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DISTURBANCE PHEROMONES IN THE CRAYFISH *Orconectes virilis*

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Abstract—The reactions of individual crayfish to the introduction of waters from tanks containing other individuals were recorded to test for the release of chemicals by stressed crayfish. Female *Orconectes virilis* and male *O. rusticus* did not show responses to stressed crayfish. Male *O. virilis* responded differently to undisturbed and disturbed male crayfish (conspecific and heterospecific). Responses to waters from tanks which contained disturbed individuals were similar whether the source of disturbance was aggressive, predatory, or thermal. Chemical(s) involved appear to persist for at least one hour at room temperature.

Key Words—Crayfish, *Orconectes virilis*, Crustacea, pheromones, stress, heterospecific responses, threshold effects, predation, thermal stress.

INTRODUCTION

Alarm pheromones and Schreckstoff are widely known in aquatic systems (Pfeiffer, 1963; Sleeper et al., 1980). In crayfish, reactions to water in which conspecific males were aggressively interacting have been reported for both *Procambarus acutus* (Thorp and Ammerman, 1978) and *Orconectes virilis* (Hazlett, 1985).

The possibility was raised (Hazlett, 1985) that the chemicals given off by aggressing crayfish were not specific to that situation but rather were given off whenever an animal was disturbed. The following experiments test the idea that such chemicals are given off in a variety of situations and that conspecifics detecting these chemicals would respond in a similar manner irrespective of the source of disturbance to the emitting crayfish.

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If the above hypothesis is validated, it would complicate the testing of any chemical detection system since disturbed and undisturbed emitters of any pheromones would present different arrays of potential signals. Clearly it would be advantageous to be able to detect if others in the area (conspecific and heterospecific individuals) are stressed in some way.

METHODS AND MATERIALS

The crayfish observed were hand-caught individuals of *Orconectes virilis* and male *O. rusticus* (no female *O. rusticus* were found at this time). The crayfish used as potential sources of chemicals were individuals of *O. virilis*, *O. rusticus*, and *O. propinquus*. The *Orconectes rusticus* were collected from a stream emptying from the southwest corner of Carp Lake near Paradise, Michigan. It should be noted that *O. rusticus* and *O. virilis* have overlapped in distribution only in recent times and are not believed to have cooccurred over evolutionary time (Capelli and Magnuson, 1983). The *O. propinquus* and most of the male *O. virilis* used were collected from the Maple River south of Pellston, Michigan. Most of the female *O. virilis* and a few males were from the north end of Burt Lake. All crayfish were sexually mature except one male *O. virilis* used as a source animal (see below). The tests were conducted in the Lakeside Laboratory of the University of Michigan Biological Station, Pellston, Michigan, during July and August 1984.

The basic method of data acquisition was similar to that outlined by Hazlett (1985). The postures of the crayfish recorded are associated with aggression/predator defense (raised postures), submission/resting (lowered postures), or general investigation of the habitat (neutral postures). For details see Hazlett (1985). Animals to be observed were placed in individual 10-gallon aquaria (50 × 26-cm bottom dimensions) which were visually isolated from each other. The water in each tank was 15 cm deep and was continually stongly aerated. Crayfish were allowed to acclimate to the observation aquaria for 48 hr prior to testing. Individuals of *O. rusticus* in particular continued to behave as if agitated (continual locomotion, frequent aggressive displays without known stimulation, climbing walls of aquaria) after only 24 hr acclimation, so a standard of two days was used for all animals to be observed or used as an undisturbed source animal. Cardboard blinds with a small viewing hole covered the sides of each aquarium.

Observations were always carried out between 1230 and 1700 hr. During a 10-min observation period, water was introduced into the aquarium in the corner farthest from the crayfish at a rate of 44 ml/min by a Masterflex brand peristaltic pump (model 7543-30, Cole Parmer) with a model 7015-20 head. The behavior of the test crayfish was categorized continuously into one of three positions (lowered, neutral, raised) for each of three body parts (cephalothorax or

body, chelipeds, and abdomen). The number of seconds during which each body part was in each position was recorded on an Esterline Angus chart recorder. The recorder and the pump were on a separate, movable table to minimize the possibility of vibrations from the equipment affecting the animals. Ten replicates of each test condition were run except for observations on female *O. virilis* ($N = 11$ for all three conditions) and the self-water condition (see below) for both *O. rusticus* males ($N = 9$) and *O. virilis* males ($N = 51$).

