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Thermal impairment of reproduction is differentially expressed in maiden and repeat spawning Atlantic salmon

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ABSTRACT

Groups of maiden or repeat spawning Atlantic salmon were maintained during vitellogenesis in austral autumn at either 14 °C or 22 °C through until April when all fish were transferred to a spawning temperature of 8 °C. There was no difference in body weight within groups for maidens and repeats, with repeats being consistently larger than maidens, no difference in condition factor amongst groups, but consistently higher gonad weight in repeats than maidens. Gonadosomatic index (GSI) and follicle diameter were suppressed in both maidens and repeats at 22 °C, with the effect being most marked in repeat spawners. Relative fecundity (egg kg^{-1}) determined from ovarian tissue samples also showed depression in repeats at 22 °C. Fish from both age classes held at 22 °C had a higher proportion of atretic follicles. Plasma levels of estradiol-17β (E_2) were strongly depressed in both maidens and repeats exposed to 22 °C throughout autumn but there was some evidence of recovery amongst maiden fish by late April. A similar effect was seen on plasma testosterone (T) levels. Plasma cortisol levels were generally low and typical of levels in unstressed fish indicating that stress did not account for the inhibitory effects observed. Hepatic *zona pellucida* protein gene expression was significantly inhibited in both maiden and repeat spawning fish reared at 22 °C, but with some evidence of recovery after temperature reduction to 8 °C. Hepatic vitellogenin (Vtg) gene expression was also lower in both maiden and repeat spawning fish exposed to 22 °C and this was accompanied by reduced plasma Vtg levels in maidens, but not repeats at 22 °C. Maidens at 14 °C began ovulating first followed by repeats at 14 °C, then repeats at 22 °C followed by maidens at 22 °C. There was reduced fertility in maidens at 22 °C relative to both maidens and repeats at 14 °C, whereas repeats at 22 °C showed intermediate fertility between 14 °C fish and 22 °C maidens. Survival to the eyed egg stage was highest in maidens at 14 °C, significantly suppressed at 22 °C in maidens, and at intermediate levels in repeats at both temperatures. This suggests that repeat spawning Atlantic salmon may be more robust in the face of thermal insult which combined with their larger size and egg production, could make their use desirable under production situations where there was any threat of exposure to higher than normal temperature.

1. Introduction

It is increasingly clear that climate change will strongly affect aquatic poikilotherms including fish, and this will be variously expressed through changes in community composition and structure, changes in species range, local and perhaps widespread extinctions of some species (reviewed in Graham and Harrod, 2009). A reasonable expectation is that salmonids will be particularly susceptible due to their distributional preferences for cooler water (e.g. Reddin et al., 2000); Welch et al., 1998a,b), and the fact that the complex anadromous life histories of many salmonid populations potentially expose them to thermal stress at a succession of critical life history stages (reviewed by Jonsson and Jonsson, 2009). Increases in temperature above optimal levels can have inhibitory effects on a range of biological processes including feeding and growth, behaviour, smoltification, disease resistance and reproduction (Battaglene et al., 2008; Graham and Harrod, 2009; Jonsson and Jonsson, 2009; Pankhurst and King, 2010; Steinum et al., 2008). A point of particular sensitivity appears to be reproductive development in females whereby inappropriately elevated temperatures impair or retard ovarian steroidogenesis – particularly 17β-estradiol (E_2) synthesis – and the subsequent hepatic synthesis of vitellogenin (Vtg), oocyte growth and development, oocyte maturation and ovulation, and egg fertility and survival (reviewed in Pankhurst and King, 2010).

Management strategies in culture situations are currently limited to thermal protection of broodstock during critical stages of vitellogenesis (Pankhurst and King, 2010); however, this requires significant infrastructure for thermal management and is not always

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possible in some aquaculture operations. A limited set of studies has examined the scope for endocrine therapy as a protectant during or after exposure to high temperature, mainly examining the value of treatment with synthetic analogues of gonadotropin releasing hormone (GnRHa). Treatment of an Australian cultured stock of Atlantic salmon (*Salmo salar*) with GnRHa was ineffective at maintaining fertility at high temperature but did restore fertility when applied in combination with a graded reduction in temperature (King and Pankhurst, 2004a). Similar experiments using longer release profile formulations of GnRHa in northern hemisphere stocks did result in maintenance of ovulation at elevated temperatures, but still with reduced egg survival at high temperatures (Vikingstad et al., 2008). The implication is that hormone therapy is only likely to be partially effective at offsetting the damaging effects of exposure to high temperature, although the more direct effects of gonadotropin or steroid therapy remain to be investigated (Pankhurst and King, 2010).

