | 0.06 | *0.203* |
| **SFR** | 1.81b | 0.04 | 1.90ab | 0.02 | 2.01a | 0.05 | ***0.029*** |
| **FCR** | 0.76 | 0.01 | 0.76 | 0.01 | 0.78 | 0.01 | *0.267* |
| **Feed/g fish** | 131.24b | 3.11 | 142.88ab | 4.69 | 155.54a | 4.70 | ***0.019*** |
| **9-14** | **TGC** | 4.27b | 0.03 | 4.57a | 0.02 | 2.74c | 0.05 | ***<0.001*** |
| **SGR** | 1.92b | 0.03 | 2.00a | 0.01 | 1.28c | 0.02 | ***<0.001*** |
| **SFR** | 1.57b | 0.03 | 1.66a | 0.02 | 1.12c | 0.01 | ***<0.001*** |
| **FCR** | 0.85 | 0.01 | 0.86 | 0.01 | 0.89 | 0.01 | *0.066* |
| **Feed/g fish** | 310.26b | 8.01 | 348.41a | 8.97 | 215.76c | 1.95 | ***<0.001*** |
| **15-18** | **TGC** | 3.97b | 0.05 | 3.68b | 0.14 | 4.47a | 0.07 | ***0.003*** |
| **SGR** | 1.46ab | 0.03 | 1.34b | 0.08 | 1.60a | 0.04 | ***0.037*** |
| **SFR** | 1.27b | 0.03 | 1.20b | 0.03 | 1.41a | 0.02 | ***0*.006** |
| **FCR** | 0.88 | 0.01 | 0.91 | 0.04 | 0.90 | 0.01 | *0.680* |
| **Feed/g fish** | 267.81 | 4.62 | 269.68 | 11.12 | 267.61 | 1.21 | *0.974* |
| **19-22** | **TGC** | 4.19 | 0.05 | 3.80 | 0.16 | 3.74 | 0.12 | *0.076* |
| **SGR** | 1.58 | 0.02 | 1.43 | 0.07 | 1.45 | 0.04 | *0.104* |
| **23-29** | **TGC** | 2.84 | 0.03 | 2.66 | 0.08 | 2.88 | 0.05 | *0.072* |
| **SGR** | 0.91a | 0.01 | 0.85b | 0.02 | 0.96a | 0.01 | ***0*.010** |

**Calculations of growth parameters**

Feed/g fish was calculated as:

E.Q. 1

The specific feeding rate (SFR) was calculated as:

E.Q. 2

The feed conversion ratio (FCR) was calculated as:

E.Q. 3

The thermal growth coefficient (TGC) was calculated as:

E.Q. 4

Where W1 and W2 are initial and final weights respectively, and ΣT the sum of day degrees.

The specific growth rate (SGR) was calculated as:

E.Q. 5

Where W1 and W2 are initial and final weights, respectively, and d is number of feeding days.

Gonadosomatic Index (GSI, %) was calculated as:





Supplementary Figure 1. TGC of maturing and immature Atlantic salmon males exposed to constant daylength (LL) (A) and short-to-long daylength (SL) (B) for 18 weeks in brackish water. Before all groups were exposed to seawater and similar decreasing daylength for 11 weeks (19-29). Data presented as mean and SEM. \* Denote significant differences between maturing and immature male fish within treatment.

# ***Supplementary information regarding analysis of steroid hormones in serum***

**Chemicals**

LC grade methyl tert-butyl ether (MTBE) and LC-MS grade methanol was purchased from Merck, Zinc sulfate heptahydrate (ZnSO4\*7H2O) from Acros Organics, LC-MS grade formic acid from Thermo Scientific, Milli-Q grade water was produced by a Millipore system.

The analytes 17a-OH-progesterone (**1**), testosterone (**2**), progesterone (**3**), 21-deoxycortisol (**4**), 11-deoxycortisol (**5**), corticosterone (**6**), and cortisol (**7**) were purchased from Cerilliant Corporation; androstenedione (**8**) from LGC Germany; 4-androsten-11B-OL3-17-dione (**10**), 4-androsten-3-11-17-trione (andrenosterone, **11**), 4-androsten-17B-OL3-11B-dione (11-ketotestosterone, **12**) from Steraloids Inc

The isotope labelled analytes D8-17a-OH-progesterone (**1\***), D3-testosterone (**2\***), D9-progesterone (**3\***), D8-21-deoxycortisol (**4\***) D5-11-deoxycortisol (**5\***), D8-corticosterone (**6\***) were purchased from Cerilliant Corporation; D4-cortisol (**7\***) from IsoScience; D3-androstenedione (**8\***) from TRC Canada; D4-4-androsten-11B-OL3-17-dione (**10\***), and D10-4-androsten-3-11-17-trione (**11\***) from Cambridge Isotope Laboratories, D3-4-androsten-17B-OL3-11B-dione (**12\***) from Cayman Chemical Group.