Each test animal was observed under three to four different test conditions, always including "self-water," but there was only one test per day per individual. The order of test conditions for any individual was random and at least three different conditions were tested on any day of observation. However, the testing of some conditions was completed earlier in the summer and others later, thus introducing the possibility of seasonal complications. Water introduced to an observation crayfish came from either the observed crayfish's own aquarium (self-water, control) or a visually isolated source aquarium containing 9 liters of water (20 cm deep) which was continually aerated and contained one or more crayfish. Several stones were used to fashion a shelter in the source aquarium and the resident was allowed 48 hr acclimation prior to use in any tests. In the self-water tests, both the input and output ends of the tubing were quietly introduced into the observed crayfish's aquarium and no observations were done if the crayfish showed any reaction to the introduction of the tubes.

Male *Orconectes rusticus* were observed during the introduction of three sources of water: (1) self-water, (2) an undisturbed, isolated male *O. rusticus* in the source aquarium, and (3) aggressing male *O. rusticus*. In condition 3, a second male *O. rusticus* was added to the source aquarium 15 sec prior to the start of observations of the test animal. The two males were of similar size and usually were very actively aggressing.

Female *O. virilis* were also observed during the introduction of three types of water: (1) self, (2) an undisturbed, isolated female *O. virilis* in the source aquarium, and (3) two aggressing female *O. virilis*.

In addition to self-water, the conditions under which male *O. virilis* were observed in the first set of tests (1-11) were as follows:
(1) undisturbed, isolated male *O. virilis;* (2) undisturbed, isolated female *O. virilis*, and (3) undisturbed, isolated male *O. rusticus*.

Agonistically Stressed Crayfish: These included (4) two aggressing male *O. virilis*; (5) two aggressing female *O. virilis*; (6) two aggressing male *O. rusticus*; and (7) two aggressing male *O. propinquus*.

Test conditions 1–7 were designed to see if all aggressing crayfish give off chemicals to which male *O. virilis* respond.

Abiotic Stress: These included (8) one heat-stressed male *O. virilis*. About 15 min prior to introducing source water, aquarium heaters started increasing the temperature of the source tank (initially 20–21 °C). When the temperature had increased by 8 °C, observations started and the elevated source aquarium

temperature was maintained during the observation period. The tubing carrying the source water was immersed in ice water and when the water was introduced to the observation aquarium it was elevated only 1.5–2.5°C and caused no measurable change in the temperature of the water in the observation aquarium. Heat was used as an easily controlled abiotic form of environmental stress which crayfish could encounter either naturally or as a result of human perturbations.

Predator-Stressed Crayfish: These included (9) one predator-stressed male *O. virilis*. The source aquarium was placed on the movable table holding the Esterline Angus recorder (both for easier access by the "predator" and to control for any possible substrate vibrations). The source crayfish was chased and prodded with an aquarium net by the observer during the observation period. Either the predator-defensive Aufbäumreflex or solid pinch on the net was elicited at least once every 30 sec.

(10) One predator-stressed male *O. propinquus* was subjected to conditions similar to nine, except there were no stones serving as a shelter in the source aquarium, thus allowing easier access by the "predator" to the source crayfish.

(11) Five predator-stressed male *O. propinquus* were subjected to the same conditions as in 10 except there were five individuals in the source aquarium and all were agitated.

In addition to the above tests, the responses of male *O. virilis* were recorded during the introduction of source aquarium water in two additional sets of tests.

Aged Water tests: (12) Forty-five minutes prior to the start of an observation period, one liter of water was scooped out of a source aquarium containing an undisturbed male *O. virilis*. This was set aside for 45 min and then used as the source water for introduction via the peristaltic pump. A given male *O. virilis* and its source aquarium was used only once per day for undisturbed male water.

