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Reproductive cues in *Panulirus ornatus*

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Abstract Two experiments were performed to assess the effect of photoperiod and temperature on spawning of *Panulirus ornatus*. In experiment 1, sexually mature lobsters taken from the wild during summer were held at one of two photoperiods, winter (13 Light:11 Dark) and summer (14.5 Light:9.5 Dark). Additionally, lobsters were also exposed to either summer (29°C) or winter (24°C) average water temperatures. Spawning was significantly greater when animals were exposed to summer photoperiod than to winter photoperiod, irrespective of temperature. Although a higher percentage of lobsters spawned when placed under a higher temperature, this trend was not statistically significant. In experiment 2, sexually mature lobsters were taken from the wild during winter and exposed to the same two photoperiods as in experiment 1, at a summer equivalent temperature of 29°C. Breeding started earlier and was more successful at the summer photoperiod. Time to first breeding was 17 weeks after exposure to summer photoperiod, compared with less than 1 week in experiment 1, and did not occur until individuals had moulted. Moulting occurred in 81% of lobsters, primarily after an increase in temperature to 29°C. The time between moulting and mating was varied and there was no significant difference in moult frequency between the two experimental photoperiods. After the lobsters had moulted, breeding success was reached earlier if photoperiod was lengthened. Results suggest photoperiod is the primary cue for the onset of gonad maturity and mating activity, with temperature playing a less important role. Physiological rest and possibly a moult may be required between breeding seasons before spawning can occur. Furthermore, temperature may be an important cue for pre-reproduction moulting.

Keywords *Panulirus ornatus*; photoperiod; temperature; breeding; moulting

INTRODUCTION

In marine lobsters, the relative importance and magnitude of temperature and photoperiod that induce gonad development, differs between species according to natural environment and breeding season (Muesy & Payen 1988). For example, Chittleborough (1976) identified temperature alone as the primary stimulus for gonad development in *Panulirus cygnus* which has a distinct spring/summer breeding season. This is in contrast with *Panulirus japonicus* which depends on both photoperiod and temperature to cue the onset of gonad maturation (Matsuda et al. 2002). Although temperature was important, gonad development in *P. japonicus* was retarded at photoperiods of less than 12.5 h of light per day. Findings were similar for *Panulirus argus* for which gonad development and mating behaviours were significantly enhanced in animals held at longer day-length, irrespective of temperature (Lipcus & Herrnkind 1985). Breeding in many lobster species has been controlled in captivity through the manipulation of environmental factors, in particular photoperiod and temperature (Lipcus & Herrnkind 1985, 1987; Matsuda et al.

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2002). To be able to successfully breed *P. ornatus* in captivity it is important that the breeding cues are first properly identified for this species.

Panulirus ornatus is known to take part in a mass annual summer breeding migration from the Torres Strait, across the Gulf of Papua to Yule Island (Moore & MacFarland 1984). However, breeding populations of *P. ornatus* on the north-east Queensland coast are non-migratory (Bell et al. 1987). In both instances, breeding is nonetheless seasonal with berried females reported in summer months when photoperiod is at its annual longest and water temperature at its highest level.

This paper aims to assess the use of photoperiod and/or temperature by *P. ornatus* as cues to the onset of breeding.

METHODS

To determine the effects of photoperiod and temperature on spawning in *P. ornatus*, wild-caught mature lobsters were exposed to different combinations of these factors. As the reproductive condition of lobsters brought into captivity for experimentation would likely influence the response to controlled environmental stimuli, lobsters were exposed to winter conditions of water temperature and photoperiod (as measured from their typical natural environment), before the application of experimental conditions for a period of 4 weeks. To mimic photoperiods that *P. ornatus* would be exposed to in nature, mean summer (14.5 Light (L) : 9.5 Dark (D)) and a mean winter (13L:11D) photoperiods were used. These were chosen as they represent nautical day lengths as recorded at Cockburn Reef (11°49′E 143°21′S) on the east coast of Queensland, from where experimental animals were collected and where breeding individuals had been found (Anon. 2002b). Similarly, water temperature equivalent to summer (29 ± 0.3°C) and winter (24 ± 0.3°C) in this location were also used (http://www.auslig.gov.au/geodesy/astro/ (accessed 2002)).

