The effects of photoperiod and temperature on embryonic diapause termination in the hermit crab *Pagurus nigrofascia*

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Received 18 May 2020; Accepted 5 October 2020 Responsible Editor: Satoshi Wada

doi: 10.3800/pbr.16.79

Abstract: The intertidal hermit crab *Pagurus nigrofascia* reproduces in March in Fukuoka, Japan. The embryos remain in diapause until October, begin to develop in November, and most broods hatch in December. This study examined the effects of photoperiod and temperature on embryonic diapause termination (EDT) in both laboratory and field conditions. Females with diapause eggs were maintained in the laboratory under three different photoperiod/temperature regimes: constant (14L/10D, 22°C), decreasing photoperiod condition (from 12L/12D to 9.8L/14.2D, 22°C), and decreasing temperature condition (14L/10D, from 22°C to 10°C). The EDT date was compared among four groups: the above three experimental groups and the field group. The dates at which >50% of females possessed eggs after the EDT were November 10 (constant), October 30 (decreasing photoperiod), September 27 (decreasing temperature), and November 4 (field). The mean duration until EDT was 82 days (constant), 67 days (decreasing photoperiod), and 36 days (decreasing temperature). Diapause was significantly shorter under both the decreasing photoperiod and the decreasing temperature conditions compared to the constant. Thus, EDT was strongly affected by temperature but weakly altered by photoperiod. This study is the first to report the influence of photoperiod and temperature on EDT in decapod crustaceans.

Key words: embryonic diapause termination, intertidal hermit crab, *Pagurus nigrofascia*, photoperiod, temperature effects

Introduction

Pagurus nigrofascia is a hermit crab that inhabits intertidal boulder shores and has only been recorded in a few sites in Japan (Komai 1996). In Hakodate, northern Japan, *P. nigrofascia* females produce a brood in May and incubate it for 9 months until the eggs hatch in February (Goshima et al. 1996). This 9-month incubation period is particularly long for hermit crabs inhabiting intertidal areas (Wada et al. 2000, 2005). In Fukuoka, southern Japan, females produce a brood in March, and the eggs hatch in December after the 9-month incubation period (Mishima & Henmi 2008). Eight months after spawning, the eggs become dormant in the blastula stage (yolk-filled, with-

out a blastodisc), a strategy known as embryonic diapause (Mishima & Henmi 2008).

The term ‘diapause’ has been widely used in entomology but had not been applied to crustaceans until recently (Stross & Hill 1965). Most crustacean species exhibiting embryonic diapause belong to the classes Branchiopoda, Maxillopoda, and Malacostraca (Hairston & Cáceres 1996). The crayfish *Astacus astacus* has a diapause of 3–4 months, and the eggs show no change between the blastoderm formation and the endomesodermal embryo (Cukerzis 1988). Wear (1974) reported egg diapause in the spider crab *Hyas coarctatus* and the masked crab *Corystes cassivelaunus*. The snow crab *Chionoecetes opilio* in the Gulf of St. Lawrence, Canada, has two distinct diapause periods: the first occurs during the gastrula stage and lasts for 6 months, and the second ensues during eye-pigment formation and lasts for 3–4 months (Moriyasu & Lanteigne 1998). There is

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also a 1-month diapause during the reduced yolk stage in *C. opilio* in the eastern Bering Sea (Webb et al. 2007).

The hermit crab *P. alatus* inhabits the Mediterranean Sea at depths of 350–450 m, and ovigerous females occur throughout the year, except during March. The eggs are without eye pigments from April to September during a possible diapause stage (Mura et al. 2006). Ovigerous *P. proximus* females are found from December to August in the subtidal zone of Vostok Bay in the Sea of Japan, and their embryos remain at an early developmental stage until May (Kornienko et al. 2019). Among the intertidal decapod crustaceans, only *P. nigrofascia* are known to have an embryonic diapause stage, and this stage occurs during summer ('aestivation'), as opposed to winter ('hibernation'), which is observed in the aforementioned freshwater or deep-sea decapods.

