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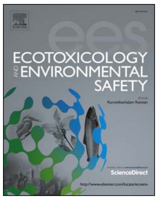

Transgenerational effects of ocean warming on the sea urchin *Strongylocentrotus intermedius*

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ABSTRACT

Transgenerational effects, which involve both selection and plasticity, are important for the evolutionary adaptation of echinoderms in the changing ocean. Here, we investigated the effects of breeding design and water temperature for offspring on fertilization, hatchability, larval survival, size, abnormality and metamorphosis of the sea urchin *Strongylocentrotus intermedius*, whose dams and sires were exposed to long-term (~15 months) elevated temperature (~3 °C above ambient) or ambient temperature. There was no transgenerational effect on fertilization and metamorphosis of *S. intermedius*, while negative transgenerational effects were found in hatchability and most traits of larval size. Dam and sire effects were highly trait and developmental stage dependent. Interestingly, we found *S. intermedius* probably cannot achieve transgenerational acclimation to long-term elevated temperature for survival provided their offspring were exposed to an elevated temperature. The present study enriches our understanding of transgenerational effects of ocean warming on sea urchins.

1. Introduction

The Intergovernmental Panel on Climate Change (IPCC) global assessment indicated that global mean seawater temperature will increase 2–4.5 °C by 2100 (IPCC, 2013). Ocean warming is one of the most prominent environmental changes, since it impacts both marine organisms (Dixon et al., 2015) and humans (Allison and Bassett, 2015). Elevated water temperature has been well documented to negatively affect growth (Wolfe et al., 2013), behavior (Brothers and Mcclintock, 2015), reproduction (Vilchis et al., 2005) and development (García and Clemente, 2015) of marine invertebrates with poor mobility in the inter-tidal and shallow seawater. Thus, it is essential for them to make adaptive and/or plastic changes to ocean warming to avoid mass mortality or even extinction.

Empirical evidence shows that animals can evolve more rapidly in changing environments than we previously thought (Singh et al., 2015) because evolution depends more on existing genetic and epigenetic variation than new mutations (Lande and Shannon, 1996). Marine invertebrates with a short generation period have a strong potential for adaptive evolution in response to ocean warming in a number of generations (Chen and Stillman, 2012). Marine invertebrates with a long generation period, on the other hand, require short-term resilience to ocean warming before evolutionary responses may occur (Foo and Byrne, 2016). However, transgenerational effects of ocean warming are poorly understood in marine invertebrates with long generation periods (Foo and Byrne, 2016; Ross et al., 2016).

Sea urchins are ecologically important key species in structuring marine benthic communities, both as grazers and prey (Pearse, 2006). Although sea urchin species are found in the deep sea (Pawson and Pawson, 2013), better known sea urchin species are distributed in intertidal and shallow seawaters off the coast (Lawrence, 2013), and potentially could be impacted by ocean warming. The sea urchin *Strongylocentrotus purpuratus* shows a strong capacity for local adaptation in response to ocean acidification (Pespeni et al., 2013). This indicates that genetic and/or epigenetic variation can be a source of adaptation of sea urchins against ocean changes in future. However, it remains largely unknown whether sea urchins have transgenerational selection and/or acclimation in ocean warming, which probably potentiates their adaptive evolution (Ghalambor et al., 2015). Recently, Suckling et al. (2015) found that fertilization, hatchability, larval survival and abnormality did not significantly decrease in the sea urchin *Sterechinus neumayeri* whose parents were exposed to 17 months of elevated temperature. However, sire/dam effects and a number of fitness related traits (for example, larval size and metamorphosis) were not involved in their study. Further, Suckling et al. (2015) investigated transgenerational effects only at an optimal seawater temperature for offspring. This consequently makes the transgenerational selection and/or acclimation to ocean warming largely untested in sea urchins.

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Strongylocentrotus intermedius, which is found in seawaters off the coasts of northern Japan, Korea, Far East Russia and northeastern China, is a representative cold, shallow seawater sea urchin species with a limited range of distribution, poor mobility, and a long generation period of ~2 years (Agatsuma, 2013). Generally, the optimal temperature is below 20 °C for *S. intermedius* (Chang et al., 1999; Agatsuma, 2013). Mass mortality occurred when juvenile *S. intermedius* were exposed to water temperature over 23 °C (Hokkaido Central Fisheries Experimental Station, 1984). Our previous study indicates that long-term exposure to elevated temperature significantly impacted a number of traits of *S. intermedius* (Chang et al., 2016). Considering the ecological importance and susceptibility to high water temperature, *S. intermedius* is a good research model to investigate the transgenerational effects of marine invertebrates to ocean warming.

The main purposes of the present study are to investigate 1) whether embryos and larvae of *S. intermedius* show significantly positive or negative transgenerational effects if their dams and/or sires are exposed to long-term elevated temperature; 2) whether the offspring fitness of *S. intermedius* shows significant sire and/or dam effects in accordance to their long-term thermal experience; 3) whether transgenerational effects of *S. intermedius* differ when offspring are exposed to ambient or elevated temperature; 4) whether transgenerational effects are useful for aquaculture of *S. intermedius*; 5) whether the present study can provide valuable information into climate change biology of marine invertebrates with a limited distribution in cold shallow seawaters.

