Title

Studies on the reproductive biology of the mud crab, Rhithropanopeus harrisii: effects of sex ratio and stocking density on egg production

Authors
Morgan, SG
Goy, JW
Costlow Jr, JD

Publication Date
2023-12-11

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Author(s): Steven G. Morgan, Joseph W. Goy, John D. Costlow and Jr.

Source: *Journal of Crustacean Biology*, Vol. 8, No. 2 (May, 1988), pp. 245-249
Published by: {brill} on behalf of The Crustacean Society
Stable URL: http://www.jstor.org/stable/1548316
Accessed: 02-04-2015 12:23 UTC

JOURNAL OF CRUSTACEAN BIOLOGY, 8(2): 245-249, 1988

EFFECT OF DENSITY, SEX RATIO, AND REFRACTORY PERIOD ON SPAWNING OF THE MUD CRAB *RHITHROPANOPEUS HARRISII* IN THE LABORATORY

Steven G. Morgan, Joseph W. Goy, and John D. Costlow, Jr.

ABSTRACT

Adult mud crabs, *Rhithropanopeus harrisii,* were held in the laboratory for 14 months at densities of 20 or 40 crabs per 0.5 m^2 and at female : male ratios of 2:1, 4:1, and 6:1. Females spawned for 9 months during the calendar year and entered a refractory period from November through January. Field populations in North Carolina spawn for only 5 months of the year. Spawning increased with increasing crab density and female : male ratio. Ovigers were most abundant in habitats containing 40 crabs at a 6:1 sex ratio (8.3 ovigers per week), and were least abundant in habitats containing 20 crabs at a 2:1 ratio (2.8 per week). However, the highest proportion of crabs spawned in habitats containing 20 crabs at a 6:1 sex ratio. Neither density nor sex ratio affected female mortality. Crabs at the lowest sex ratio (2:1) and highest density (40 crabs) produced larvae that survived poorly (45% survival to first crab stage). However, larval viability was good at all other densities and sex ratios. Maintaining crabs at high densities and female : male sex ratios in the laboratory, even during a portion of the nonbreeding season, augmented egg production. These methods can facilitate studies of reproduction and development of *R. harrisii.*

Temperate brachyuran crabs generally breed only in summer. However, the mud crab *Rhithropanopeus harrisii* Gould and the stone crab *Menippe mercenaria* Say have been induced to mate and spawn during winter under laboratory conditions (McConaugha *et al.,* 1980; Goy *et al.,* 1985). *Rhithropanopeus harrisii* is a small, euryhaline crab that occurs in estuaries from New Brunswick, Canada, to Veracruz, Mexico, and northeastern Brazil. The species also has been introduced to Coos Bay, Oregon, San Francisco Bay, California, northwest Europe, and the Black Sea (Williams, 1974). Perhaps there have been more studies of the reproductive and developmental biology of *R. harrisii* than of any other crab. The nearly 100 laboratory studies of the larval biology of *R. harrisii* include investigations on temperature and salinity responses (Costlow *et al.,* 1966), toxicity (Laughlin *et al.,* 1983), chromatophores (Pautsch, 1967), molting (Freeman and Costlow, 1984), regeneration (Freeman, 1983), biochemistry (Sanders *et al.,* 1984), predation (Morgan, 1987), larval behavior (Forward *et al.,* 1986), and larval release (Rittschoff *et al.,* 1985). The common, robust adults spawn frequently and are easy to collect and maintain. The larval development of *R. harrisii* is brief, taking approximately 17 days to the first crab stage at 25°C, and larval survival is consistently high at about 85–90% (Costlow *et al.,* 1966).

Individuals of *Rhithropanopeus harrisii* spawn repeatedly from April to September in North Carolina estuaries. Females may oviposit up to four times after a single mating, requiring 3–7 days to spawn again after hatching (Morgan *et al.,* 1983). *Rhithropanopeus harrisii* was induced to spawn into December by maintaining them in the laboratory at 24°C and under a 12:12 h day : night photoperiod (Goy *et al.,* 1985). In this study, we investigated the duration of reproductive activity of *R. harrisii* throughout the year under laboratory conditions, and the effect of density and female : male sex ratio on spawning. Both maximization and efficiency of spawning are considered. The highest reported natural densities of *R. harrisii,* 40 crabs/m^2 (Odum and Heald, 1972), is between the densities used in this study.