(13) For 0-min *O. rusticus* aggressing males, two male *O. rusticus* were placed in a source aquarium and allowed to aggressively interact for 10 min. They were then removed and water from the now-empty aquarium used for introduction.

(14) For 15-min *O. rusticus* aggressing males the conditions were the same as 13 except that 15 min passed between removal of the *O. rusticus* and the start of observations.

(15) For 45-min *O. rusticus* aggressing males the conditions were the same as 13 and 14 except that 45 min passed between removal of the *O. rusticus* males and the start of observations.

Water from aggressing male *O. rusticus* was used in conditions 13–18 because of the strong responses of male *O. virilis* to aggressing male *O. rusticus* water and the lack of any response to undisturbed *O. rusticus* water, thereby eliminating any complication of responses to sex pheromones.

During observations, test animals usually showed no observable response to the introduction of water of any condition during the first several minutes.

Moreover, it seemed that once a test animal was behaving in a given manner, it persisted even after the introduction of water had ceased. To see if this was due to a threshold effect, the following tests were run:

(16) For 2-min active *O. rusticus* aggression, water from a source aquarium containing two aggessing male *O. rusticus* was introduced to an observation aquarium containing a male *O. virilis* for 2 min and the pump then turned off. Observation continued for 8 min without the introduction of any water.

(17) For 4-min active *O. rusticus* aggression conditions were the same as 16 except that 4 min of water introduction was followed by an additional 6 min of observation without water introduction.

(18) For 10-min active *O. rusticus* aggression conditions were the same as six.

All statistical analyses for differences in responses were by one-way ANOVAs of the number of seconds spent by crayfish in the various postures under the different test conditions. Since there was a set number of seconds (600) in an observation period and the three positions were exhaustive (the crayfish had to be in one of them), only two postures of each body part can be treated as independent. The neutral and raised postures were chosen for analysis as they were the best indicators of responsiveness in earlier tests (Hazlett, 1985), although the netural postures were infrequent in that study due to the test conditions utilized.

ANOVAs with pair-wise comparisons were run separately on the responses of female *O. virilis*, the responses of male *O. rusticus*, and the responses of male *O. virilis* to (a) conditions involving female *O. virilis* as a potential source, (b) all other conditions in the first set of tests, (c) aged water tests, and (d) threshold tests. Only those pair-wise comparisons that addressed biologically interesting questions were examined.

In just a few cases, the equality of variance assumption was not met by untransformed data, and so nonparametric analyses were also run wherever possible. In every case the Kruskal-Wallis scores were associated with probability values similar to those from the ANOVA analyses. Overall comparisons between treatments were either clearly insignificant ($P > 0.10$) or very significant (<0.001) by both tests. For ease of reading, only the ANOVA results will be mentioned.

RESULTS

The time spent in the various postures by males of *Orconectes rusticus* during observation periods is shown in Table 1. There was no significant variation among treatments in any of the postures (overall F values associated with $P > 0.10$).

Responses of female *Orconectes virilis* are shown in Table 2. Although the time spent in raised body positions appeared to be higher when undisturbed

TABLE 1. MEAN NUMBER OF SECONDS (±SD) SPENT IN VARIOUS POSTURES BY MALE *Orconectes rusticus* DURING 10-MIN OBSERVATION PERIODS WHILE DIFFERENT SOURCES OF WATER WERE INTRODUCED

Source condition	Body neutral	Chelipeds neutral	Abdomen neutral	Body raised	Chelipeds raised	Abdomen extended
Self-water	118 (124)	70 (72)	47 (47)	120 (195)	112 (196)	109 (205)
Undisturbed male *O. rusticus*	104 (121)	114 (138)	75 (111)	152 (169)	139 (171)	117 (166)
Aggressing male *O. rusticus*	111 (104)	130 (131)	68 (69)	90 (135)	82 (114)	60 (120)
Probabilities associated with *F* values	0.96	0.54	0.74	0.79	0.73	0.71

