

THE INFLUENCE OF SOCIAL ENVIRONMENT
ON SEX DETERMINATION IN HARLEQUIN SHRIMP
(*HYMENOCERA PICTA*: DECAPODA, GNATHOPHYLLIDAE)

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A B S T R A C T

Harlequin shrimp, *Hymenocera picta*, are monogamous and pair-bonding, and are usually found in isolated singles and pairs in the field. The apparent rarity of this species in their habitat and high levels of aggression between conspecifics suggests the possibility of some sort of plasticity in their primary sex determination. In this study, the influence of social environment upon primary sex determination in *H. picta* was examined experimentally in the laboratory. Naïve juveniles were placed in three novel social environments: paired juveniles, single juveniles, and adult/juvenile pairs. Spacing behavior and the onset of external differentiation were observed during long-term experiments for each social treatment. Gonadal development was also observed. Spacing behavior of paired shrimp reflected the sexual composition of social groups; high intrapair distance (IPD) for same-sex pairs and low IPD for opposite-sex pairs. Sex determination results were not so clear. Two of the three paired juvenile replicates displayed phenotype frequencies different from those expected from a random sample of gonochoristic organisms with a 1:1 sex ratio. However, the third replicate and the combined frequencies did not show any statistical difference from the hypothetical random sample. Furthermore, single juveniles and those paired with adults expressed sex in nearly 1:1 ratios, regardless of the sex of adult conspecifics. Histological examination of juvenile shrimp confirmed that sex was determined as early as four weeks after larval metamorphosis. Therefore, social environment had no clear effect upon sexual phenotype expressed. However, single females attained puberty at a significantly greater age and larger size than did females paired with males. Hence for *H. picta*, social environment had a measurable effect on the timing of female puberty. This is the first demonstration of this phenomenon in decapod Crustacea.

The phenomenon of environmental sex determination (ESD) (Bull, 1983) is well documented in many teleost fish families in the form of some sort of sequential hermaphroditism (Atz, 1964; Smith, 1975; Yogo, 1987). The best such examples in fish involve the social environment and its effect on sex (e.g., Fricke and Fricke, 1977; Moyer and Nakazono, 1978; Warner, 1982). In crustaceans, however, there appears to be little direct evidence of social control of functional sex. The best known examples occur in parasitic bopyrid isopods and protandrous hermaphrodite shrimp of the family Pandalidae. In bopyrid examples, the first individual to reach a host becomes female and may cause subsequent conspecifics to become male (Reverberi, 1944; Reinhard, 1949; Charniaux-Cotton *et al.*, 1983). In pandalid shrimp, the timing of male-female sex change is thought to be influenced by the demographics of their populations (Charnov, 1982). However, the mechanism of such a system is in doubt (Bergström, 1997).

Examples of hermaphroditism in the malacostracan Crustacea are relatively uncommon and usually are in the form of protandrous sex change. Hermaphroditism is known from many species in a few subgroups of isopods and tanaidaceans (Charniaux-Cotton, 1975; Policansky, 1982). However, only approximately 42 species of decapod crustaceans are thought to be hermaphroditic, and 35 of these are caridean shrimp (see lists in Carpenter, 1978; Policansky, 1982; Bauer, 2000) (and for additional examples see Kagwade, 1981; Sukumaran, 1981; Bauer, 1986; Nakashima, 1987; Gherardi and Calloni, 1993; Gavio *et al.*, 1994; Rudolph, 1995; de Almeida and Buckup, 1997, 2000; Bauer and Holt, 1998; Fiedler, 1998). Furthermore, at least two hippolytid carideans in the genus *Lysmata* display a form of simultaneous hermaphroditism (Bauer and Holt, 1998; Fiedler, 1998). Because marine decapods share many of the same ecological and habitat selection pressures with teleost fishes, it is surprising to find so few examples in this diverse

group of crustaceans. Perhaps past research has not focused closely on the best crustacean candidates for sex change.

Harlequin shrimp, *Hymenocera picta* (Dana, 1852), are small caridean shrimp that may be ideal candidates for studies on plastic sex determination. They are found in shallow coastal waters (1–30 m) of the tropical Indo-Pacific region but are apparently uncommon throughout their range (personal observation; Debelius, 1984). The shrimp are hardy in captivity and are popular aquarium pets, but only eat asteroid echinoderms (Wickler, 1973; Debelius, 1984). Males and females are sexually dimorphic and form monogamous pair bonds that are maintained via mate-guarding behavior by males (Wickler, 1973; Seibt and Wickler, 1979). Both males and females are aggressive towards individuals of the same sex, and males will readily fight each other to death in an encounter. Also, adults are not known to change sex. Mature females spawn 100–5,000 eggs every 18–26 days, concurrent with their molt cycle (Seibt and Wickler, 1979; Kraul and Nelson, 1986). *Hymenocera* larvae pass through twelve planktonic zoeal stages and reach the settlement stage in 5–6 weeks (Kraul and Nelson, 1986; Fiedler, 1994). Postlarvae begin eating seastars five days after metamorphosis (Fiedler, 1994).

Given the relative rarity of these animals, their high intraspecific aggression, the dispersal of their prey, and the low probability of larval survival, it seems likely that a mechanism ensuring access to the opposite sex would have evolved. This might be accomplished via the ability to follow distant chemical cues from the opposite sex or a mechanism of environmentally (socially) regulated juvenile sex determination. Harlequin shrimp certainly fit the criteria for one of three models for the evolution of hermaphroditism proposed by Ghiselin (1969). The Low Density Model implies that there is an advantage in “being able to mate with any member of the species, where the probability of encountering a suitable mate is low” (Ghiselin, 1969: 200). This model suggests the evolution of simultaneous hermaphroditism or labile juvenile sex determination in marine animals, like harlequin shrimp, with limited postlarval dispersal. In this study, I assess the presence of such mechanisms in juvenile *Hymenocera picta*. These experiments examine social influence on the primary sex determination of this species.