2. Materials and methods

2.1. Parental sea urchins and their temperature treatments

The source of parental sea urchins and methods of seawater treatments were fully described in our previous report (Zhang et al., 2017) with some differences, which are summarized as follows:

Juvenile *S. intermedius* were transported from Dalian Haibao Fishery Company to the laboratory at Dalian Ocean University on March 5, 2015. Seawater temperature treatments lasted for ~15 months (from July 8, 2015 to October 19, 2016) before the transgenerational experiments. Ambient temperature (group L) was that of filtered seawater pumped from the field. It was measured daily. High temperature (group H) was set at ~3 °C higher than the ambient temperature, an increase relevant to the predicted increase in seawater temperature. In order to avoid mass mortality of *S. intermedius*, 23 ± 0.5 °C was set as the upper limit for group H (Hokkaido Central Fisheries Experimental Station, 1984), and thus 20 ± 0.5 °C for group L, although field water temperatures were over 20 °C in a few days in summer. Water temperatures of groups L and H were allowed to fluctuate during the temperature treatments regulating by a seawater temperature control and recirculation system (Huixin Co, China) (Fig. 1a). Water salinity and pH value were irregularly measured during the parent acclimation using a portable water quality monitoring meter (YSI, USA). They were 30.10–32.98‰ and 7.84–8.35, respectively. *S. intermedius* did not spawn before the breeding experiments at either ambient or elevated temperature. When the breeding experiments were carried out, the seawater temperatures were ~18 °C and ~21 °C in groups L and H, respectively. Thus, ~18 °C and ~21 °C were the temperatures used for all following fertilization assays.

2.2. Experimental design for transgenerational effects

S. intermedius of groups L and H were used as parents for the following breeding experiments. To detect potential transgenerational effects, the experiments had the following design: 4 breeding designs × 2 offspring temperatures × 4 replicates. The experimental design is shown in the conceptual diagram of Fig. 2, left. To analyze the potential sire and/or dam effects, we made a simple breeding design of the North Carolina II (Lynch and Walsh, 1998), which mated N_S sires with N_D dams for parental males and females from groups L and H to generate $N_S \times N_D$ offspring families (Foo and Byrne, 2016). In the breeding design, group A refers to dams from group H crossed with sires from group H, group B refers to dams from group L crossed with sires from group L, group C refers to dams from group H crossed with sires from group L and group D refers to dams from group L crossed with sires from group H (Fig. 2, left). To investigate the effects of water temperature on offspring, *S. intermedius* of all breeding groups (A, B, C and D) were exposed to the ambient and elevated temperatures (~3 °C above ambient), respectively, consequently establishing eight experimental groups (designated as HA, HB, HC, HD, LA, LB, LC and LD). HA and LA represent the offspring of group A exposed to high and ambient temperatures, respectively. The groups HB, HC, HD, LB, LC and LD were designated accordingly (Fig. 2, left). Ambient temperatures for offspring (16.5 °C to 18.5 °C, Fig. 1b) were among the optimal temperature (15.2 °C to 18.5 °C) for fertilization, hatchability, larval development and metamorphosis of *S. intermedius* (Wang and Chang, 1997).

Spawning of sea urchins was induced by the injection of 1 mL KCl (0.5 M). Sperm were collected dry and eggs were collected in filtered seawater. To investigate the effect of water temperature for offspring, we kept dams' genital pores being submerged while spawning alternately in two separated bottles with seawater at ~18 °C and ~21 °C, ensuring approximately the same amount of eggs in each bottle. Spawning time was limited to 30 min to ensure gamete quality. Collected eggs were filtered and transferred to 3 L bottles at the appropriate temperatures using a fine silk net (mesh size: 106 µm). According to the mating design, 500 mL of egg suspension (~ 0.1 million eggs) were collected from the well mixed seawater in the 3 L bottle and fully mixed with ~50 µL dry sperm for fertilization. According to the method of Suckling et al. (2015), our preliminary experiment indicated that this egg: sperm ratio is optimal for the fertilization of *S. intermedius*. The egg: sperm ratio was kept approximately the same across the experiment, although sperm concentration was not calculated. The breeding experiments described above were exactly repeated using different dams and sires four times from October 19, 2016 to October 23, 2016 (N = 4), establishing 16 families at each of the two offspring temperatures (Fig. 2, left).

2.3. Larval and juvenile cultures

Fertilized eggs were accordingly transferred into 32 separated cylindrical cages (226 cm^2 × 25 cm, ~5 L). They were washed twice to remove excess sperm two and four hours after fertilization. The embryo density was ~20 ind/mL. After measurement of fertilization and hatchability, hatched blastulae in the upper 3/4 of the seawater were collected to remove dead and unhatched blastulae at the bottom of the cages. They were transferred to 32 separated tanks of ~20 L (length × width × height: 32 cm × 20 cm × 30 cm) for further larval and juvenile culture (Pespeni et al., 2013). These tanks were bathed at L and H temperatures throughout larval development (Fig. 1b). All tanks were constantly aerated. Hatching density for all tanks was adjusted 30 h after fertilization to 0.3–0.5 ind/mL. Larvae in all tanks were fed with the microalga *Chaetoceros gracilis* three times a day in the weakly aerated seawater at concentrations from 1×10^4 to 7×10^4 cells·mL^{-1}.day^{-1}. About two thirds of the seawater was changed each day using a fine silk net (mesh size: 48 µm). Fertilization, embryo and larval development were under natural photoperiod (~0–500 lx) using sand filtered seawater (30.21–32.01‰ salinity and 7.93–8.18 pH). Same methods and similar conditions were used for larval and juvenile cultures in all experimental groups to avoid the involvement of the non-experimental factors.