Very few studies have tried to clarify the control mechanisms of diapause in decapods. Westin & Gydemo (1986) showed that the duration of egg development decreased from 9 months to 4 months under an experimental high-temperature condition in the noble crayfish *Astacus astacus*, and in some *Chionoecetes* cold water crabs, diapause is directly related to temperature (Moriyasu & Lanteigne 1998, Webb et al. 2007). It is not yet evident whether environmental factors other than temperature affect the duration of diapause in decapod embryos, although photoperiod has been shown to affect embryonic diapause termination (EDT) in many other crustaceans (Stross 1969, Miller et al. 1991, Alekseev & Starobogatov 1996). In the flagellates, moreover, photoperiod and temperature are two major factors affecting cyst germination (Bravo & Anderson 1994, Imai & Yamaguchi 2012), although cyst formation is markedly different from embryonic diapause. The onset of embryonic diapause in *P. nigrofascia* may be controlled by intrinsic factors since the eggs become dormant at early stage; whereas the termination of diapause could be controlled by intrinsic and/or external factors. In this study, we examined the influence of external factors, specifically photoperiod and temperature, on EDT in *P. nigrofascia*.

Materials and Methods

Incubation conditions

We conducted the experiment from August 22 to December 29, 2007 (129 days). *P. nigrofascia* were collected on August 21, 2007, from Wajiro beach (33°40'N, 130°25'E), Fukuoka, Japan. Each ovigerous female ($n=120$) was individually placed into a 300 mL beaker containing 200 mL of seawater and randomly divided into the three experimental groups ($n=40$ crabs each). Each group was housed in a separate incubator (BITEC-300, Shimadzu Rika Institute Co., Ltd., Japan) that was illuminated using a straight daylight fluorescent tube (20 W) placed on the interior ceiling. The seawater in each beaker was changed weekly, and the hermit crabs were fed

crushed Hikari Crest (Kyorin Co., Ltd., Japan). The incubators had photoperiod/temperature regimes as follows (see Figs. 1 & 2): the constant condition (Group 1) was maintained on a constant 14L/10D light schedule at 22°C throughout the experiment; the decreasing photoperiod condition (Group 2) began on a 12L/12D light schedule, which was gradually reduced to 9.8L/14.2D, and maintained at 22°C throughout the experiment; the decreasing temperature condition (Group 3) was maintained on a 14L/10D light schedule initially at 22°C and gradually reduced to 10°C. Differences in duration until EDT among

Fig. 1. The photoperiod regime in three experimental groups: Group 1 (constant), Group 2 (decreasing photoperiod condition), Group 3 (decreasing temperature condition) and in the field (data from National Astronomical Observatory of Japan).

Fig. 2. The temperature regime in three experimental groups: Group 1 (constant), Group 2 (decreasing photoperiod condition), Group 3 (decreasing temperature condition) and in the field.

Fig. 3. The percentage of females with each egg stage in Group 1 (constant), Group 2 (decreasing photoperiod condition), and Group 3 (decreasing temperature condition). The number of days from August 22 is shown in parentheses.

experimental groups (Group 1–3) were evaluated using Cox proportional-hazards regression analysis (Cox, 1972, Yamada et al. 2018) using R ver. 4.0.2 (<http://cran.r-project.org/>). The analysis included experimental groups and dates (nested within groups) as explanatory variables.

Ecological basis of incubation conditions

In Fukuoka, the day length varies from 14.4 hours (at summer solstice) to 9.9 hours (at winter solstice) (National Astronomical Observatory of Japan). At the start of the experiment on August 22, the day length was approximately 14 hours, and therefore we housed Groups 1 and 3 under a 14 h constant light regime. Group 2 was adjusted from 12 hours of light per day, which corresponds to the autumnal equinox, to 9.8 hours, which approximates that of the winter solstice.

