Aquaculture 303 (2010) 77–85

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Out-of-season gamete production in *Strongylocentrotus droebachiensis*: Photoperiod and temperature manipulation

Nicole T. Kirchhoff *, Stephen Eddy, Nicholas P. Brown

Center for Cooperative Aquaculture Research, University of Maine, Franklin, ME 04634, USA

ARTICLE INFO

Article history:
Received 13 November 2009
Received in revised form 19 February 2010
Accepted 2 March 2010

Keywords:
Spawning
Gametogenesis
Photoperiod
Sea Urchin
Temperature
Strongylocentrotus droebachiensis

ABSTRACT

The natural spawning of *Strongylocentrotus droebachiensis* is limited to late winter into early spring. In order to reduce the cost of urchin production, out-of-season gamete production is necessary. This study attempted to condition broodstock for out-of-season gamete production through the use of two different photoperiod and temperature regimes: a Constant Spring and an Advanced year; hypothesized to induce maturation through suspension or advancement of the gametogenic cycle, respectively. Viable gametes were successfully produced out-of-season with fertilization and hatch rates equal to or surpassing the levels recorded for wild urchin's in-season. In addition, specific fecundity was significantly higher in out-of-season conditioned urchins compared to the in-season wild urchins. Histological techniques proved that gametogenesis was both suspended and advanced through manipulation of the photoperiod and temperature. In addition, several important observations were made regarding reproductive physiology in the green sea urchin, the implications of which are important to the economic development of the green sea urchin aquaculture industry and management of wild stocks.

1. Introduction

Out-of season conditioning of sea urchins began in the 1960s (Fuji, 1967) in an attempt to increase market value through enhanced gonad yield. While gonad enhancement is an important step towards the development of sea urchin aquaculture, economical gamete production is also necessary (Harris et al., 2003; Luis et al., 2005; Walker et al., 1998). Research began on out-of-season gamete production in the green sea urchin, *Strongylocentrotus droebachiensis* (O.F. Müller, 1776) in the 1990s (Walker et al., 1998). However researchers have so far been unsuccessful in promoting out-of-season vitellogenesis and gamete production in this species.

One of the biggest hindrances to aquaculture development of the green sea urchin is the seasonality of natural spawning, which limits production to between four and seven months a year. Annual spawning of *S. droebachiensis* typically occurs in the spring, from February to May (Cocanour and Allen, 1967; Falk-Peterson et al., 1983; Himmelman, 1978; Larson et al., 1980; Meidel and Scheibling, 1998a; Munk, 1992; Oganesyan, 1998; Vadas et al., 1989). In central and northeastern Maine, where this study occurred, peak spawning takes place in March (Vadas and Beal, 1999), yet urchins can be induced to spawn from late-January to early-July (Oganesyan, 1998). The ability to manipulate spawning season would enable commercial sea urchin hatcheries to utilize facilities more efficiently.

The trigger of gametogenesis, and more specifically vitellogenesis, for *S. droebachiensis* is still under debate and has been intensely researched in recent years. Since each species of sea urchin has been found to have its own environmental or chemical cue, this field is still extremely underdeveloped. Three environmental factors are universally cited as important to the reproductive cycle: diet, photoperiod, and temperature (McBride, 2005). Giese (1959) was the first to suggest photoperiod to be a control of gametogenesis. It has been determined that short days (or the autumnal equinox) in the annual cycle are what triggers the initial stage of gametogenesis in some species such as *Strongylocentrotus purpuratus* (Bay-Schmidt and Pearse, 1987; Pearse et al., 1986), *S. droebachiensis* (Dumont et al., 2006; Walker et al., 1998),and *Eucidaris tribuloides* (McClintock and Watts, 1990). Walker and Lesser (1998), using the autumnal equinox equivalent photoperiod, produced mature *S. droebachiensis* males, but failed to produce mature females. Subsequently, they suggested that temperature is an important modulator of vitellogenesis in green sea urchins (Walker and Lesser, 1998). Wild observations on *S. droebachiensis* suggest that gametogenesis coincides with a drop in temperature from mid-December to January and spawning correlated with the rapid rise of seawater temperature in the spring (Stephens, 1972; Vadas et al., 1999). Seawater temperature has been found to be an important cue for out-of-season gametogenesis in some sea urchin species: *Anthocidaris crassispina* (McBride, 2005), *Hemicentrotus pulcherrimus*, and *Pseudocentrotus depressus* (Yamamoto et al., 1988). Each of these species lives in areas with large annual