TABLE 2. MEAN NUMBER OF SECONDS (±SD) SPENT IN VARIOUS POSTURES BY FEMALE *Orconectes virilis* DURING 10-MIN OBSERVATION PERIODS WHILE DIFFERENT SOURCES OF WATER WERE INTRODUCED

Source condition	Body neutral	Chelipeds neutral	Abdomen neutral	Body raised	Chelipeds raised	Abdomen extended
Self-water	157 (144)	87 (79)	57 (100)	52 (95)	45 (91)	13 (32)
Undisturbed female *O. virilis*	127 (99)	97 (120)	61 (91)	113 (157)	99 (48)	74 (130)
Aggressing female *O. virilis*	135 (128)	83 (85)	47 (65)	55 (73)	57 (67)	14 (21)
Probabilities associated with *F* values	0.84	0.92	0.92	0.37	0.47	0.13

conspecific water was introduced, there were no significant differences among treatments for any of the postures (overall F values associated with $P > 0.10$).

The responses of male *O. virilis* under all the conditions tested are shown in Table 3. Comparisons of the time spent in the various postures by treatment yielded very significant effects of treatment in every case (overall F values associated with $P < 0.0001$; between 21 and 29% of the variance explained by treatment effects).

The time spent in the various postures by male *O. virilis* did not differ among treatments when female *O. virilis* were in the source aquarium except for one comparison. When two females were interacting aggressively, more time was spent in the body neutral posture than during control periods ($P = 0.04$). All other comparisons were clearly insignificant. In the case of undisturbed, isolated male *O. rusticus* and two male *O. propinquus*, there were no significant differences in any of the postures when compared to control periods.

The time spent in postures by male *O. virilis* when undisturbed male conspecific (UMC) water was introduced was significantly different from control periods for only one posture (abdomen extended) (Table 4). However, two of those tests were inadvertently run with a source male *O. virilis* which was form II and probably sexually immature. If the responses of male *O. virilis* to only form I (sexually active form), undisturbed, male conspecifics are considered ($N = 8$), there are differences in the time spent in all three neutral postures compared to control periods. If the responses to UMC water aged 45 min are considered, clear differences appear, especially in the greater time spent in the raised postures (Table 4), compared to either control periods or the unaged UMC tests.

The responses of male *O. virilis* to water from aggressing male conspecifics was different from controls only in a greater time spent in the body neutral position ($P = 0.030$). Responses to water from one predator-stressed *O. propinquus* were different from controls in all three neutral positions ($P = 0.029$, 0.014, and 0.020 for body, cheliped, and abdomen).

Four other test conditions (predator-stressed male *O. virilis*, five predator-stressed male *O. propinquus*, aggressing male *O. rusticus*, and heat-stressed male *O. virilis*) were very similar in the responses elicited. For all four conditions, (a) all three neutral positions were very significantly different from control periods (all $P < 0.001$), (b) in almost all there were no differences from control periods in the time spent in the raised positions ($P > 0.10$, except for aggressing *O. rusticus* which did elicit an increase in raised postures compared to control periods), and (c) very few differences among the responses shown under these four test conditions. The latter is shown in Table 5, in that among the 36 pairwise comparisons possible, the only differences were greater raised time with aggressing *O. rusticus* than with predator-stressed conditions and less time in neutral postures with heat-stressed compared to predator-stressed *O. virilis*. The

differences among these four conditions are not large compared to the differences between each of the four and the control period.

The responses of male *O. virilis* to aged water in which male *O. rusticus* had been aggressing did not differ from the responses shown to unaged water (containing aggressing *O. rusticus*). The overall F values comparing the four treatments (unaged, aged 0 min, 15 min, and aged 45 min *O. rusticus* aggressing males) were all associated with $P > 0.10$, except the body raised posture (overall $P = 0.0853$) for which the 15- and 45-min aged water was different from the 0-aged water ($P = 0.036$ and $P = 0.046$).