To determine the incubation temperature, we considered our data in 2004, which showed that the monthly mean temperature at the study site (lower intertidal) peaked at 27°C in August. Unfortunately, we could not sustain such a high temperature due to the difficulty of maintaining the water conditions in the beakers. Instead, we determined a temperature for Groups 1 and 2 of 22°C, which corresponded to a mid-October temperature at the field site. We reduced the temperature of Group 3 to 10°C, which corre-

lates to field conditions in December.

Determination of embryonic diapause termination

We determined the egg developmental stages by holding the shells with tweezers under a stereomicroscope and waiting for the hermit crabs to emerge and reveal their pleopods with eggs. The egg stages (A–E), determined based on Wada et al. (1995), were measured on September 16, October 8 & 21, November 4 & 18, and December 1, 16 & 29 (see Fig. 3). We defined Stage F as partly hatched broods, Stage G as completely hatched broods, and EDT as broods at Stage B (yolk absorption and germinal disc appearance).

Comparison with the field and recording temperatures

To confirm egg development in the field, we collected *P. nigrofascia* females on Wajiro beach on August 12, September 30, October 28, November 25, and December 24, 2007. We recorded the egg stage of each female and measured the seawater temperature at the lower tidal zone every hour using temperature data loggers (Stowaway Tidbit, Onset Computer Co., MA, USA). The mean temperature was calculated every 2 weeks (see Fig. 2).

Results

Egg development and EDT in the experimental groups

Egg development in the experimental groups is shown in Fig. 3. In each group, the number of crabs varied because of death, loss of eggs, or crabs not emerging from their shells. For instance, in Group 1 on October 21, we observed that 28 crabs had Stage A eggs, 3 crabs had lost the majority of their eggs, and 9 crabs did not emerge from their shells. In Group 1, most crabs attained EDT during the experimental period despite the constant photoperiod/temperature conditions, but 2 crabs remained in Stage A on December 29, 2007. In Group 2, all crabs attained EDT by November 18, 2007, and in Group 3, all crabs attained EDT between September 16 and October 8, 2007.

We defined the EDT date for each female as the midpoint between the last day of Stage A and the first day of Stage B. The mean duration until EDT excluding censored data was 81.6 days in Group 1 ($n=22$), 67.4 days in Group 2 ($n=23$) and 36.0 days in Group 3 ($n=38$). Durations until EDT were significantly different among all experimental groups ($df=2$, $\beta=0.913$, Wald statistics=42.86, $p<0.001$). The duration until EDT in Groups 2 and 3 were also significantly different from that of Group 1 (Group 2: $z=1.12$, $p<0.001$; Group 3: $z=5.13$, $p<0.001$).

Percentage of EDT in the experimental groups and the field

The percentage of EDT in each treatment group and in the field is shown in Fig. 4. The dates when more than half of females possessed eggs after EDT were November 10 in Group 1, October 30 in Group 2, September 27 in Group

Fig. 4. The percentage of embryonic diapause termination (EDT) in three experimental groups: Group 1 (○; constant), Group 2 (□; decreasing photoperiod condition), Group 3 (▲; decreasing temperature condition) and in the field (●).

3, and November 4 at the field site. The diapause dates of Groups 1 and 2 were almost similar to that at the field site. In contrast, the embryonic diapause of Group 3 was terminated more than a month earlier than in the field.

Discussion

To our knowledge, this is the first study to experimentally test the influence of photoperiod and temperature on EDT in decapod crustaceans. We found that temperature was an obligatory condition of diapause termination for *P. nigrofascia* embryos, and that photoperiod influenced EDT to a lesser extent. In intertidal areas, temperature fluctuations are unpredictable, and photoperiodism may act as an additional influence for the timing of egg development. However, the degree to which environmental factors such as temperature and photoperiod affect EDT can vary among regions or seasons. Some organisms may respond to relative changes as opposed to absolute values of these variables, or these factors may interact with one another. For example, *P. nigrofascia* females produced broods in May in Hakodate and in March in Fukuoka, but EDT occurs in November at both locations, despite differences in climate and latitude (Goshima et al. 1996, Mishima & Henmi 2008). In Group 1 in this study, however, most crabs attained EDT despite the constant light and temperature conditions. This can indicate the presence of region-specific and endogenous long-range timers.