METHODS AND MATERIALS

Adult mud crabs were collected occasionally from the Neuse River estuary, North Carolina, from April to August 1980 and April through May 1981, and placed in a large (60 × 180 × 50 cm) holding tank. Artificial "habitats" were stocked on 1 May 1980 at densities of 20 or 40 crabs (29 or 58/m^2), and at female : male ratios of approximately 2:1 (14:7, 27:13), 4:1 (16:4, 32:8), and 6:1 (18:3, 35:6). Habitats were off-white copolymer tubs (58.5 × 58.5 × 63.5 cm) equipped with under-

Table 1. Spawning, mortality, and larval viability of overwintered *Rhithropanopeus harrisii* maintained at various stocking densities and sex ratios.

Density (crabs/habitat)	20			40		
Sex ratio (females : males)	2:1	4:1	6:1	2:1	4:1	6:1
Mean spawning/week						
Number ovigerous	2.8 ± 1.4	4.2 ± 1.9	5.7 ± 1.1	5.4 ± 2.2	7.5 ± 3.1	8.3 ± 2.1
Per cent ovigerous	20 ± 10	26 ± 12	32 ± 6	20 ± 8	23 ± 10	24 ± 6
Mean mortality/week (missing or dead)						
Number females	2.7 ± 2.0	2.4 ± 2.6	2.5 ± 2.7	3.5 ± 2.8	4.8 ± 3.6	5.3 ± 3.4
Per cent females	20 ± 14	15 ± 16	14 ± 15	15 ± 12	15 ± 11	15 ± 10
Number males	1.2 ± 1.2	0.2 ± 0.4	0.5 ± 0.8	1.7 ± 1.9	0.6 ± 0.8	0.7 ± 0.6
Per cent males	17 ± 17	4 ± 9	16 ± 28	13 ± 15	8 ± 10	12 ± 11
Larval viability						
Number hatches reared	13	11	11	4	10	9
Mean % survival to megalopa	91 ± 6	88 ± 22	88 ± 6	61 ± 24	84 ± 15	88 ± 19
Mean % survival to crab	89 ± 7	86 ± 23	87 ± 9	45 ± 21	74 ± 18	84 ± 24
Mean number days to megalopa	12.5 ± 0.9	12.7 ± 1.1	12.5 ± 0.7	13.0 ± 0.4	12.4 ± 0.7	12.4 ± 0.5
Mean number days to crab	19.0 ± 1.1	18.9 ± 1.4	19.0 ± 1.4	18.6 ± 0.8	18.1 ± 1.0	17.6 ± 0.5

gravel filters, air pumps, and submersible heaters. A 4 cm deep layer of crushed and unbroken oyster shell was placed in each habitat. A 14:10 h day : night photoperiod was provided by 4 fluorescent bulbs placed 1 m above the bottom of each habitat. Water temperatures were maintained at 30 ± 1°C, and salinities were kept at approximately 12‰. The habitats were cleaned and the water was changed biweekly. Crabs were fed weekly on Ralston Purina Marine Ration no. 25 and daily on San Francisco Bay brand (lot no. 3288) *Artemia*. The diet was occasionally supplemented with minced clams and fish.

The habitats were checked weekly from 8 May 1980 until 2 July 1981. Ovigerous crabs were removed and placed individually in 8 cm diameter culture dishes containing 12‰ sea water until their eggs hatched. Gravid females and missing or dead crabs were replaced with crabs of the same sex and approximately the same size to maintain the initial sex ratios and densities. These replacements came from crabs collected from the field and maintained in the large holding tank, as well as from gravid females that had previously hatched their eggs.

Fifty zoeae from each hatch were reared under a 12:12 h day : night photoperiod. Ten zoeae were placed in each of five 3.5-cm diameter bowls filled with sea water of 30°C and 20‰, which was changed daily. Larvae were fed daily with an excess of freshly hatched San Francisco Bay brand (lot no. 3288) nauplii of *Artemia*. Larval molts and deaths were recorded daily until the zoeae metamorphosed to the first crab stage.

Results were compared by analysis of variance (ANOVA). When there was not a significant interaction between the main effects, the Student-Newman-Keuls (SNK) means comparison procedure was used to test for significant differences between the sex ratio treatments.

Results

In 1980 and 1981 the breeding seasons of *R. harrisii* in the Neuse River estuary lasted for approximately five months, from mid-April to the end of September. Breeding by crabs in the laboratory was extended by approximately four months. Crabs stopped breeding in the laboratory from 5 November 1980 until 6 January 1981. The number of gravid females declined gradually from mid-September through October before crabs entered the refractory period. One crab produced eggs on 6 January 1981, but a steady supply of ovigerous females was not obtained until 27 January 1981.