For quality control (QC) of analytes **1-8** the CE-IVD MassChrom® Steroids panels were purchased from Chromsystems. For compound **10**, **11** and **12** there were no external quality control samples available.

**Sample preparation**

A seven-point calibration curve ranging from for 0.13 to 130 nM for **1,2,3,5,6,8**;0.013 to 13 for **4**; 2.08 to 2080 for **7**, and 0.025 to 25 for **10,11,12** was prepared in MeOH:water (1:1). The isotope labelled analytes were used as internal standards (IS) and were mixed in a concentration of 30 nM in ultrapure H2O.

Extraction was done on a Tecan Fluent 780 liquid handler. 70 ul of sample, calibration standard or QC samples were transferred to a 96 well plate (Sarstedt 1.2 mL polypropylene) whereafter 60 ul IS-mix was added and 110 ul 0.1 M ZnSO4:MeOH (1:1) for protein precipitation. After shaking at 1000 RPM (BioShake, QInstruments) for 2 minutes 500 ul MTBE was added and the samples were shaken at 1300 RPM for 3 minutes for metabolite extraction. The plates were centrifuged (Hettich universal 320 R) for 10 minutes at 1600 RPM and the upper MTBE phase was transferred to a new plate. The solvent was evaporated under a stream of nitrogen (Techne sample concentrator) while kept on 35 °C. Finally, the sample was reconstituted in 60 ul of 70% MeOH with 0.1% formic acid.

Retention time (RT), multiple reaction monitoring (MRM) transitions, cone voltage and collision energy used for the different analytes.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **nr** | **Analyte** | **RT (min)** | **Quantifier MRM transitions (m/z)** | **Qualifier MRM transitions (m/z)** | **Cone (V)** | **Collision (eV)** |
| 1  1\* | 17a-OH-progesterone  D8-17a-OH-progesterone | 6.74  6.66 | 331.1>97  339.1>100 | 331.1>109 | 40 | 23/23  23 |
| 2  2\* | testosterone  D3-testosterone | 6.31  6.26 | 289.1>97  292.1>97 | 289.1>109  292.1>109 | 40 | 24/24  24/24 |
| 3  3\* | progesterone  D9-progesterone | 8.37  8.27 | 315.1>109  324.1>113 | 315.1>97  324.1>100 | 40 | 23/23  23/23 |
| 4  4\* | 21-deoxycortisol  D8-21-deoxycortisol | 4.77  4.69 | 347.1>121  355.4>113 | 347.1>311  355.4>319 | 20 | 21/21  18/21 |
| 5  5\* | 11-deoxycortisol  D5-11-deoxycortisol | 4.99  4.93 | 347.1>109  352.1>113 | 347.1>97  352.1>100 | 40 | 23/24  24/24 |
| 6  6\* | corticosterone  D8-corticosterone | 4.77  4.69 | 347.2>329  355.2>337 | 347.2>121  355.2>125 | 20 | 18/20  21/21 |
| 7  7\* | cortisol  D4-cortisol | 3.63  3.60 | 363.1>121  367.1>121 | 363.1>327  367.1>331 | 40 | 25/14  25/14 |
| 8  8\* | androstenedione  D3-androstenedione | 5.78  5.73 | 287.1>109  290.1>109 | 287.1>97  290.1>100 | 40 | 23/23  23/23 |
| 10  10\* | 4-androsten-11B-OL3-17-dione  D4-4-androsten-11B-OL3-17-dione | 4.22  4.18 | 303.2>267  307.2>270 | 303.2>285  307.2>289 | 40 | 18/18  21/21 |
| 11  11\* | 4-androsten-3-11-17-trione  D10-4-androsten-3-11-17-trione | 3.46  3.41 | 301.2>257  311.2>125 | 301.2>121  311.2>265 | 40  40 | 23/23  23/23 |
| 12  12\* | 4-androsten-17B-OL3-11B-dione  D3-4-androsten-17B-OL3-11B-dione | 3.89  3.86 | 303.2>121  306.2>121 | 303.2>259  306.2>262 | 40 | 23/23  23/23 |