MATERIALS AND METHODS

In order to examine the effect of social environment on the primary sex determination of *Hymenocera picta*, a series of five experiments was carried out in which postlarvae were placed into artificial social situations in the laboratory. In the “Paired Juveniles” experiment (replicates I, II, and III), pairs of postlarvae were isolated and observed closely over a period of several months. In the “Single Juveniles” experiment, postlarvae were isolated individually and monitored. In the “Adult/Juvenile Pairs” experiment, single postlarvae were isolated with one adult shrimp. In the “Histology/Morphology Pairs” experiment, postlarval pairs were isolated and sacrificed at regular intervals in order to examine morphological characteristics of primary sex determination. The three social contexts (juvenile conspecific, adult conspecific, and no conspecific) were intended to simulate the potential social environments that *Hymenocera picta* postlarvae experience in the field. All the experiments are explained in detail below.

Experiment One: Paired Juveniles

In this experiment, I assessed the presence of socially regulated Environmental Sex Determination (ESD) during the juvenile phase of *Hymenocera picta* by observing the behavior and differentiation of isolated juvenile pairs. Under optimal ESD, we would expect that an isolated pair of juvenile shrimp would mature as a male/female pair. Additionally, I expect that spacing behavior would reflect the sexes of the pairs as occurs in *H. picta* adults. Seibt (1980) observed artificial groupings of adult *H. picta* in the laboratory and found that individuals of the opposite sex were found in closer proximity than those of the same sex.

Three replicates of this experiment were performed and designated as replicates I, II, and III. All three replicates were conducted at the Hawaii Institute of Marine Biology under similar conditions.

Replicate I.—Forty-two *Hymenocera picta* postlarvae, approximately two weeks postmetamorphosis, were obtained from the Waikiki Aquarium. Individual shrimp were randomly selected, by drawing numbers from a hat, and placed into twenty pairs. The two extra shrimp were held in reserve and not used. Shrimp pairs were isolated in small (8 l) plastic buckets. The buckets were arranged in two wet tables and provided flowing sea water via a polyvinyl chloride (PVC) pipe frame in each wet table. Buckets had holes drilled approximately 4 cm under the rim to facilitate water flow. Each bucket was provided with its own aeration source. Shrimp were fed small (approximately 1 cm) pieces of seastar (*Linckia* spp.) *ad libitum* during the experiment. Decaying food and detritus were siphoned from the buckets as needed.

Pairs were monitored for seven months (1 July 1991–8 February 1992). The data recorded during the experiment included the Intra-Pair Distance (IPD), total length (TL), and secondary sex characteristics. These data were recorded daily during the first month of the experiment and at increasingly longer intervals (2–7 d) in the remaining six months. I measured IPD with a transparent, flexible ruler, recording the linear distance separating two shrimp between the bases of their respective antennae. Total length (TL) was recorded weekly by carefully removing shrimp from their containers with a clear glass beaker that was then placed above an opaque plastic ruler. The TL was recorded as the linear distance between the tips of the rostrum and the uropod. Although carapace length (CL) would have been a more reliable indication of size, measuring TL was most

expedient and less stressful to these delicate shrimp. The onset date of maturity (appearance of secondary sex characters) was determined by carefully examining the ventral abdomen of shrimp for female characteristics. Characteristics of female puberty included the presence of white or red pigment and eggs on pleopods. Although males possess morphologically distinct pleopods, they remain transparent. This made it difficult to record the presence of structures such as the appendix masculina on living individuals. Therefore, male puberty was recorded as the date at which an individual was able to fertilize its partner (if female). The sex of morphologically indistinct individuals was inferred by placing them with adults of known sex and observing their stereotypical behavior (Wickler and Seibt, 1970). Unusual or obviously agonistic behavior was also noted throughout the experiment.

Replicate II.—For this replicate, postlarvae were obtained from a clutch reared by the author (for larval rearing protocol, see Fiedler, 1994) at the Hawaii Institute of Marine Biology. Forty experimental postlarvae were randomly selected from a total of 86 healthy individuals 2–4 weeks past metamorphosis. The experimental procedure was identical to replicate I with the following exception: IPD was measured every 5–7 d throughout the experiment, which ran for approximately 6 months (4 July 1992–28 December 1992).

Replicate III.—For this replicate, forty postlarvae were obtained from a clutch of 61 reared by the author (as above). These postlarvae metamorphosed 1–4 weeks prior to the experiment. The experimental procedure was identical to replicate I, with the following exceptions. First, IPD was recorded every 1–3 days for approximately the first three months and at increasingly longer intervals (3–11 days) for the rest of the experiment. Second, the experiment ran for more than eight months (25 May 1995–28 January 1996).

Experiment Two: Single Juveniles

As a control and comparison for paired juvenile development, postlarvae were isolated singly and observed as above. If sex determination in this species is socially regulated ESD, I hypothesize that single shrimp experiencing the same social environment (no conspecific) would differentiate as the same sex (either all males or females). An alternative hypothesis is that the presence of no conspecific represents no social environment, and individuals will mature as males or females in equal proportions.

Twenty postlarvae were randomly chosen from the same clutch used for Paired Juveniles, replicate II. Shrimp were maintained in buckets, as above, in a single large wet table. The experiment duration was 11 months (4 July 1992–15 June 1993). All individuals were observed (same schedule as Paired Juveniles II) for presence of secondary sex characteristics (female) and measured (TL) when sex could be judged. The sex of all individuals was verified behaviorally at the completion of the experiment.

Experiment Three: Adult/Juvenile Pairs

In the field, it is unlikely that juveniles encounter only juvenile conspecifics. To simulate such natural conditions, newly metamorphosed individual juveniles were randomly paired and isolated with sexually mature adult individuals. Cues from adult shrimp may have a more pronounced effect upon the ESD of juveniles. If this is the case, juveniles should predominantly mature as the opposite sex of their adult partners.

Sixteen postlarvae were randomly selected from the same clutch used for Paired Juveniles, replicate III. Half of these were individually paired with adult male *Hymenocera picta*. The other half were individually paired with adult females. Nine of the adults were wild-caught, whereas seven others were laboratory-reared (some left over from Paired Juveniles replicate II). The animals were maintained and observed as in the Paired Juveniles experiment, with the following exceptions. First, experimental buckets were larger (12 l) and each was provided with a standard shelter (~5 cm length of 3.8-cm diameter PVC pipe half) as a refuge for the small juveniles. Second, IPD and shrimp TL were measured on the same schedule as Paired Juveniles, replicate III. Third, the onset of juvenile maturity was assessed by determining the presence of external female sex characteristics or fertility of partner's eggs. In the cases where juvenile sex was "unknown," shrimp were sexed behaviorally. The duration of this experiment was approximately seven months (24 May 1995–31 December 1995).