More clutches were spawned by crabs maintained at a higher density and female : male ratios than the lower ones (Tables 1, 2). The number of clutches produced per week was significantly different (SNK, $P < 0.05$) for all sex ratios tested. Approximately eight females spawned per week at the higher combinations of density and sex ratio (40 crabs, 4:1 and 6:1), whereas only about three spawned per week at the lowest (20 crabs, 2:1). Approximately 20–30% of the females spawned per week (Table 1). Females stocked at the two higher female : male ratios (6:1, 4:1) produced a significantly greater (SNK, $P < 0.05$) proportion of clutches than those at the lower sex ratio. The highest proportion of crabs spawned at the lowest density (20 crabs) and highest sex ratio (6:1).

Mortality of females (2.4–5.3 per week) was greater at the higher density (40 crabs), but the percentage of dead females (14–20% per week) was not significantly different between treatments (Tables 1, 2). Sex ratio did not have a significant effect on female mortality (Tables 1, 2).

The pattern of male mortality was similar to that of females. Mortality of males was greater at the higher density (although not quite significant at $P < 0.05$) than at the lower density, and the percentage of dead males was similar between the two densities (Tables 1, 2). However, mortality of males was greatest (SNK, $P < 0.05$) at the lowest female : male ratio (2:1), and the proportion of dead males in habitats stocked at the low sex ratio was significantly greater (SNK, $P < 0.05$) than at the intermediate (4:1) but not at the highest (6:1) sex ratio (Tables 1, 2).

Larval viability was usually good regardless of the crab density or sex ratio at which the clutches were spawned (Table 1). However, crabs at the lowest sex ratio (2:1) and highest density (40 crabs) produced larvae that survived poorly (Table 2). Larvae from all treatments required similar lengths of time to metamorphose to megalopae, but the larvae of crabs that were maintained at the higher density (40 crabs) took less time to metamorphose to first crab stage than those at the lower density (Tables 1, 2).

Discussion

The length of the breeding period of *R. harrisii* was nearly doubled by inducing crabs to spawn in the laboratory later in the fall and earlier in the spring. The breeding season of the Neuse River population of *R. harrisii* extends from mid-April to late September, but in the laboratory crabs spawned for an additional four months and entered a refractory phase only from November through January.

The onset of the refractory period of *R. harrisii* overwintered in the laboratory during the present study is similar to that found for a subtropical population of *R. harrisii* in the St. Johns River estuary, Florida, although zoeae have been collected as late as November in Florida (Tagatz, 1968). How-

Table 2. ANOVA of spawning, mortality, and larval viability of overwintered *Rhithropanopeus harrisii* maintained at various stocking densities and sex ratios. Percentages arcsin-tranformed; * indicates significance at $P < 0.05$.

Source of variation	*d.f.*	*MS*	*F*	*P*
Number, ovigers				
Density	1	161.528	43.357	<0.001*
Sex ratio	2	61.277	16.448	<0.001*
Interaction	2	1.090	0.292	0.747
Error	83	3.726		
Percentage, ovigers				
Density	1	0.026	1.638	0.204
Sex ratio	2	0.102	6.465	0.002*
Interaction	2	0.020	1.279	0.284
Error	83	0.016		
Number, dead females				
Density	1	82.669	10.661	0.002*
Sex ratio	2	1.878	0.242	0.785
Interaction	2	8.655	1.116	0.332
Error	83	7.755		
Percentage, dead females				
Density	1	0.003	0.059	0.809
Sex ratio	2	0.012	0.225	0.799
Interaction	2	0.048	0.928	0.400
Error	83	0.051		
Number, dead males				
Density	1	3.933	3.725	0.057
Sex ratio	2	8.811	8.344	<0.001*
Interaction	2	0.184	0.174	0.840
Error	83	1.056		
Percentage, dead males				
Density	1	0.020	0.229	0.634
Sex ratio	2	0.304	3.412	0.038*
Interaction	2	0.041	0.459	0.634
Error	83	0.089		
Percentage, survival to megalopa				
Density	1	0.138	3.036	0.087*
Sex ratio	2	0.025	0.553	0.579
Interaction	2	0.171	3.765	0.030*
Error	52	0.045		
Percentage, survival to crab				
Density	1	0.497	10.612	0.002*
Sex ratio	2	0.074	1.579	0.216
Interaction	2	0.261	5.570	0.006*
Error	52	0.047		
Duration to megalopa				
Density	1	0.028	0.041	0.840
Sex ratio	2	0.144	0.213	0.808
Interaction	2	0.753	1.113	0.336
Error	52	0.677		
Duration to crab				
Density	1	11.505	8.751	0.005*
Sex ratio	2	0.415	0.316	0.731
Interaction	2	0.853	0.649	0.527
Error	52	1.315		