* Corresponding author. Tel.: +1 61488474304; fax: +1 61363243804.
E-mail address: Nicole.kirchhoff@umit.maine.edu (N.T. Kirchhoff).

0044-8486/$ – see front matter
doi:10.1016/j.aquaculture.2010.03.003

fluctuations of seawater temperature (Sakairi et al., 1989; Yamamoto et al., 1988) much like the green sea urchin of Maine. A highly nutritious diet is also important for successful gametogenesis in *S. droebachiensis* (Ebert, 1968; Lemire and Himmelman, 1996; Minor et al., 1997; Thompson, 1982; Vadas, 1977), including various species kelp, their preferred diet (Ebert, 1968).

This study describes attempts to achieve out-of-season gamete production in *S. droebachiensis* using two different photoperiod/temperature manipulations. The first follows research on *Paracentrotus lividus* (Luis et al., 2005), using Constant Spring conditions of central to northeastern Maine: 12 L:12 D and 6 ± 0.3 °C. It was hypothesized that this technique should maintain the ripe condition of both males and females indefinitely, without triggering a new cycle of gametogenesis. The second conditioning treatment employed an Advanced regime, altered so that by mid November, the apparent photoperiod and temperature will be that of mid April, i.e. advancement of 6 months. It was hypothesized that this second technique should stimulate premature initiation and completion of gametogenesis in both sexes.

2. Materials and methods

2.1. System design

Two separate broodstock conditioning systems were designed and constructed, each with a separate water-treatment system contained within a 10′ × 9′ insulated, light proof enclosure. Each system consisted of 4 × 100 l holding tanks, a 2 m^3 sump/moving bed biofilter with plastic media, a ¾ HP circulating pump, a 35 µm cartridge filter, and a 40 W U.V. sterilizer. Lighting for each system was provided by an 8′ double fluorescent, T1 daylight fitting (0.580 W/m^2) fitted with electric timers to control the photoperiod. Each system was also thermostatically controlled with a 1 kW heater and a 1HP chiller. Water flowed into each holding tank via a spray bar mounted at one end of the tank and left via a surface drain at the other end. From the holding tanks, water drained directly into the aerated biofilter below, then pumped through the heater/chiller system, through the cartridge filter and U.V sterilizer before returning to the holding tanks. Mortality, water temperature, and dissolved oxygen were measured daily and water quality parameters (dissolved ammonia, nitrite, nitrate, pH, alkalinity, and salinity) were assessed weekly.