To elucidate the effects of light and temperature on breeding, two photoperiod treatments (summer and winter) were applied to sexually mature and active lobsters maintained at summer or winter (24°C and 29°C) (experiment 1). To determine if time of year (i.e., season) also influenced breeding, the above photoperiod treatments were also applied to seasonally sexually inactive lobsters maintained, in winter, at a summer temperature of 29°C (experiment 2).

Both experiments were conducted in six 2000 litre round polyethylene tanks supplied with semi-recirculated sea water, within an environmentally controlled room. Three tanks were applied to each of the two treatments within a separate recirculation system. Each system was connected to a 2000 litre sump. Water in each system was recirculated continually at the rate of 330 litre/h^{-1} providing a total replacement of water in each tank 4 times per day.

Water from each tank drained to the sump through a screen to collect larger solids, and was then pumped through bead and fluidised bed filters, a protein skimmer and a heat pump before delivery back to the tanks. Water temperature for each of the two systems was controlled using Aqua hort™ heat exchange units and logged in each system by Gemini Tinyview™ temperature loggers.

Light was applied by single 20-watt halogen waterproof lights mounted on the wall of each covered tank. Maximum light levels of an intensity of 110 lux were used to simulate typical light levels found in the natural environment of *P. ornatus*. Light was remotely controlled by a Clipsal Pty Ltd C-Bus™ home automation system which ramped tank light levels up and down at specific programmed times. Sunrise and sunset were programmed to occur over 17 min each daily within each tank. Light was monitored and logged by Stowaway SLA-08™ light loggers.

Each tank was equipped with a freestanding PVC table shelter measuring 600 mm × 800 mm and standing 250 mm high. A 1 m^2 strip of plastic mesh (10 mm mesh size) was suspended from the edge of each tank and weighted so as to partially cover an area of the tank wall and floor to enable spawning females to hang vertically, as an aid for oviposition (Berry 1970).

Previous studies have shown that in wild breeding populations, females with carapace lengths (CL) of >100 mm comprise the majority of reproductive animals (MacFarlane & Moore 1986). Consequently, only females >100 mm and <130 mm CL were used in these experiments. Similarly, as mating success has been demonstrated to improve with increased male relative to females size (Berry 1970), only males >130 mm CL were used. In the wild, at recognised breeding areas for *P. ornatus*, males tend to be larger and females more abundant (ratio of males to females is 1:1.5 or greater) (MacFarlane & Moore 1986). Lobsters for the experiments were chosen accordingly. Seven adult lobsters, two male and five female were assigned to each tank (male to female ratio of 1:2.5). Animals were selected from commercial landings from Cockburn Reef (11°49′E

143°21′S), north-eastern coast of Australia in October 2002 and 2003 and April 2003.

Individual weight, CL, and sex were recorded at the time of stocking to the experimental tanks. Lobsters were identified using individually numbered tags, consisting of small round pieces of waterproof paper glued to the base of the rostrum using Locktite 454™ instant adhesive. Moulted individuals were recognised during daily tank checks as those without a tag, and based on the tagged exuvium were identified (by patterns between the frontal horns) and re-tagged.

Lobsters were stocked to the experimental tanks and then conditioned to average winter temperature and photoperiod by incrementally adjusting water temperature to 24°C ± 0.3°C and photoperiod to 13L:11D over a period of one week to stimulate them to a non-breeding condition. For experiment 1, this involved a reduction from ambient summer conditions, and for experiment 2, from ambient winter conditions. Lobsters were held in these conditions for 4 weeks. For initiating the treatment effect in both experiments, photoperiod was increased immediately from the winter (13L:11D) condition to summer (14.5L:9.5D) photoperiod in half of the experimental tanks. For experiment 1, water temperature was maintained at either 24°C ± 0.3°C or increased to 29°C ± 0.3°C overnight. For experiment 2, the water temperature in all tanks was increased overnight to 29°C ± 0.3°C at this point.