Experiment Four: Morphology/Histology Pairs

If the sex determination of *Hymenocera picta* juveniles is plastic or labile, there may be evidence of bipotentiality in juvenile gonads and in some external secondary sex characteristics. Therefore, histological examination of paired juveniles sacrificed during the putative maturation period was undertaken.

Ten pairs of juveniles were randomly selected from remnants of the clutch used for the Paired Juveniles, replicate II and Single Juveniles experiments. Pairs were isolated and maintained in buckets in the same manner used in the Paired Juveniles experiment. Behavioral and spacing observations were not made during this experiment. Over the course of the experiment (5 July 1992–19 September 1992), pairs were selected at random at approximately 7-d intervals (beginning 23 July 1992) and sacrificed for histological examination. Sacrificed shrimp were fixed in 10% seawater Formalin. Fixed shrimp were examined and measured (TL and CL) under a dissection microscope. The appendages, abdomen, and carapace were then excised from fixed specimens. The remaining cephalothorax was embedded in paraplast media following decalcification (with Fisher brand CalEx), dehydration (EtOH), and clearing (toluene). The entire cephalothorax was used in order to observe the location and morphology of gonoducts. In all cases, 8 μ m transverse serial sections were made for detailed observation of the morphology and histology of the entire gonad. Sections were mounted on glass slides, stained with Harris progressive hematoxylin and eosin (Galigher and Kozloff, 1971), and examined with a compound microscope.

Data Analyses

All statistical analyses were conducted using the software package "StatView v.5.01" on a Macintosh PowerPC computer. For all experiments, observed frequencies of expressed sexes were compared with expected frequencies in a Chi-Square Goodness of Fit test. Expected frequencies used were generated extrinsically to reflect the 1:1 male/female sex ratio observed in the field. In this way, I determined whether the observed frequencies are significantly different from those expected from gonochoristic animals without ESD.

I compared the overall average IPDs for all pair types in each Paired Juveniles and Adult/Juvenile Pairs experiment in a one-way ANOVA. In this way, I determined whether group composition (pair types) had a significant effect upon Intra-Pair Distance. Multiple pair-wise comparisons were

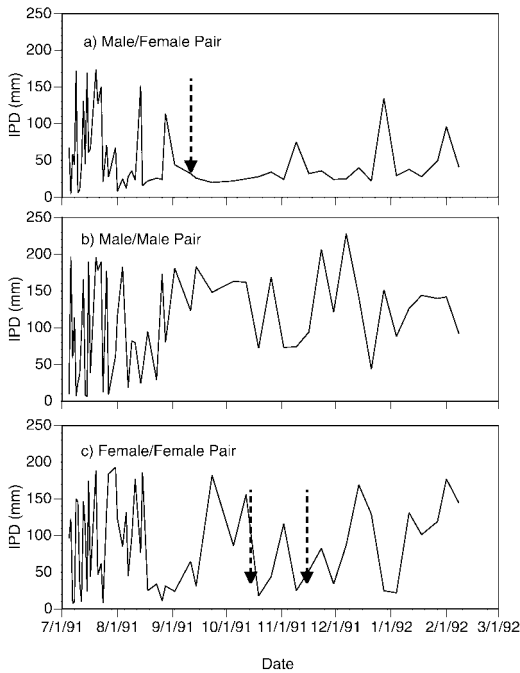


Fig. 1. Representative IPD over time for each pair type. Graphs of Intra-Pair Distance (IPD) for three different pair types over the course of replicate I of the Paired Juveniles experiment. Arrows represent the dates of female puberty onset.

made using a Scheffé's *post hoc* test because of unequal sample sizes and concerns about data normality.

Using a one-way MANOVA, age and size of female puberty was compared between isolated individuals, those paired with males, and those paired with other females. Again, a *post hoc* Scheffé's test was employed to determine differences between "treatments" for both puberty size and age.

RESULTS

Experiment One: Paired Juveniles

Sexes of Pairs.—In replicate I, 10 pairs differentiated into male/female pairs (MF), nine became male/male pairs (MM), and one pair matured as female/female (FF). A 1:1 sex ratio (based on unpublished field collections) was used to generate expected frequencies (50% MF, 25% MM, 25% FF) for a Chi-Square goodness of fit test (GOF). The GOF test applied to replicate I counts indicated a significant deviation from expected counts (Chi-Sq. = 6.40, *df.* = 2, *P* = 0.041).

In replicate II, 14 pairs differentiated as MF, none as MM, and five as FF. One pair could not be judged, as one shrimp died and was too deteriorated to examine. These data indicated that the observed combinations deviated signif-

icantly from expected values (Chi-Sq. = 6.90, *df.* = 2, *P* = 0.032).

In replicate III, 12 pairs differentiated as MF, three as MM, four as FF, and one pair suffered early mortality and could not be determined. The resulting Chi-Square GOF analysis showed that observed counts did not deviate significantly from expected values (Chi-Sq. = 1.42, *df.* = 2, *P* = 0.491).

I applied the same analysis for combined data from all three Paired Juveniles experiments. The total counts were 36 MF, 12 MM, and 10 FF pairs. The GOF test indicated that counts were not statistically different from expected values (Chi-Sq. = 3.52, *df.* = 2, *P* = 0.172).

Intra-Pair Distance and Sex of Shrimp.—Representative IPDs for MF, MM, and FF pairs over the duration of the experiments are graphed in Fig. 1. For each experiment, IPDs of MF pairs were typically low or became lower following female puberty. Same-sex pairs (MM and FF) remained highly spaced over the entire experiment but varied more. Mean IPDs for each replicate (I, II, and III) were analyzed to determine if pair type (MF, MM, FF) can account for variance in mean IPD. Hence, ANOVA was performed with pair types (MF, MM, FF) as an independent variable and average IPD of each pair as the dependent variable. Then, a *post hoc* Scheffé's test was performed to determine which pair types differed. The minimum criterion for statistical significance was $P \leq 0.01$ in both the ANOVA and Scheffé's tests. In each experiment, the pair types accounted for most of the variation in IPD (I: *F*-value = 30.1, *df.* = 2; II: *F*-value = 122.01, *df.* = 1; III: *F*-value = 28.54, *df.* = 2; actual $P < 0.0001$ for I, II, III). *Post hoc* Scheffé's tests for each experiment (Fig. 2a–c) showed that MF IPDs were lower than MM (actual $P < 0.0001$). The MF and FF IPDs were significantly different in replicates II and III (actual $P < 0.0001$ and $P = 0.0018$, respectively) but not in replicate I (actual $P = 0.15$). The FF and MM IPDs did not differ significantly in any replicate.

