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Advanced ovarian development of Murray cod *Maccullochella peelii peelii* via phase-shifted photoperiod and two temperature regimes

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ABSTRACT

The timing and characteristics of reproductive development in adult female Murray cod exposed to a simulated seasonal photothermal cycle (14:45 to 09:45 daylight h; 12–26 °C) (CONTROL) were compared to the development of females exposed to a three month phase-shifted (advanced) seasonal photothermal cycle (PHOTOTHERMAL) and to females exposed to a three month phase-shifted (advanced) photoperiod cycle in combination with constant temperature (19.5 °C) (PHOTOPERIOD). Females in PHOTOTHERMAL and PHOTOPERIOD treatments reached maturity up to three (June) and four (May) months in advance of CONTROL fish (October), respectively. Biannual maturation was also observed in four PHOTOPERIOD females (13%). Mean ovary diameter and relative fecundity of mature females were similar between treatments ($p>0.05$), and viable eggs were produced in all groups (100% ovulated; 14.02%–39.12% mean survival to hatching). Ovary diameters and plasma levels of E_2 and T in phase-shifted females remained at basal levels and/or were significantly reduced ($p<0.05$) relative to CONTROL fish throughout the early to mid phases of the maturation period. However, rapid increases in plasma T (0.54–4.39 ng ml^{-1}) and ovary diameter (20.0–42.4 mm) in the 60 to 90 days preceding the onset of maturity in phase-shifted females revealed a capacity of Murray cod to accelerate development processes to compensate for earlier delays in photo-responsiveness. Low levels of E_2 that persisted throughout the maturation period of PHOTOTHERMAL and PHOTOPERIOD females did not appear to greatly affect ovarian growth. The successful maturation of photoperiodically-manipulated females under constant temperature demonstrates an alternative approach for influencing maturation patterns in Murray cod that may improve the versatility and cost-effectiveness of broodstock conditioning procedures.

1. Introduction

The Australian Murray cod *Maccullochella peelii peelii* is a large endemic freshwater fish with a distinct annual reproductive cycle and seasonally-defined spawning period (Rowland, 1998). Gonad development is initiated in austral autumn (March) and is generally completed by September as water temperatures approach 20 °C (Gooley et al., 1995; Newman et al., 2007).

Murray cod are currently the basis of a small, but emerging domestic and international aquaculture industry (Ingram et al., 2005). Intensive production methods for this species are developed (Ingram and De Silva, 2004), however, the confined spawning period and the current dependency of hatcheries on spawn harvested from earthen ponds, means that the present supply of seed stock commonly suffers from a lack of continuity and inconsistent production. The use of modified seasonal cycles of photoperiod and water temperature to advance the timing of broodstock maturation and spawning (reviewed by Bromage et al., 2001) appears to offer the greatest potential towards overcoming current restraints to Murray cod gamete production. However, the adaptation of these methods to this species is unrefined and is yet to be formally evaluated.

Various alterations to seasonal photothermal cycles have been employed to advance reproduction in a number of fish species, including uniformly compressed cycles, phase-shifted cycles, and strategic combinations of constant short and long days (e.g. Blythe et al., 1994a; Tate and Helfrich, 1998; Davies and Bromage, 2002). Photoperiod is generally acknowledged as the principal regulatory component in each of these regimes (Bromage et al., 2001), however, many environmental control protocols have ensured that both photoperiod and temperature cycles are modified concomitantly so that synergistic interaction between the two factors is maintained. Preserving the natural light-temperature interaction would theoretically enable conditions during gonad development to be optimised. However, in temperate zones, modifying high-amplitude seasonal temperature cycles so that they are in synergy with altered

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photoperiod cycles demands considerable heating and cooling of broodstock holding water and as a consequence, photothermal control of reproduction typically requires specialised thermal conditioning facilities that are often limited to their specific function. Therefore, the development of modified photoperiod cycles in conjunction with constant temperatures should enable individual rearing systems to facilitate more than one farming activity (e.g. division of broodstock to separate lighting regimes; egg/larval rearing; grow-out production; etc.) and thereby improve the versatility and cost-effectiveness of commercial operations. Nevertheless, the adoption of such alternative thermal regimes must first ensure that gonad function and photo-responsiveness of fish are not adversely impacted.

The present study quantified the effects of phase-shifted seasonal phototherm (incl. cycling temperature) and phase-shifted seasonal photoperiod (incl. constant temperature) on the timing of maturation and reproductive performance of female Murray cod. Temporal changes in ovary diameter (via ultrasound) and levels of plasma gonad steroids (17β-oestradiol [E_2] and testosterone [T]) were monitored throughout the reproductive cycle to indicate changes in oocyte development (Hobby and Pankhurst, 1997; Newman et al., 2008a).