Experimental conditions (post conditioning) were maintained until a majority had spawned (3 weeks for experiment 1). In experiment 2, at 16 weeks post-conditioning, the photoperiod for the winter, short day treatment was increased to 18L:6D to impose a large photoperiodic increase in an attempt to shock the animals into breeding. Females were examined on a weekly basis to check for spawning activity.

At the end of the experiments, weights and CL were re-recorded. Animals from experiment 1 that had not spawned were dissected and the ovaries macroscopically staged from 1–4 as follows.

Stage 1: Immature. Ovaries white, flattened dorso-ventrally.
Stage 2: Developing. Ovaries pink to pale orange, noticeably enlarged.
Stage 3: Ripe. Ovaries bright orange to red, greatly enlarged.
Stage 4: Spent. Ovaries white, yellow or pale pink, often with a few enlarged ova retained from stage 3 at overall lobe extremities (often indistinguishable from stage 1).

pH, salinity, dissolved oxygen, ammonia, and nitrite were measured and recorded weekly and more frequently if outside the desirable range. Food provided to the experimental lobsters consisted of live and frozen pipis *Plebidonax deltoides*, frozen green mussels *Perna canaliculus*, and squid *Loligo opalescens* provided once per day after 1500 h. Animals were fed at the rate of 3% body weight per day consistently for all tanks (approximate maximum food intake from pilot study).

The proportion of females within each treatment that had spawned was analysed by application of a two-way ANOVA using Genstat 5th ed. (Lawes Agricultural Trust). Data was first ArcSin transformed to normalise the distribution. Additionally, ANOVA was used to asses differences in weight and CL between both sexes and tanks.

RESULTS

Experiment 1

Of the original female stock held at 24°C, 30% unexpectedly produced unfertilised eggs during the conditioning period and were excluded from the trial. As a consequence, only four of the six proposed replicates were run with unspawned animals repositioned into four tanks to maintain the initial stocking density. A similar phenomenon was observed for the 29°C trial, however more animals were conditioned which, therefore, enabled the stocking of the full six available tanks. Three weeks after the application of the two photoperiod treatments, significantly more females had spawned under the summer photoperiod (14.5L:9.5D) than at the winter photoperiod (13L:11D) ($P = 0.030$) irrespective of temperature (Fig. 1). Temperature slightly increased breeding success, however not significantly ($P = 0.380$) (Fig. 1). Dissections of the remaining animals found significantly greater ovary development in animals held under summer photoperiod ($P = 0.004$) (Fig. 2).

There was no significant difference in weight within or between tanks for each sex. Males were significantly heavier and had significantly longer CL than females within each tank ($P = 0.001$). Neither CL nor weight was found to have affected breeding ($P = 0.324$ and 0.176 respectively).

Experiment 2

Moulting rate increased dramatically 4 weeks after the conditioning period was completed, i.e., after the temperature had been increased to 29°C (Fig. 3). No

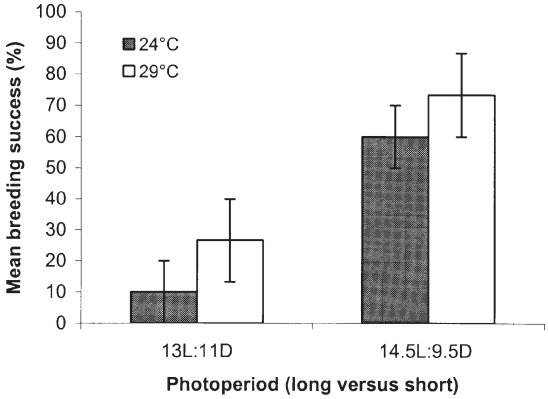

Fig. 1 Percentage of lobsters (*Panulirus ornatus*) (female) spawning in each tank under two photoperiod treatments: short day (13L:11D) and long day (14.4L:9.5D) at either summer or winter average temperature.