Alekseev & Starobogatov (1996) reviewed many studies of diapause in marine, freshwater, and terrestrial crustaceans and concluded that photoperiod affected the timing of EDT in certain species (1 Anostraca species, 2 Notostrepta species, 2 Cladocera species, and 3 Calanoida spe-

cies). Stross (1969) reported that EDT occurred at day lengths longer than 20 hours in an arctic population of cladoceran *Daphnia middendorffiana*, although the EDT percentages were affected by the culture density. In these crustaceans, however, females do not directly affect the induction and termination of diapause because their eggs are released into the water immediately after spawning. In contrast, in Pleocyemata (a suborder of decapod crustaceans), the fertilized eggs are incubated until hatching. Females of *P. nigrofascia*, a species under that suborder, incubate their eggs for 9 months in Fukuoka (Mishima & Henmi 2008), and during the summer, they move to the upper intertidal zones and estivate under boulders (Mishima & Henmi 2008). Therefore, diapause in Pleocyemata is expected to be greatly affected by the physical environment of incubation sites.

Few available studies have examined embryonic diapause in hermit crabs. In *P. alatus* on the Mediterranean Sea bottom (350–450 m depth), all ovigerous females were shown to have eggs without eye pigments from April to September (Mura et al. 2006). Their diapause period is similar to that of *P. nigrofascia* (March–November) in Fukuoka, but the diapause timing is likely only affected by temperature, unlike that of *P. nigrofascia*, as sunlight does not reach these depths. Ovigerous *P. proximus* females were found from December to August in the subtidal areas of Vostok Bay, Sea of Japan, but the embryos remained at an early developmental stage until May (Kornienko et al. 2019), as in this region, the surface water temperature decreases to negative values in winter months and reaches 10°C in May. Additionally, the length of embryonic diapause varies among individuals, ranging from the absence of diapause to up to 5 months of diapause. This relates to the proportion of ovigerous females increasing from December to May, and as a result, the larval release being concentrated in June. In Hakodate, ovigerous *P. proximus* females were found from January to July (7–21°C), with no embryonic diapause (Wada & Mima 2003). Thus, the environmental signal for EDT may be temperature in *P. proximus*.

Anger (2001) suggested that embryonic diapause is an adaptation that alters the duration of the incubation period to synchronize the timing of larval release with the period of abundant primary production. In *P. nigrofascia*, the mass release of larvae occurs mainly in December in Hakodate and Fukuoka (Goshima et al. 1996, Mishima & Henmi 2008), whereas in *P. proximus*, it occurs in early June in Vostok Bay (Kornienko & Korn 2016). The timing of larval release differs between species but may match the season of phytoplankton blooms, which differ based on water temperature. Petersen & Anger (1997) suggested that the embryonic diapause of the high-latitude majid *Hyas araneus* coordinates hatching with plankton production; such traits should be expected to evolve at high latitudes.

Although our study site was not at a high latitude, we presume that *P. nigrofascia* have evolved their long incu-

bation period and embryonic diapause with the advance in upper intertidal zones. Females of this species widely distribute into the intertidal zones during spring, and most produce broods in March (Mishima & Henmi 2008). During summer, females retain diapause eggs, and travel to the upper intertidal areas to estivate under boulders. They return to lower areas in autumn and release larvae in December. Thus, *P. nigrofascia* has adapted to the upper intertidal areas, where the environmental conditions are severe, by adopting the special life history traits of estivation and embryonic diapause. The present study sheds light on the influences of photoperiod and temperature on EDT in the hermit crab *P. nigrofascia*. However, the estivation in the intertidal areas is a unique trait even among crustaceans; therefore, further studies are required to clarify the effects of environmental conditions on the induction and termination of embryonic diapause. In addition, such studies help to clarify the adaptive significance of diapause.

Acknowledgements

We thank Akiyo Maenaka and Keiko Shiohira for their assistance in the laboratory. We also thank two anonymous reviewers for their valuable comments on the manuscript.

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