Experiment Two: Single Juveniles

Sexes of Individuals.—In the Single Juveniles experiment, eighteen of twenty individuals survived and could be sexed. Of these, ten individuals were judged as male, and the remaining eight were female. These results do not

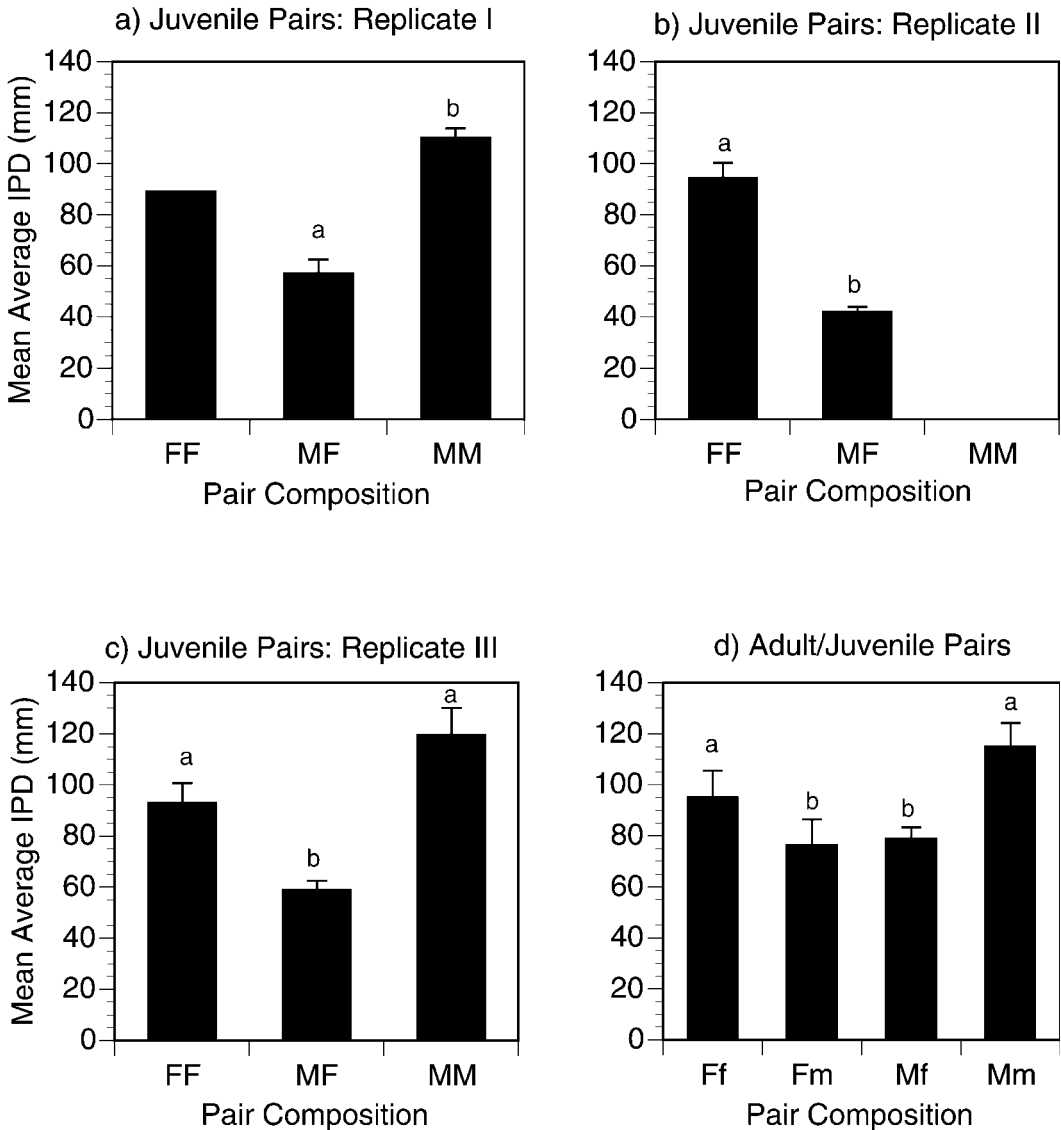


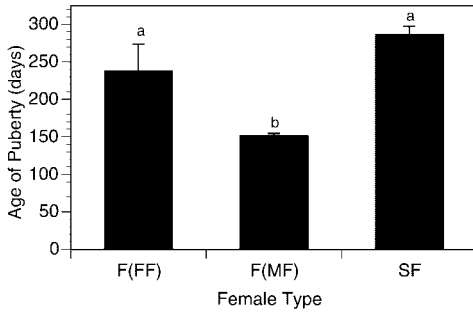
Fig. 2. Comparisons of average IPD for all pair types. Bars designated with different letters are significantly different at $P \leq 0.01$ level, as determined by a Scheffé's test (all pair-wise comparisons). Error bars represent one standard error of the mean. For a, b, and c: FF = matured as female/female, MF = matured as male/female, and MM = matured as male/male. For d): Ff = adult female and maturing female, Fm = adult female and maturing male, Mf = adult male and maturing female, Mm = adult male and maturing male.

indicate a significant deviation from a 1:1 sex ratio (Chi-Sq. = 0.22, $df. = 1$, $P = 0.637$) for isolated individuals. Therefore, there is no support for the hypothesis that shrimp maturing alone (under identical social context) are likely to differentiate as one sex or the other. This alone, however, does not rule out the effect of social environment on expressed sex.