significant difference in frequency of moulting was evident between winter and summer photoperiod (P = 0.505, n = 42). When no spawning occurred after 16 weeks, but the bulk of lobsters moulted, it was postulated that a pre-reproduction moult was necessary before reproduction could be triggered. In an attempt to cue the onset of reproduction the winter (13L:11D) treatment was increased to 18L:6D (Fig. 3). Breeding occurred first in the animals held in the summer photoperiod treatment, 17 weeks after the end of conditioning (Fig. 3). Breeding began 2–3 weeks later for populations exposed initially to winter photoperiod.

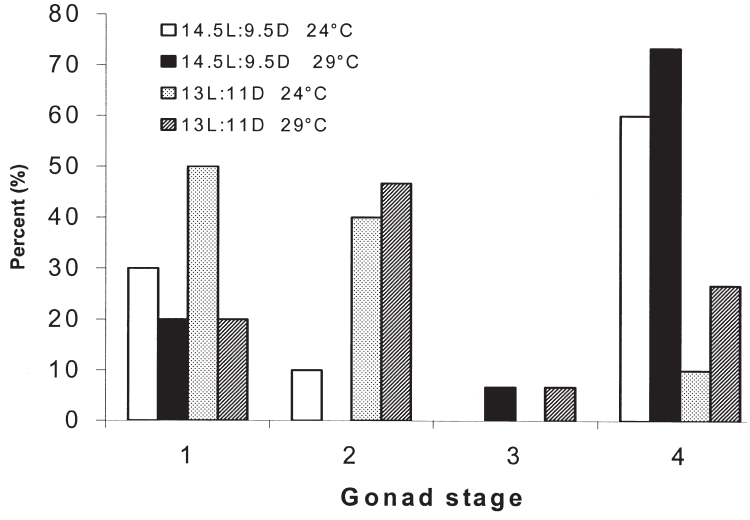

Fig. 2 Stages of ovary development from animals held under long or short day lengths at both temperatures (%). Spawned animals were assumed to be at a spent (stage 4) condition and recorded as such. Stages are described under methods.

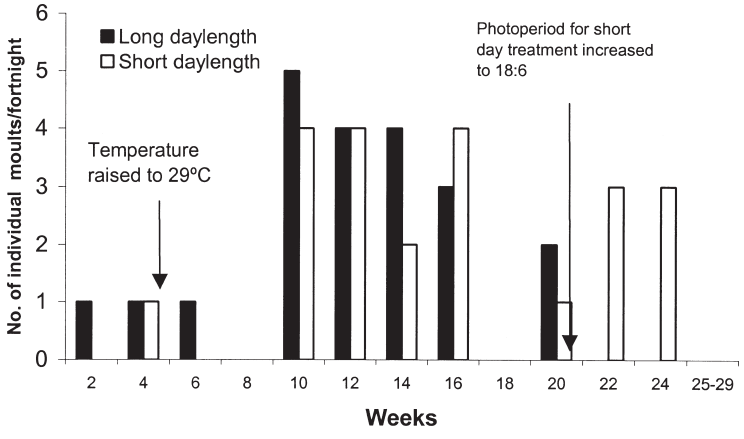

Fig. 3 Overall number of moults per fortnight through the experiment for each treatment (long and short photoperiod). Conditioning period ended after 4 weeks, at which time water temperature was raised to 29°C.

DISCUSSION

Panulirus ornatus is known to take part in migration to breeding aggregation sites during summer (Moore & MacFarlane 1984; Bell et al. 1987). Breeding is seasonal with berried females reported within summer months annually. Seasonally changing environmental parameters may be used by this species as cues to aid synchrony of migratory behaviour/breeding. However possibilities are numerous and may include, for example, seasonal changes in water temperature, photoperiod, or other abiotic stimuli (Herrnkind 1980). Temperature and photoperiod were tested in this experiment. As breeding occurs in *P. ornatus* populations when photoperiod is at its annual longest and water temperature at its highest, different combinations of these factors were assed as cues for breeding in *P. ornatus*.

