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Weakly synchronized larval release maintained in the presence of predatory fishes

Leif K. Rasmuson [a,*], Jessica L. Couture [b], Steven G. Morgan [c]

[a] University of Oregon, Oregon Institute of Marine Biology, PO Box 5389, Charleston, OR 97420, United States
[b] University of California Santa Barbara, Bren School of Environmental Science & Management, 2400 Bren Hall, Santa Barbara, CA 93106-5131, United States
[c] University of California Davis, Department of Environmental Science and Policy, Bodega Marine Laboratory, PO Box 247, Bodega Bay, CA 94923, United States

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ABSTRACT

Many marine species minimize predation during hatching or spawning by releasing larvae or gametes synchronously during nocturnal spring ebb tides. Propagules are then rapidly transported away from high densities of predatory fishes into deeper waters under the cover of darkness. Females also suspend foraging in the presence of predators, but it is unknown whether they are able to delay releasing larvae. In a previous study, we found that larval release is only weakly synchronized to the safe period in a cold upwelling region, although the study was conducted on outdoor seawater tables in the absence of tides that reinforce endogenous rhythms. In this study, we experimentally determined whether larval release 1) is better synchronized in the field and 2) delayed in the presence of predatory fishes. Larval release was weakly synchronized to tidal amplitude, tidal and diel cycles, occurring from late flood to late ebb tide on both intermediate and spring tides during twilight as well as darkness. Weak synchrony likely arises because cold temperatures extend incubation of externally brooded embryos, increasing exposure to environmental variation. Larval release was not delayed in the presence of predatory fishes; nor was refuge use or other behaviors of females altered by fishes. Behaviors were not affected by predators presumably because larvae are already being released near the safe period from refuges. Our results likely apply to other cold regions of the world, but it remains to be determined whether predators alter the activities of nonovigerous female and male shore crabs, which may not spend as much time in refuges as ovigerous females, potentially resulting in cascading effects on communities.

1. Introduction

Reproduction by many species of coastal marine animals occurs when predation on adults or offspring is minimized relative to diel, tidal and tidal amplitude cycles (Morgan, 1995; Morgan and Christy, 1995; Thurman, 2004). Reproduction occurs at night when parents and offspring are least visible to predators (Hovel and Morgan, 1997; Morgan, 1990). It also occurs near high tides and especially spring high tides, when intertidal species can release larvae or gametes from the safety of refuges (Christy, 1986; Morgan, 1996a; Morgan and Christy, 1995) and larvae or gametes are transported by ensuing ebb tides to deeper waters where fewer predators occur (Hovel and Morgan, 1997; Johannes, 1978; Morgan, 1995). Thus, reproduction by many crabs and fishes is synchronized to coincide during the safest phases of all three environmental cycles: nocturnal, spring, and high tides (Morgan, 1995; Thurman, 2004).

In upwelling regimes, larval release by crabs appears to be only weakly synchronized (high variance around the mean) to the safe period (Christy, 2003, 2011; Kerr et al., 2012; Morgan et al., 2011). Cold upwelled water and exposure to variable temperatures during low tide increase the incubation time of embryos, which in turn, likely increases variation in the timing of hatching. In warmer upwelling regions, female crabs may adjust the timing of mating, regulate their depth in burrows or choose the width of burrows during courtship to compensate for variation in development rates of embryos arising from small changes in temperature (Christy, 2011; Christy et al., 2001; Kerr et al., 2012; Reaney and Backwell, 2007). However, these compensatory behaviors may be less effective when cold temperatures lengthen incubation periods, especially for species that live in depressions under rocks rather than in burrows (Christy, 2003; Morgan et al., 2011).