2.2. Establishing the photo-thermal conditioning regime

From March 1st to 15th 2006, each conditioning system was stocked with reproductively ripe wild adult (>40 mm test diameter) *S. droebachiensis*. Two hundred and seventy four urchins with a mean test diameter of 66.4 ± 1.0 mm were stocked into the Constant Spring system (Constant Spring group 1) and 251 urchins with a mean test diameter of 64.4 ± 1.1 mm were stocked into the Advanced system (Advanced System Group). The urchins were obtained by divers from three locations in Penobscot Bay, Maine, during a typical commercial harvest. Urchins were collected from a variety of bottom types, kelp, cobble or ledge, at depths between 5 and 15 m. They were transported moist to the Center for Cooperative Aquaculture Research (CCAR) within 2 h from landing at the dock and were randomly distributed among the two conditioning systems. After a one month acclimation period, the Constant Spring system was held at a constant photoperiod of 12 L:12 D and a temperature of 6 °C ± 0.3 °C. The Advanced system received an advanced photoperiod and temperature regime adjusted so that by the 19th of November 2006, the apparent ambient temperature and photoperiod would be similar to that of the 10th of April in the northeastern Gulf of Maine, i.e. an advancement of 6 months (Fig. 1). An additional 50 urchins were stocked into the Constant Spring system in March 2007 and conditioned in the same method (Constant Spring group 2). The urchins were fed to satiation weekly with fresh, locally harvested kelp (75% *Laminaria saccharina*, 15% *Laminaria digitata*, and 10% *Ulva lactuca*; approximately 10% dry matter), about 4.5 kg wet weight per system per week. Fecal pellets were siphoned biweekly and tank sides scoured twice per month in each conditioning system.

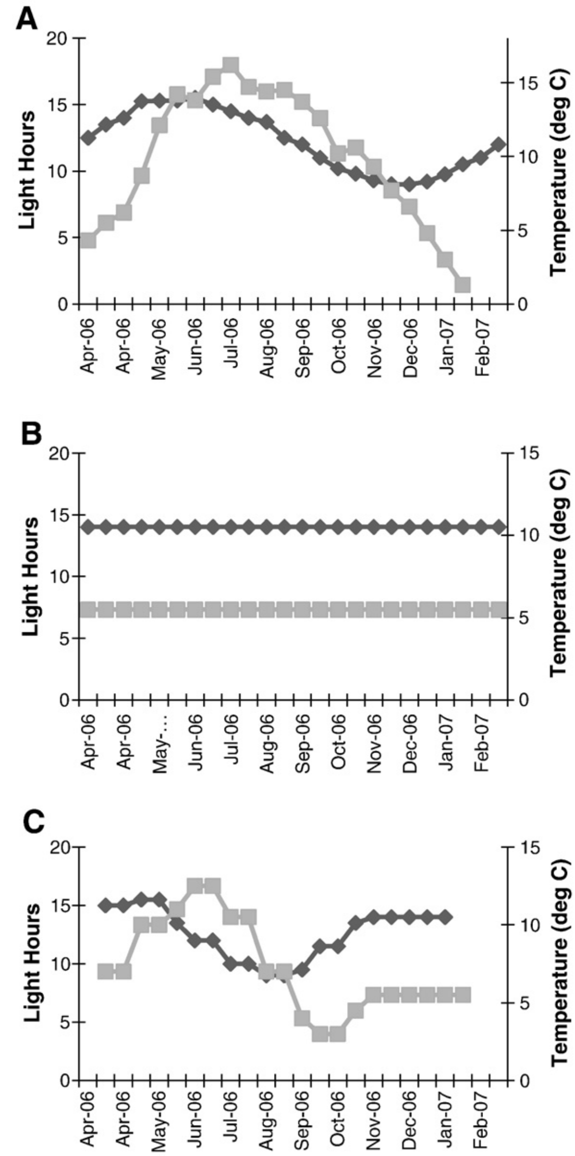

Fig. 1. Photoperiod and temperature conditions. (A) Ambient conditions in Penobscot Bay (B) Constant Spring conditioning system and (C) Advanced conditioning system. Temperature is denoted in grey, light hours in black.

2.3. Sampling

Samples were taken periodically from each system and from the field for morphological examinations, spawning trials, and histology/stereology (Table 1). For all samples, test diameter was measured using a ruler to the nearest 0.1 mm and the total body weight was measured to the nearest 0.1 mg. In addition, gonad weights were collected on all spawning and histology samples. The gonads were removed, rinsed with saltwater, blotted dry, and weighed to the nearest 0.1 mg. Whereas gonad weights and derived gonad indices are affected by test diameter, standardization needs to be performed prior to analysis (Harrington et al., 2007). Adjusted gonad weight (AGW) was calculated.