Results from experiment 1 implicate photoperiod as a significant cue for breeding in *P. ornatus*. Photoperiod is used to cue processes such as diapause, hibernation, breeding, and migration in many species of animals including marine invertebrates, resulting most probably from annual reliability (Herrnkind 1980; Gwinner 1981; Olive 1995). Findings from this study compare with other similar studies which have also determined photoperiod to be an important environmental cue to breeding in other palinurid species (Lipcus & Herrnkind 1985, 1987; Matsuda et al. 2002).

Why photoperiod is used as a primary cue and not temperature may be related to the occurrence of mass migrations for spawning *P. ornatus*. General trends in water temperature, although most likely important to moulting and gonad maturation, are subject to significant variation within season or local geographic area. Although mass spawning populations of *P. ornatus* are restricted within the species distribution, local variations in water temperature across this range, especially at small isolated reefs, may not allow for synchronised timing of the migratory and spawning event. However, photoperiod as a feature of latitude would be expected to vary little over the range of this species and as such may be a more reliable cue, allowing for breeding synchrony across the population (Olive 1995).

Temperature appears to be of less significance. However, it is possible that this was shown to be non-significant because of low sample size within this trial. Spawning rates were increased (non-significantly) and dissected gonads were further advanced in animals held under high temperatures. It is likely that temperature is in some way related to the onset of breeding. However, based on the results of this study it is probably a less important breeding cue for this species.

The absence of breeding activity in experiment 2 (17 weeks exposed to the experimental treatments) was unexpected. In all respects, experiment 2 was equivalent to experiment 1 with only one major difference: the experimental lobsters were obtained from the wild during winter (in a non-breeding condition) relative to those of experiment 1 which were obtained in summer. Although the summer photoperiod cue may be appropriate to stimulate spawning, it may only work for lobsters physiologically prepared. An important part of this may be a necessity to moult before breeding. The dramatic increase in moulting of lobsters in experiment 2 after the temperature was raised suggests that the increase in temperature may be a cue for pre-reproductive moulting. Twelve weeks after the sudden increase in moulting, spawning activity was still not evident and the photoperiod of the winter photoperiod treatment (13L:11D) was increased to 18L:6D to provide a further cue to trigger onset of reproduction. This appeared to have an effect with breeding occurring in subsequent weeks. It may be possible to somewhat hasten breeding through the induction of a moult and subsequent increase in photoperiod post moult.

The speed with which these treatments have their desired effect has important implications to the establishment of management protocols for year-round breeding. Although it may be possible to shift the reproductive season, using controlled conditions, to enable breeding out of phase with wild populations, it may not be possible to significantly condense the reproductive cycle into a period less than 12 months. Additionally, condensation of the breeding season may have consequences for larval quality. Although several other tropical Palinurid species have been shown to breed year round (Macdonald 1982; Juinio 1987; Briones-Fourzan & Lozano-Alvarez 1992), continuous breeding of the same captive lobsters, many successive times, may not generate high quality eggs and larvae. For example, it is possible that lobsters forced to breed for two or more seasons in one year may hatch larvae of lesser quality than those that undergo the usual one breeding season annually. The establishment of multiple breeding populations that are out of phase with wild season is likely to be the most effective way to generate high quality larvae year round.

The necessity for a pre-reproduction moult is well documented for many crustaceans. In lobsters,

moulting provides the female with fresh ovigerous setae on the endopods of the pleopods which are critical for attachment of the freshly laid eggs (MacDiarmid & Kittaka 2000). In establishing protocols for year-round breeding, it may be necessary to stimulate a moult before the initiation of mating and spawning.

Although this preliminary work has isolated some of the cues important to breeding, further research into the endocrinology of moulting and gonad development as well as flow-on research into effects of different breeding regimes on larval quality may provide a more complete understanding of *P. ornatus* reproduction.

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