Timing and Size of Female Differentia-

tion.—The mean ages and sizes of maturity in females were compared for females in the Paired Juveniles (replicate II) and Singles experiments (Fig. 3). Data from only these experiments were used, as they were run concurrently and from the same clutch of postlarvae. A MANOVA was used to test the null hypothesis that the treatment experienced by a given female (female in an MF pair, female in an FF pair, and single female) had no effect

a) Comparison of Puberty Age for Different Females



b) Comparison of Puberty Size for Different Females and Males

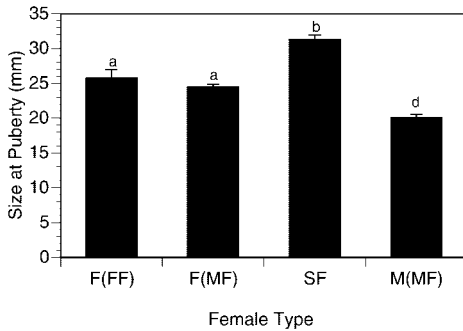


Fig. 3. Comparisons of puberty size and age for females (and males) maturing in different social environments. Bars designated with different letters are significantly different at $P < 0.01$, as determined by a Scheffé's test (all pair-wise comparisons). Error bars represent one standard error of the mean. Female types are as follows: F(FF) = females from female/female pairs, F(MF) = females from male/female pairs, SF = single females. All females were from the same clutch, but paired females were observed in the Paired Juveniles replicate II experiment, and SF were observed in the Single Juveniles experiment.

on puberty size and age. The MANOVA rejected the null hypothesis for both puberty age (F -value = 20.7, $d.f.$ = 2, $P < 0.0001$) and puberty size (F -value = 25.6, $d.f.$ = 2, $P < 0.0001$). This demonstrates that the social setting of female shrimp does indeed have an effect on the size and age of puberty. A Scheffé's *post hoc* test was performed for both puberty size and age (Fig. 3) and showed that single females (SF) matured later ($P < 0.0001$) and at a larger size ($P < 0.0001$) than females from MF pairs (F(MF)). While single females also matured at a larger size ($P = 0.0002$) than females from FF pairs (F(FF)), there was no clear difference between age of puberty ($P < 0.1818$). Furthermore, F(MF) matured at a lower age ($P = 0.0032$) than F(FF), but there was no significant difference in their puberty sizes. It should be mentioned that the second maturing

female in 3 FF pairs matured much later than the first female, much like single females. This accounted for the high variance in FF puberty size and age (Fig. 3). Male age of maturity could not be estimated accurately, as it depended on the presence of a mature female. However, a maximum estimate of male maturity size was made when possible when female pair-mates first spawned fertile eggs. Males appeared to mature at a smaller size than females in any social context (Fig. 3).

Experiment Three: Adult and Juvenile Pairs

Sexes of Pairs.—In the Adult/Juvenile experiment, only 12 of 16 juveniles paired with adults could be sexed behaviorally, functionally, or morphologically. Four individuals died prematurely and were found decomposed. Of the surviving shrimp, four juveniles paired with adult males became female, and two became male. When paired with adult females, three juveniles became males and three became females. A Chi-Square GOF test was performed, using a 1:1 sex ratio to calculate expected frequencies for each treatment. The test indicated that the observed sexes did not differ significantly from expected counts (Chi Sq. = 0.67, $d.f.$ = 3, $P = 0.881$), showing no effect of adult sex on the expressed sexes of juveniles.

IPD and Sex of Shrimp.—Intra-Pair Distances did not always decrease over time for MF pairs in this experiment (Fig. 2d). Qualitatively, it appeared that all pair types had a greater range of IPDs over the course of the experiment than for the Paired Juveniles experiment. For quantitative analysis, experimental pairs were divided into *Mf*, *Mm*, *Ff*, and *Fm* (capital letter denotes the adult sex and lower case denotes juvenile sex). Mean IPD for each of these pair types was compared using an ANOVA, which showed that pair sexes did not account for differences in IPD means (F -value = 3.75, $d.f.$ = 3, $P = 0.06$), but not convincingly. As in the Paired Juveniles experiment, there was a trend for hetero-sex pairs (*Mf*, *Fm*) to have lower IPDs. However, the differences were not statistically different.

Experiment Four: Morphology/Histology Pairs

Sexes of Pairs.—Eight of ten experimental pairs were available for weekly sacrifice during the experiment. Three shrimp disappeared from their holding containers before they could be

selected for sacrifice (one pair, and one individual). For the surviving eight pairs, two matured as MM, four as MF, and two as FF. These values did not differ from (are identical to) sex combinations expected from frequencies generated from a 1:1 sex ratio.

Morphological and Histological Examination.—Nearly 130 slides of 17 specimens were examined for microscopic evidence of sex (Figs. 4, 5). All but three possessed a clear histological indication of sex. Even the smallest and youngest specimens (19 d) showed evidence of internal differentiation. These observations were supported by the presence/absence of the male copulatory appendage, the appendix masculina.

Nearly all males possessed definite lobed testes, sperm ducts, and a terminal sperm sinus or ampulla (Fig. 4). Mature “tack-shaped” sperm were invariably observed in the sinus, duct, and often the gonad lumen of males. Females had two ovary lobes, but oviducts were difficult to discern. Oocytes within each lobe increased in size toward the lateral portion of the gonad (Fig. 5). This indicates a clear pattern of medial to lateral egg development. However, no secondary vitellogenic oocytes were observed in any specimens.

There were three histologically uncertain specimens in this experiment. One (7A) did possess structures resembling sperm duct, but no testis was evident. However, a clear appendix masculina was present on its second pleopods. This specimen appeared poorly embedded, and testicular tissue may have been inadvertently excised. The other two specimens (4A and 4B) were the oldest individuals and among the largest. The dorsal portion of serial sections of these specimens appeared to have crumbled or fallen away. This is often typical of vitellogenic oocytes, and fragments of what appear to be yolk particles were apparent. Furthermore, no appendix masculina were observed on the pleopods of these specimens. It appears that both these individuals were females. Other than the above three shrimp, there were no examples of ambiguous gonad structure and no evidence of a hermaphroditic gonad in any specimen.

DISCUSSION

Sex Determination—Environmental or Genetic?