The response of male *O. virilis* in the threshold tests showed significant variation among treatments ($P < 0.01$) for all six postures, especially in the time spent in neutral postures ($P < 0.0006$). Two minutes of aggressing *O. rusticus* water did not elicit responses different from control periods (all postures $P > 0.10$), while both 4 and 10 min of active aggressing *O. rusticus* elicited significantly more time in all three neutral positions compared to controls ($P < 0.001$) but did not differ from one another ($P > 0.10$).

DISCUSSION

Male *Orconectes virilis* reacted to waters in which other male crayfish were stressed, whether those crayfish were conspecifics or members of other species. The responses were similar in all cases and consisted of increased time spent in "neutral" postures during the observation periods.

The "neutral" label could be replaced by "intermediate," since the postures were physically between the resting/lowered postures and the aggressive/raised postures. Normally, as during the control (self-water) periods, individuals of most crayfish are relatively inactive during the midafternoon hours and are in the lowered postures unless disturbed. If the source of disturbance is strong and direct (e.g., predator attack, aggressing conspecific), the crayfish responds with raised postures. As proposed earlier (Hazlett, 1985), the intermediate (neutral) postures seem to reflect a change in alertness associated with a low level of disturbance of a less well-defined nature. The positions of the cephalothorax, chelipeds, and abdomen seem linked with an investigatory or wary mood. It would appear that male *O. virilis* can chemically detect if other male crayfish in the area have been stressed or disturbed by something and are then more attentive to stimuli in general. This response would increase the recepient's chances of detecting the source of disturbance and perhaps avoiding stressful situations.

The lack of a significantly different response by male *O. virilis* to aggressing female *O. virilis* may well result from the complications of sex phermone detection. As reported earlier (Hazlett, 1985), male *O. virilis* can chemically

TABLE 3. MEAN NUMBER OF SECONDS (±SD) SPENT BY MALE *Orconectes virilis* IN VARIOUS POSTURES DURING 10-MIN OBSERVATION PERIODS WHILE WATER FROM VARIOUS SOURCES WAS INTRODUCED

Source condition	Body neutral	Chelipeds neutral	Abdomen neutral	Body raised	Chelipeds raised	Abdomen extended
Self-water	39 (70)	21 (54)	19 (45)	4 (21)	2 (10)	2 (8)
Undisturbed male *O. virilis*	102 (107)	103 (114)	74 (103)	42 (53)	35 (39)	35 (51)
Aggressing male *O. virilis*	144 (144)	91 (95)	51 (93)	34 (98)	31 (99)	28 (91)
Undisturbed female *O. virilis*	82 (103)	54 (103)	26 (55)	9 (15)	6 (13)	1 (3)
Aggressing female *O. virilis*	139 (173)	85 (112)	61 (113)	19 (36)	22 (39)	5 (10)
Undisturbed male *O. rusticus*	66 (107)	61 (107)	57 (121)	18 (39)	20 (43)	9 (22)
Aggressing male *O. rusticus*	223 (179)	241 (191)	166 (192)	67 (118)	58 (117)	39 (92)
Aggressing male *O. propinquus*	98 (144)	77 (115)	76 (125)	18 (34)	17 (34)	10 (26)
Heat-stressed male *O. virilis*	190 (153)	124 (88)	82 (109)	26 (76)	26 (79)	25 (81)
Predator-stressed male *O. virilis*	322 (203)	240 (195)	251 (218)	7 (14)	3 (6)	1 (3)
Predator-stressed male *O. propinquus*	147 (184)	126 (165)	123 (196)	18 (47)	20 (38)	1 (3)
Predator-stressed five male *O. propinquus*	269 (212)	182 (173)	169 (160)	10 (20)	15 (23)	1 (1)