ever, Goy *et al.* (1985) found that crabs collected from the Neuse River, North Carolina, in 1979 and held at 24°C in the laboratory did not become reproductively quiescent until after mid-December. The later onset of the refractory period may have been a result of the lower temperature (24°C) at which the crabs were maintained. Goy *et al.* (1985) maintained 45 crabs per habitat (same dimensions as used in this study) at a 2:1 female : male ratio; these crabs produced a mean of 4.3 clutches per week. In the present study, 5.3 clutches were produced per week by only 40 crabs at the same sex ratio and at 30°C. The higher temperatures at which the crabs were maintained during the current study may have increased reproductive output, thereby depleting the energy reserves more quickly and initiating an earlier onset of the refractory period provided that crabs were not well fed.

Spawning can be increased simply by increasing crab density and female : male ratio in the laboratory. Thus, ovigers were most abundant in habitats containing 40 crabs at a 6:1 sex ratio (8.3 ovigers per week), and were least abundant in habitats containing 20 crabs at a 2:1 ratio (2.8 per week). However, the difficulty in maintaining artificially elevated populations in the laboratory resides in the ability to collect females, since the sex ratio of natural populations in the Neuse River estuary is 1:1. Investigators encountering difficulty in collecting females should consider maintaining laboratory populations at the highest sex ratio (6:1) and lowest density (20 crabs) due to the high spawning efficiency.

Spawning was greatest when males were least abundant, perhaps because a few males can mate with many females, and because males apparently kill females as well as each other. Males are larger and more aggressive than females, which would be particularly vulnerable to attack while molting. The mortality rate of females may have increased as the density of males increased. The increase due to the concomitant rise in the number of females was thereby offset by the higher mortality rate. Mortality of females also was greater at the higher density simply because more females were present; the percentage of dead females was not dependent on the stocking density. Because the proportion of dead females was similar between all treatments, mortality of females need not be considered when selecting the density and sex ratio for a laboratory population of crabs. However, mortality of crabs might be reduced and spawning of females increased if additional food and refuge were provided.

The mean survival and duration of larvae of *R. harrisii* in the present study were comparable to those of larvae hatched from crabs that spawned in the wild during the normal breeding season (Costlow *et al.*, 1966). Thus, the viability of embryos and larvae was not affected adversely by maintaining crabs in the laboratory over a long period of time. However, low survival of larvae was observed for crabs maintained at the highest density and lowest female : male sex ratio. The poor survival may have been an artifact of the low number of hatches reared (four), but Goy *et al.* (1985) also obtained low survival (69%) for *R. harrisii* maintained at a similar density and sex ratio. It is possible that the combination of crowding and the high abundance of males in this treatment resulted in more stressful behavioral interactions for females, lower food intake, and less yolk for embryos. The duration of larval development to megalopa was similar between treatments, but inexplicably development to first crab stage took longer for those larvae spawned by females in the least crowded conditions.

The maintenance of a reproducing population of *R. harrisii* in the laboratory is efficient and economical. Egg production can be increased by stocking crabs at high densities with a greatly disproportionate number of females. Furthermore, experimentation on the reproductive and developmental biology of temperate populations of crabs need not be restricted to summer months. The beginning and end of the refractory period may be manipulated by controlling photoperiod and temperature. Such manipulation may be useful for studies of the endocrinology of decapod reproduction.

Acknowledgement

We thank John Christy for commenting on the manuscript.

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RECEIVED: 27 January 1987.
ACCEPTED: 5 October 1987.

Addresses: (SGM) Duke University Marine Laboratory, Beaufort, North Carolina 28516 and (current address) Smithsonian Tropical Research Institute, Box 2072, Balboa, Republic of Panama; (JWG) Duke University Marine Laboratory, Beaufort, North Carolina 28516 and (current address) Department of Biology, Texas A & M University, College Station, Texas 77843; (JDC) Duke University Marine Laboratory, Beaufort, North Carolina 28516.

ANNOUNCEMENT

"Les Pylochelidae ou 'Pagures symétriques' (Crustacea Coenobitoidea)" by J. Forest, Muséum National d'Histoire Naturelle, Paris, has been published (1987) in Résultats du Campagne MUSORSTOM, 3.A, volume 137, pages 1–254, with 9 plates. ISSN 0078-9747.

This publication is available from: Editions du Muséum, 38 rue Geoffroy Saint-Hilaire, 75005 Paris, France. The price is 300 FF.