The early experiments (Paired Juveniles, replicates I and II) indicated that pairs of shrimp

matured in combinations outside what would be expected from gonochoristic organisms with genetic sex determination and Mendelian sex alleles. Taken alone, these data suggest that some factor (such as social environment) in the experiment influenced the sex outcome of individuals. If, however, sex of conspecifics was an important factor in sex determination, we would expect to see the results skewed highly towards MF pairs. There was some indication of this in replicate II (14 MF, 5 FF, 0 MM). This can be examined by partitioning the observed frequencies into two groups (14 hetero-sex and 5 same-sex pairs) and performing a Chi-Square GOF test with expected values for hetero-sex : same-sex as 1:1 (Zar, 1984). This analysis shows that the number of MF pairs is responsible for the nonconformity of the data to expected values.

If genetic sex determination (GSD) was predominant, what could explain the results in replicates I and II? It is possible that the sex ratio of postlarvae was skewed prior to the experiment. In replicate I, shrimp were taken from a very large (>1,000) clutch of postlarval siblings. There was no record of sex ratio from the entire batch or whether the available postlarvae were a representative sample of the clutch. Male postlarvae could have been predominant because of higher larval survival for males or nonrandom removal from the rearing tanks. In contrast, all available animals from one clutch were used in Paired Juveniles replicate II, the Singles, and the Histology experiments. A female-biased sample could explain results of replicate II but not the Singles and Histology experiments where there was a clear 1:1 sex ratio. Therefore, it is possible that an environmental sex determination (ESD) effect was present for replicate II of the Paired Juveniles experiment, as there is not another likely explanation available. A re-analysis of the data supports this possibility. If the data for replicates I and II are analyzed in a GOF test using the sex ratios observed in each trial (M:F = 7:3 and 7:12, respectively), we see that the observed sex combinations in replicate II do not conform to expected combinations ($P = 0.039$) from the experimental sex ratio. This suggests some environmental effect on the outcome of replicate II, but the specific source is not clear.

For the rest of the experiments, the results closely mirrored expected values. Paired juveniles in Paired Juveniles replicate III and the Morphology/Histology Pairs experiments con-

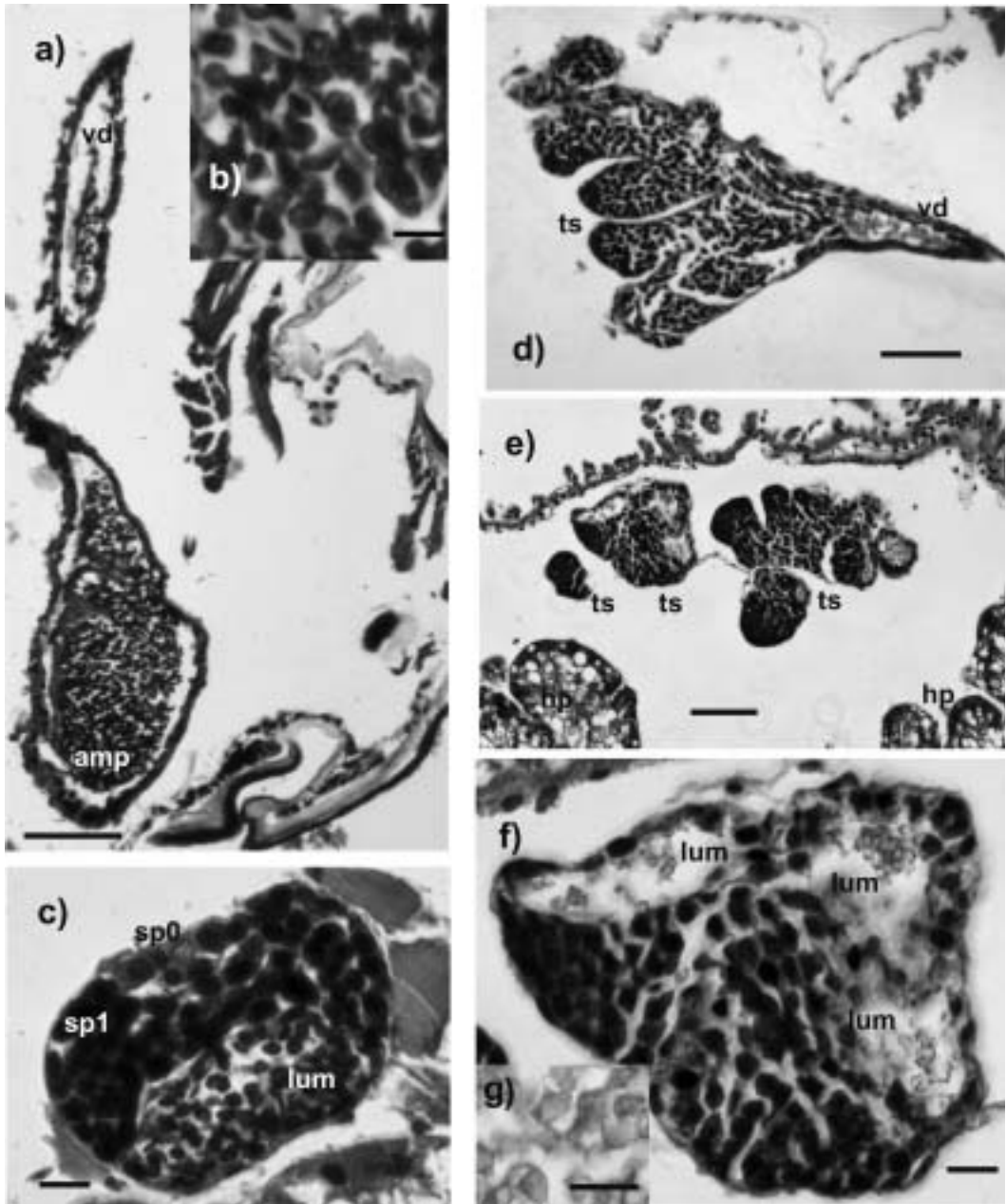


Fig. 4. Reproductive histology of male *Hymenocera picta* from the Morphology/Histology Pairs experiment. a) Terminal ampulla and distal vas deferens of 3.8 mm CL male. b) Close-up of mature sperm in terminal ampulla. c) Cross section of small testis lobe, same individual. d) Section of large testis lobe and proximal vas deferens of 5.0 mm CL male. e) Several testis lobes from same individual. f) Close-up of one testis lobe from previous photo. Obvious lumen with sperm cells. g) Sperm in lumen from previous photo. Abbreviations: vd = vas deferens, amp = ampulla, sp0 = spermatogonia, sp1 = spermatozoa, lum = lumen, ts = testis, hp = hepatopancreas. Scale bars are as follows: 100 μ m in a, d, and e; 20 μ m in c and f; 10 μ m in b and g.