45-min aged water						
undisturbed male						
O. virilis	167 (99)	147 (97)	148 (116)	183 (168)	144 (143)	139 (128)
Probabilities	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Aged water tests						
0-min aged water						
aggressing						
O. rusticus	349 (119)	307 (145)	208 (180)	51 (56)	43 (56)	35 (47)
15-min aged water						
aggressing						
O. rusticus	181 (208)	131 (166)	141 (204)	3 (8)	3 (7)	1 (1)
45-min aged water						
aggressing						
O. rusticus	260 (210)	185 (157)	201 (187)	6 (14)	11 (27)	5 (12)
Probabilities	0.227	0.120	0.404	0.085	0.209	0.230
Threshold Effects Tests						
2-min active						
aggressing						
O. rusticus	102 (165)	54 (104)	58 (130)	0	0	0
4-min active						
aggressing						
O. rusticus	228 (158)	143 (141)	126 (138)	12 (24)	12 (30)	0
10-min active						
aggressing						
O. rusticus	180 (166)	139 (137)	136 (187)	49 (109)	41 (90)	28 (83)
Probabilities	<0.001	<0.001	<0.001	0.032	0.018	0.075

TABLE 4. PROBABILITIES ASSOCIATED WITH *F* VALUES FROM ANOVA PAIR-WISE COMPARISONS FOR RESPONSES OF MALE *O. virilis* TO UNDISTURBED MALE *O. virilis* WATER (AGED AND UNAGED) AND CONTROL PERIODS

Comparison	Body neutral	Chelipeds neutral	Abdomen neutral	Body raised	Chelipeds raised	Abdomen extended
Self vs. all UMC unaged	0.201	0.055	0.229	0.070	0.086	0.044
Self vs. form I UMC unaged	0.011	0.000	0.005	0.081	0.079	0.058
Self vs. aged UMC	0.009	0.003	0.005	0.000	0.000	0.000
Unaged vs. aged UMC	0.305	0.419	0.215	0.000	0.000	0.000

TABLE 5. PROBABILITIES ASSOCIATED WITH *F* VALUES FROM ANOVA PAIR-WISE COMPARISONS OF TIME SPENT IN VARIOUS POSTURES BY MALE *O. virilis* UNDER FOUR DIFFERENT TEST CONDITIONS

Comparison	Body neutral	Chelipeds neutral	Abdomen neutral	Body raised	Chelipeds raised	Abdomen extended
Aggressing *O. rusticus* vs. predator-stressed *O. virilis*	0.124	0.992	0.154	0.024	0.027	0.075
vs. predator-stressed *O. propinquus* (5)	0.475	0.286	0.958	0.032	0.083	0.070
vs. heat-stressed *O. virilis*	0.605	0.034	0.155	0.120	0.192	0.539
Predator-stressed *O. virilis* vs. predator-stressed *O. propinquus* (5)	0.407	0.291	0.170	0.907	0.626	0.973
vs. heat-stressed *O. virilis*	0.040	0.035	0.004	0.478	0.359	0.243
Heat-stressed *O. virilis* vs. predator-stressed *O. propinquus* (5)	0.219	0.289	0.140	0.552	0.666	0.230

distinguish between male and female conspecifics, and the response to undisturbed females is to assure submissive postures, the latter being part of courtship behavior (Ameyaw-Akumfi, 1979). If males' responses to sex pheromones are stronger than responses to disturbance pheromones, demonstration of release of the latter by females would be complicated.

The lack of a response by male *O. virilis* to water containing two male *O. propinquus* was clearly related to the low level of (aggressive) stress of the *O. propinquus*. In every replicate, the two *O. propinquus* spent the majority of the test period quietly resting in different parts of the source aquarium, not interacting, whereas when two male *O. virilis* or *O. rusticus* were placed in the source tank they interacted aggressively the vast majority of the testing period. Thus this situation actually tested for responses to apparently undisturbed male *O. propinquus*, and there were no differences between that condition and control periods. Male *O. virilis* showed no observable responses to water containing undisturbed male nonconspecific crayfish (*O. propinquus* and *O. rusticus*), a result similar to that reported for (*O. rusticus*) by Tierney and Dunham (1984).

The responses of male *O. virilis* to water containing one predator-stressed *O. propinquus* just were significantly different from control periods. However, water from five predator-stressed *O. propinquus* did elicit a clear response, thus male *O. virilis* do react to chemicals from stressed individuals of that species. The weak response to the single stressed *O. propinquus* condition is probably related to the size of *O. propinquus* used as a potential source. This is a smaller species in general, and the mean cephalothorax length of the source individuals of *O. propinquus* was 26 mm compared to 41 mm, 40 mm, and 44 mm for male *O. virilis*, female *O. virilis*, and *O. rusticus*, respectively. In the test situation used, one crayfish that size may not have been able to produce enough of the purported chemical(s) to pass the detection threshold of the system in many of the replicates.