Although the proximal and ultimate factors regulating reproductive synchrony have been well studied in crabs, it remains unknown whether females can delay releasing larvae in the presence of predators (Morgan, 1995). Predators are well known to affect foraging by many animals with cascading nonconsumptive indirect effects on communities (Schmitz et al., 2004; Trussell et al., 2006; Werner and Peacor, 2003). Further, in some species, such as snails and amphibians, the presence of predators induces embryos to hatch raising the possibility that crustacean embryos may potentially be able to chemically detect predators (Blaustein, 1997; Miner et al., 2010; Warkentin, 2011a, 2011b). Females even may delay releasing larvae in the presence of predators if embryos that are ready

* Corresponding author. Tel.: +1 541 888 2581; fax: +1 541 888 3250.
E-mail address: Rasmuson@uoregon.edu (L.K. Rasmuson).

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to hatch can be postponed. Predatory fishes abound along shorelines, and females, embryos and larvae all may be vulnerable during larval release (Hovel and Morgan, 1997; Morgan, 1990; Yoklavich et al., 1991).

We determined the effectiveness of intertidal crabs to regulate the timing of larval release in cold upwelling regimes in the presence of predators. Our previous study showing that larval release by several species of crabs was weakly synchronized to the safe period in a cold upwelling regime was conducted on outdoor seawater tables in the absence of tides, and it is possible that larval release could be more synchronous in natural populations where tides reinforce endogenous rhythms (Morgan et al., 2011). To test this hypothesis, we determined the timing of larval release relative to the safe period in the field. We then experimentally determined whether the presence of predatory fishes substantially delays larval release in the laboratory. We tested two species of fishes, one that preys on adult crabs and a second species that feeds on larvae, to determine whether predation on adults or larvae cause females to delay the release of larvae. We concurrently observed whether fishes altered foraging and other behaviors of females to determine whether ovigerous females were generally threatened by these fishes.

We chose the lined shore crab, *Pachygrapsus crassipes* (Randall 1839), because this high intertidal species should release larvae more synchronously relative to the safe period than species that live lower on the shore (Morgan, 1995; Morgan and Christy, 1995; Thurman, 2004). *P. crassipes* inhabits exposed rocky shores and estuaries where it primarily feeds on algal films and spawns from April through September brooding embryos for 16–29 d (Hiatt, 1948). Average carapace width of female and male *P. crassipes* is 40 and 47 mm, respectively, and adult density varies among estuaries (Morgan et al., 2006). The predator on adults was the cabezon, *Scorpaenichthys marmoratus* (Ayers 1854), which feeds on *P. crassipes* and other benthic invertebrates and fishes (O'Connell, 1953). The planktivore was the three-spined stickleback, *Gasterosteus aculeatus* (Linnaeus 1758), which preys on *P. crassipes* larvae and other zooplankton (Rasmuson and Morgan, 2013).

2. Materials and methods

2.1. Timing of larval release

We determined the timing of larval release by *P. crassipes* relative to the lunar, tidal amplitude, tidal and diel cycles for 30 d (June 14–July 13, 2006) in the field. Ovigerous *P. crassipes* ($n = 60$) were captured by hand and placed in a mesocosm that was secured to the substrate near a large population of *P. crassipes* in Bodega Harbor, California. Crabs that released larvae during the first 15 d of the study were replaced with 30 ovigerous females to ensure that sufficient numbers of ovigers were present to release larvae during the second half of the study. Seawater temperature ranged from 9.4 to 15.8 °C with a mean temperature of 11.8 °C during the study.