trasted sharply with Paired Juveniles replicates I and II. Furthermore, combined data from all the Paired Juveniles experiments generated no significant relationship. Also, single shrimp showed no tendency to mature as one or the

other sex. It is possible that sex cues from juveniles were incomplete or weak, and this effect could be multiplied with the combination of juveniles. Hence, we might expect a stronger effect from adults reared with juveniles. In the

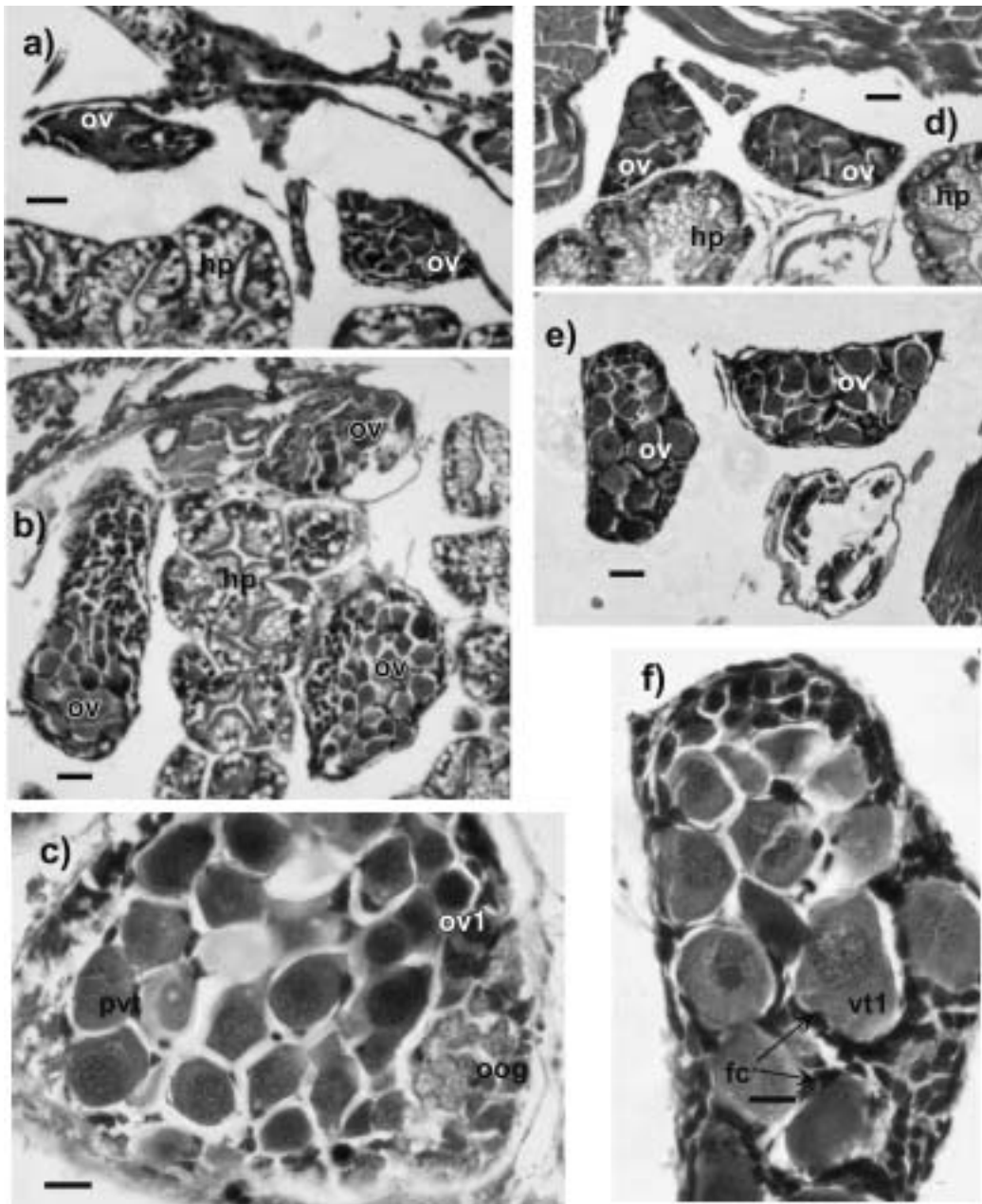


Fig. 5. Reproductive histology of female *Hymenocera picta* from Morphology/Histology Pairs experiment. a) Ovary lobes of 4.0 mm CL female with only previtellogenic ova. b) Three ovary lobes in more anterior section from same individual. c) Close up of one lobe from 3.6 mm CL female, showing progression of egg development. d) Ovary lobes with large previtellogenic oocytes from 4.6 mm CL female. e) More anterior section of ovaries of same individual. f) Close-up of ovary lobe from previous photo. Note a few cells in primary vitellogenesis, surrounded by follicular cells. Abbreviations: ov = ovary, oog = oogonia, ov1 = early oocyte, pvt = previtellogenic oocyte, vt1 = oocyte in primary vitellogenesis, fc = follicular cell. hp = hepatopancreas. Scale bars are as follows: 50 μm in a, b, d, and e; 20 μm in c and f.

tanaidacean *Heterotanais oerstedii*, for example, the sex of adult conspecifics has a direct impact upon larvae reared in isolation (Bückle-Ramirez, 1965). Here, the larvae mature as the sex

opposite of the adults kept with them. In the present study, this effect is convincingly refuted by the results of the Adult/Juvenile experiment, where available sex cues should

have been strongest. All these factors point to one conclusion: sex for harlequin shrimp is predominantly fixed and unalterable during juvenile and adult life. The histological data provide even further support for this conclusion. Hence, clear evidence for ESD in this species is lacking from these experiments. Even as excellent candidates for Ghiselin's "Low Density Model" (1969) of hermaphroditism, other mechanisms must be invoked to explain how these sparsely distributed organisms ensure the availability of suitable mates. This is comparable to an experimental study on the mole crab *Hippa pacifica* (see Haley, 1979), which refuted theoretical assertions that this species should display protandric hermaphroditism (Wenner, 1972).