An unexplored area is the effect of thermal stress on different age classes of fish, with the majority of farmed salmon being from maiden or first-spawning broodstock. The first cycle of reproduction in maiden spawning Atlantic salmon covers the initial period of reproductive maturation or puberty. Studies on other species suggest that puberty is associated with an increase in hypothalamic GnRH mRNA and this is followed by FSH expression and secretion and increased levels of steroid hormones (Campbell et al., 2006; Okuzawa, 2002). In addition, the recently discovered G-coupled protein receptor GPR54 and its ligand the Kisspeptin have been shown to have an important role in initiating GnRH secretion (reviewed in Taranger et al., 2010). Blockades in the endocrine cascade prior to puberty have been identified at the levels of GnRH and FSH synthesis, and gonadotropin receptor binding at the gonadal level in a variety of species including salmonids (Gur et al., 2000; Nocillado et al., 2007; Okuzawa, 2002). The main effect of this appears to be the occurrence of a 'dummy run' with only partial endocrine activation and gonadal response, in the season before the first spawning period occurs, with the phenomenon being described in striped bass (*Morone saxatilis*) (Holland et al., 2000), masu salmon (*Oncorhynchus masou*) (Amano et al., 1992) and rainbow trout (*Oncorhynchus mykiss*) (Prat et al., 1996). In other species full 'endocrine maturity' may also not occur until the second spawning season. In both snapper (*Pagrus auratus*) (Cleary et al., 2000) and striped bass (Holland et al., 2000) relative ovarian size and plasma levels of gonadal steroids are lower in the first than the second spawning seasons. Cultured Atlantic salmon stocks in Tasmania are farmed towards the upper limit of their thermal tolerance range with the result that growth occurs at a faster rate, and developmental milestones are reached at a younger age than in their northern hemisphere counterparts, with most fish maturing as grilse after a single winter at sea (at 3 years of age) compared with 2 sea-winters in the northern hemisphere (King and Pankhurst, 2003). Rapidly maturing southern hemisphere Atlantic salmon does not appear to display the dummy run phenomenon. However, part of the inhibitory effect of elevated temperature could arise from its effect on grilse showing endocrine immaturity of the type described above for snapper and striped bass.

A key step in the maturation process is the production of E_2 by the developing ovarian follicle. E_2 is transported in the bloodstream to the liver where it binds to estrogen receptors (ER) in the hepatocyte cytoplasm. The E_2–ER complex in turn acts as a promoter for expression of the gene or genes coding for Vtg (reviewed in Watts et al., 2003), which is then sequestered into the developing oocyte through a process of receptor-mediated endocytosis (Tyler et al., 2000). The second important effect of E_2 is to stimulate hepatic synthesis of precursors of three structural proteins (collectively termed ZP) that will form the *zona pellucida* of the developing oocyte, and subsequently the chorion of the mature egg (Tyler et al., 2000). The genes coding for ZP are highly sensitive to stimulation by E_2 and ZP appear rapidly in the plasma soon after hepatic exposure to estrogens (Berg et al., 2004; Celius et al., 2000; Fujita et al., 2004). Disruption of E_2 synthesis and subsequent E_2–ER binding in the Tasmanian stock of Atlantic salmon is accompanied by chorionic abnormality, poor fertility and reduced embryonic survival (Pankhurst and King, 2010), suggesting that part of the effect may result from disruption of the expression of important E_2-inducible genes.

The present study examined whether the effect of thermal insult is differentially expressed in different age classes of Atlantic salmon. Maiden (3 year old) and repeat (4 year old) spawners were exposed to temperatures previously shown to inhibit reproductive development in this stock, and effects on ovarian growth and plasma levels of gonadal steroids and hepatic synthesis of Vtg were assessed. In addition, the E_2-dependent expression of genes coding for Vtg and *zona pellucida* (ZP) proteins was also measured.

2. Materials and methods

2.1. Fish husbandry and maintenance

Maiden (first spawning 2+year old fish) and repeat (second spawning 3+year old fish) cultured adult females were held at the SALTAS Wayatinah Hatchery (Tasmania, Australia) at ambient temperature and photoperiod in either 200 (maidens) or 50 (repeats) m^3 circular tanks at stocking densities of 12–18, and 24–36 kg m^{-3} for maidens and repeats, respectively until early January 2008. In January, fish were divided into treatment groups (n=28 per group) and transferred to temperature-controlled 4 m^3 tanks (14 fish per tank) under simulated ambient photoperiod. Fish were not fed from the time of transfer to the temperature controlled systems in January consistent with hatchery practice for management of this experimental stock of fish.

Treatment groups:

1. Maidens held at 14 °C;
2. Repeats held at 14 °C;
3. Maidens held at 22 °C;
4. Repeats held at 22 °C.

All fish were maintained at the nominated temperature (14 or 22 °C) until early April when all fish were exposed to a temperature ramp down over 11 days to 8 °C to induce final oocyte maturation and ovulation (King and Pankhurst, 2000). Temperature profiles for the two temperature regimes are shown in Fig. 1.

2.2. Sampling protocol

Fish from both maiden and repeat groups were sampled on the 31st August and 2nd November 2007, and 7th January 2008 to cover the initiation of vitellogenesis for each age class (Samples 1 to 3), and after introduction to the controlled temperature regimes on the 14th February 2008 (Sample 4), 28th March (Sample 5) and 25th April (Sample 6). Seven fish were sampled from each group at each sample time, leaving 7 fish from each treatment to proceed through to ovulation and stripping, after the final destructive sample in April.

For sampling, fish were netted from the holding tanks, terminally anaesthetised in Aqi-S ™ (Crop & Food, New Zealand), weighed, measured and then blood sampled by caudal puncture using pre-heparinised syringes fitted with 22 G needles. Blood plasma was centrifuged at 12,000 *g* for 3 mins, and stored frozen at −20 °C for later measurement of plasma hormones. Ovaries were excised, weighed and portions allocated to 50 mL-pots containing teleost saline or 10% neutral buffered formalin for fecundity estimation and follicle measurement, and histology, respectively. Segments of liver were transferred to 1–2 mL of RNA Later ™ (Qiagen, Germany) to stabilise mRNA for later measurement of gene expression. Samples were held overnight at 4 °C, then stored at −20 °C.