The mesocosm ($1 \times 0.5 \times 0.5$ m) was constructed of plastic. A lid was constructed of clear Plexiglass covered with Vexar mesh (0.5 cm) to allow sunlight to enter and heat to escape the mesocosm. Ninety two holes were drilled in the walls of the mesocosm (5-cm diameter) and were covered with Nitex mesh (135 μm) to permit water exchange while excluding most zooplankters and all predators and containing newly released larvae. Rocks amid the adjacent natural population of crabs were collected and placed inside the mesocosm to provide cover and food for these herbivorous crabs. Temperature loggers (Tidbit, Onset Inc.) were secured inside and outside of the mesocosm to ensure that temperatures inside the mesocosm were similar to ambient temperatures. A manual submersible pump was attached to the mesocosm, and seawater (240 l) was pumped every 30 min during high tides following inundation (tidal height = 1.25 m). Pumped seawater was discharged through a plankton net (333 μm) that was suspended in a bucket. Before the start of each daily sampling period, the mesocosm was pumped to clear any larvae that might have been released between observation times. Mechanical difficulties precluded sampling for one day (June 25). Larvae from each 30 min sample period were preserved separately in 95% ethanol, and the amount of larvae released was quantified volumetrically (ml). An average of seven (range 2–11) samples were collected during each sampling period.

Whether larval release occurred relative to the diel (24 h) cycle was determined using a G-test after categorizing data as daylight, nighttime and twilight (sunset and sunrise ± 90 min). Larval release relative to semilunar (15 d), tidal amplitude (12–17 d) and tidal (12 h) cycles in the mixed-semidiurnal tidal regime was analyzed using circular statistics, Rayleigh test. For significant peaks of larval release, we related means and standard deviations to the phasing of environmental cycles to determine the timing and degree of synchrony. During our one-month study, larval release was determined relative to two semilunar cycles, two tidal amplitude cycles, 30 diel cycles and 60 tidal cycles.

2.2. Affect of predators on the timing of larval release

We conducted a laboratory experiment to determine whether 1) the presence of predators reduces the number of female crabs that release larvae, 2) females delay larval release until after predators leave and 3) predators of females or their larvae are more likely to affect the timing of larval release. The timing of larval release was expected to occur near high tide, when all intertidal species of crabs worldwide release larvae (Morgan, 1995; Thurman, 2004). However, the precise timing of larval release varies among individuals, especially in this cold upwelling regime where larval release by *P. crassipes* is weakly synchronized to the tidal cycle (Morgan et al., 2011). The present study confirmed that peak larval release occurred within the first 3 h following high slack tides. Therefore, we expected to be able to detect a delay in the timing of larval release from the first 3 h to the last 3 h of ebb tide in the presence of predators.

The experiment was conducted during July and August 2006. Ovigerous *P. crassipes* were collected by hand during low tide from the population in Bodega Harbor, and fishes were seined from an adjacent eelgrass bed. The crabs and both species of fishes (*S. marmoratus* and *G. aculeatus*) were held separately in flow-through outdoor tanks under ambient light conditions. A simulated tidal cycle coincided with local tides to reinforce endogenous tidal rhythms and water level oscillated (±18 cm). Continuous water flow maintained ambient water temperature and oxygen levels. Tanks were filled with rocks and gravel that were collected together with female crabs to provide a familiar refuge.

Embryos of all crabs were inspected before each trial to ensure that they were fully developed and ready to be released. These females were placed into experimental tanks for observation during the morning of each trial. Because larval release by *P. crassipes* was not highly synchronous, the number of ovigerous crabs that were ready to release larvae varied from day to day ranging from three to eight crabs per tank. The number of fishes in each of three predator treatments remained constant throughout the experiment: two juvenile *S. marmoratus*, five adult *G. aculeatus* and no predators. More *G. aculeatus* than *S. marmoratus* were placed in each tank, because the latter species is smaller and more prevalent (Rasmuson and Morgan, 2013). Three replicate aquaria (110 l) were used for each of the three treatments resulting in a total of nine tanks per trial. The small tanks created a high-density environment to ensure encounters between predators and prey. Ten trials were conducted on different nights resulting in 30 replicates per treatment.

Table 1
Timing of observations and the introduction and removal of fishes to determine whether *P. crassipes* delayed releasing larvae relative to high tide or altered other behaviors in the presence of predators.

Time relative to high tide	1 h before	High tide	3 h after	6 h after
Observation period	X		X	X
Predator		Introduced	Removed	