Social Environment and Timing of Maturation

Social environment may not influence the direction of expressed sex, but there is good evidence that it influences the timing of female maturation. Single females reached puberty (differentiated externally) nearly 130 days later than females from MF pairs (Fig. 3). Single females were, on average, 22% larger than MF-pair females at the onset of puberty (Fig. 3). Therefore, single females put energy into growth at the expense of reproductive development. Female reproductive fitness is directly related to size (number of eggs produced), and eggs spawned without fertilization represent wasted resources. Harlequin shrimp females are similar to most caridean shrimp in that they generally spawn eggs with every molt (Nelson, 1991). Therefore, lone females that can forego sexual maturation and egg production until mates are present have a selective advantage over those that cannot. Conversely, females with an available mate may benefit from reproducing as quickly as possible. This would ensure some reproductive fitness in the face of high mortality rates or competition with other females for food and mates. These mechanisms may have evolved for this species as a result of their sparse distribution and limited food source (sea stars). Interestingly, the effect of social environment on the timing of puberty has not been demonstrated in any other crustacean.

It is difficult, however, to explain comparisons to females paired with other females (F(FF)). These individuals matured later than females from MF pairs (Fig. 3) but not lone females. However, F(FF) puberty sizes were not different from F(MF) but were statistically different from single females. Furthermore,

within FF pairs, one female typically matured early (on par with F(MF)), whereas the other had much delayed puberty. This manifests itself in the high standard deviation in F(FF) puberty age but not size.

What could explain these observations? Conceivably, females paired with other females should maximize their competitive abilities by outgrowing their partners. Alternatively, limited numbers of available males may select for early maturation to take advantage of any available mates. Competition for males seems unlikely, as males of this species do not provide any parental care or resources to females and can fertilize more than one if they are in close proximity (Seibt and Wickler, 1979). Also, the social system of *Hymenocera picta* has been described as male-enforced monogamy (Seibt and Wickler, 1979; Wickler and Seibt, 1981). Differences were noted in the TLs of FF pair mates at most stages of Paired Juveniles replicate II. The larger female invariably reached puberty earlier than the smaller within each pair. Also, there was evidence of agonistic interactions in FF pairs, such as missing appendages and high IPD. These larger females may reach maturity earlier than smaller ones simply because they can. In the natural habitat, females would likely disperse from each other, except if competing directly for very limited food sources. In the experimental buckets, females were forced to stay in relatively close proximity. If the cue for early female maturation is simply the constant presence of another conspecific, forced proximity may trip this switch. However, competition for food and agonistic interactions may only allow the larger individual to reach puberty at a normal schedule and size. Males were absent; therefore, unless females are competing for future available males, it is difficult to explain any selective advantage for the early maturation observed in experimental FF females.

Experimental evidence for social control of puberty timing in other decapods is lacking. The closest example comes from the freshwater prawn *Macrobrachium rosenbergii*, in which there are three ontogenetic stages of mature males (Ra'anan and Cohen, 1985; Ra'anan and Sagi, 1985). Here, the relative ratios of male morphotypes is fixed, and individuals can shift (irreversibly) to the next stage if more advanced individuals are removed from the population (Ra'anan and Cohen, 1985). This research parallels the present study in that the timing of sexual development is dependent

upon social environment. However, this is an example of social control of mature male morphotypes, not social control of the timing of puberty onset, as in the present study.

Intra-Pair Distance and the Pair Bond

For juvenile pairs, mean IPD is a reasonable indicator of the sex of pair mates, at least for MF pairs, supporting Seibt's (1980) observations on adult pairs. Close proximity is characteristic of the pair bond and "male-enforced" monogamy in *Hymenocera* (Seibt and Wickler, 1979), so it is not surprising. In the present experiment, fluctuations in IPD appear to be reduced near the time of female puberty in male/female pairs (Fig. 1). This suggests that close associations between pair-mates may not occur until females reach puberty. If this is the case, the opportunities to influence the expressed sex of juveniles may be limited to adult-juvenile interactions.

Unfortunately, the IPD data from the Adult/Juvenile experiment do not support this. Intra-pair distances appear to fluctuate over the entire course of the experiment for all pairs, with one MF exception (not shown). Furthermore, IPDs for various sex combinations cannot be distinguished statistically (Fig. 2d). Artifacts of the Adult/Juvenile experiment could account for some of the discrepancies. The buckets were larger, decreasing the chance for close interactions. Also, the shelters may have allowed juveniles to hide effectively. However, this seems unlikely, as shrimp outgrew the shelter within a few weeks of puberty.

Another possibility is that the adults are less willing to associate with small individuals. A smaller female represents a lower quality reproductive opportunity for adult males (fewer eggs per spawning). Adults do show preference and some memory for past pair-mates (Seibt and Wickler, 1979), and the most of the adults were paired with larger shrimp prior to the experiment. However, it seems unlikely that these animals could retain a memory of past partners for several months. This is the case for the stenopodid shrimp *Stenopus hispidus*, which also forms long-term monogamous pair bonds (Johnson, 1977). *Stenopus hispidus* does not show individual recognition towards past pair-mates if they have been separated for more than an entire molt cycle.

Yet another possibility is the use of food. Although juvenile/adult pairs were often observed to share the same piece of food, it

is possible that competition for food was more intense given the larger size of one pair member. However, the amount of food provided was adjusted for the size of shrimp. No data were taken on the amount of food at the time IPD was measured. Therefore, explanations for IPD differences between adult/juvenile pairs and juvenile pairs are only speculative.

CONCLUSIONS

1. A variety of artificial social environments do not appear to have a clear effect on the expressed sexes of cultured juvenile Harlequin shrimp (*Hymenocera picta*) in the laboratory.
2. Spacing behavior, in the form of Intra-Pair Distances, was indicative of the sexes of young shrimp and, occasionally, functional maturity.
3. Social environment did have an effect on the timing of female puberty, as single females matured later and at larger body sizes than females with male partners. However, single females matured in a similar amount of time as females with female partners, but at smaller sizes.

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