2. Methods

2.1. Animals and rearing conditions

Domesticated Murray cod ($n=300$; 3+ years old), reared from juveniles in a recirculation aquaculture system (RAS), were purchased from a commercial supplier (Australian Aquaculture Products, Euroa, Victoria, Australia) in 2003 and transferred to a RAS facility (Spirit of the Sea Aquaculture, SSA) in Warrnambool, Victoria, Australia. All fish were considered maidens since no prior spawning activity had been observed. Fish were reared at SSA for a further two years under a 12:12 h light:dark cycle and a RAS-moderated seasonal temperature regime (~18–26 °C) that is typical of intensive farming conditions for this species. In August, 2005, 138 fish from the original stock (5+ years old; 4548±88 g mean body weight) were randomly selected and sexed using ultrasound (Newman et al., 2008a) and a passive integrated transponder tag (PIT tag; Trovan Microchips, Keysborough, Victoria, Australia) was implanted into the dorsal musculature of each fish to identify individuals. Fish were subsequently transferred to the Deakin University Aquaculture Centre (DAC), Warrnambool, and randomly allocated to six groups of 23 fish ($n=5$–7 males and $n=15$–17 females per group) which were held in one of six 2500 l (1.90 m diameter; 0.88 m depth) circular polyethylene tanks within three adjacent, replicate indoor RAS (1 group per tank, 2 groups per RAS). Each RAS within DAC was equipped with an independent water supply and water treatment plant, as well as separate lighting (TridonicAtco Pty. Ltd., Melbourne, Australia) and water temperature control (Aquahort Ltd., Auckland, New Zealand). Water in each system was treated using a rotating drum-screen filter, a floating microbead biological filter, a degassing column, and ultraviolet light sterilization.

Upon stocking, fish within each system were held under a simulated natural photothermal regime (Fig. 1a) for approximately 4 months prior to the commencement of trials. The photoperiod cycle used in this study mimicked the annual profile for Lake Charlegrark, Victoria (36°46′S, 141°15′E; Cadwallader and Gooley, 1985), however, dawn/dusk scenarios were not replicated (i.e. abrupt lights on/off). Seasonal water temperature profiles were based on annual temperature cycles recorded in the River Murray, Waikerie, South Australia (34°11′S, 139°59′E; Department of Water Resources, unpublished data). Throughout the acclimation and subsequent trial period, fish were fed to satiation (generally 0.1–0.3% body weight day^{-1}) with a commercial salmonid maturation diet (Skretting Vitalis SA; 9 mm pellet) using automatic belt feeders. Temperature, dissolved oxygen and pH were logged continuously using automated monitoring systems, and total ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were measured every second day. Water quality parameters were within optimal ranges for Murray cod RAS-culture (Boreham et al., 2004) throughout the duration of the trial (pH, 7–9; NH_4^+,<0.01 mg l^{-1}; NO_2^-,<0.1 mg l^{-1}; NO_3^-,<50 mg l^{-1}; total hardness and total alkalinity, 300–400 mg l^{-1}; turbidity, 150 FAU) and did not vary significantly between systems.

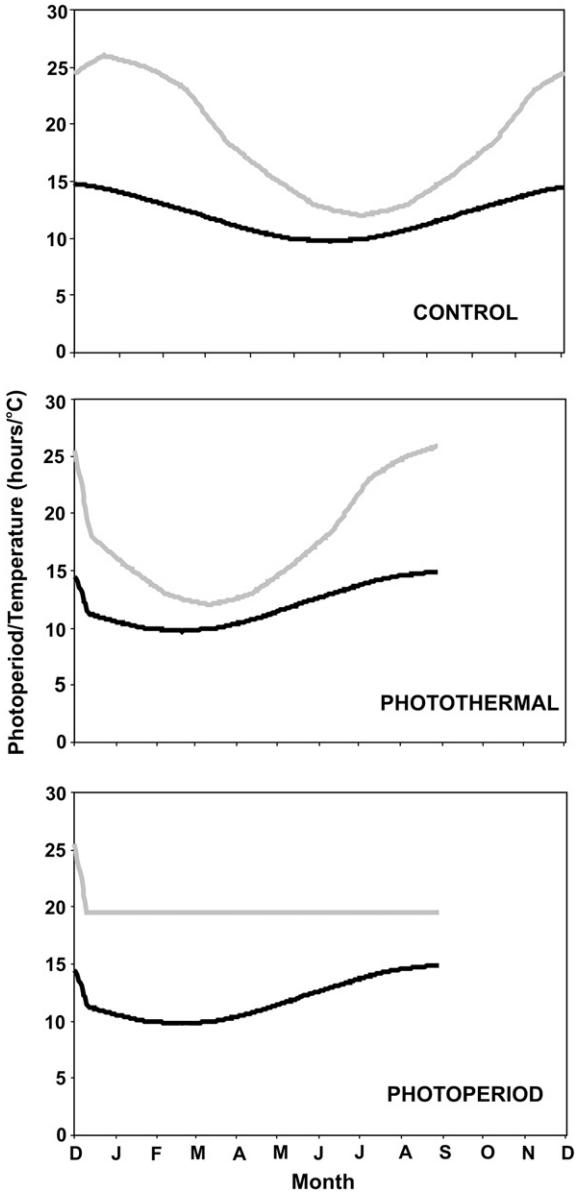

Fig. 1. Photothermal treatments (CONTROL, PHOTOTHERMAL and PHOTOPERIOD) used to condition Murray cod broodstock in this study. Photoperiod and water temperature are represented by black and grey lines, respectively.

2.2. Photothermal treatments

Manipulation of photothermal cycles was initiated at a time approximate to the austral summer solstice (late December, 2005) which corresponded with the late gonad regression phase of Murray cod (Gooley et al., 1995; Rowland, 1998; Newman et al., 2007). Each of the three RAS were exposed to separate photothermal treatments (Fig. 1) as follows: (a) CONTROL; simulated ambient photoperiod and temperature cycle; (b) PHOTOTHERMAL; three month phase-shifted