The lack of female *O. virilis* response differences to waters of different conditions is perplexing but consistent with the results from earlier tests (Hazlett, 1985). Female *O. virilis* do respond differently to waters containing conspecific vs. heterospecific males (Tierney and Dunham, 1984), but they showed no response differential to waters of differing conditions in the present study. Additional types of tests are needed such as those of Rose and Casper (1980) which did demonstrate a number of chemically mediated responses in female *Procambarus clarkii*.

The clear lack of differences in the responses of male *O. rusticus* may have been an artifact of the testing situation. Individuals of *O. rusticus* were very easily disturbed in the laboratory situation and did not appear fully acclimated to the aquaria (observation or source) even after two days. The individual *O. rusticus* observed may have been unacclimated and thus no differential in responses detectable (a "wary" crayfish cannot become "wary"). In addition, the

source water (self, isolated, and aggressing) may all have been from partially disturbed crayfish, thus the lack of a differential in responses to such waters.

As in any study, the presence or absence of differences in responses to various conditions is, of course, limited by the behavioral data taken. The nine categories used (three body parts, three positions) were chosen for their utility in recognizing responses to sex pheromones (Ameyaw-Akumfi and Hazlett, 1975; Hazlett, 1985). Obviously in every case where no response differential was detected among or between treatments, it may have been that the appropriate behavior patterns were not recorded (see Rose, 1982). Thus while the data taken indicate differentials between control and many test situations, the lack of differences among various stressed situations could be due to the limited number of patterns measured (Ameyaw-Akumfi and Hazlett, 1975; Christofferson, 1978; Gleeson, 1980). The detection of disturbance pheromones requires undisturbed animals for control periods. The lack of such controls may well lead to problems of data interpretation [see the sex pheromone discussion of Hazlett (1984), Thorp (1984), and Rose (1984)].

The responses of male *O. virilis* to undisturbed male conspecifics (UMC) were similar to that reported for this species (Hazlett, 1985), when only form I males were tested. The results of the two replicates which involved a form II source male can only be taken as suggestive that form II males do not give off a sex pheromone. It is clear, however, that even considering only the responses for form I UMC, the response level was higher in the aged UMC water tests compared to the unaged water. It is unlikely that the potency of the sex pheromone increases upon aging 45 min [although the chemical(s) involved do remain active at least that long]. A more probable explanation is the difference in time of testing. Most of the unaged UMC tests were run in June when many males had just recently molted to the sexually active form I. The aged UMC tests were conducted in August when some sexual activity by *O. vivilis* was seen in the field and laboratory. Seasonal variation in behavior has been reported for most temperate zone crayfish (e.g., Thorp, 1978).

Whatever the chemical nature of the disturbance pheromone, it seems somewhat stable. Although there seemed to be a trend towards reduced effectiveness of aggression-stressed water after 45–55 min of standing at room temperature, this was not significant.

The tests with 2, 4, and 10 min of aggressing *O. rusticus* water introduction indicated a threshold effect in two ways. The clear lack of responses to just 2 min of water introduction suggests that there is a response threshold—88 ml of water was insufficient to elicit a response while 176 ml was sufficient. This could be due to a detection threshold (insufficient concentration of molecules for sensory detection given the pattern water movement) and/or a motivational threshold. The latter refers to central nervous system influences which determine the behavior state or drive level of an animal. The fact that 176 ml of water elicited

the same responses as 440 ml of water (10 min of introduction) points toward a motivational threshold mechanism. Once sufficient information about disturbances is detected, the animal is wary for some minutes even if added input is not forthcoming. The results of Rose and Casper (1980) with female *Procambarus clarkii* also indicated a threshold effect.

The results of this study take care of several problems with an earlier study of responses to pheromones by individuals of *Orconectes virilis* (Hazlett, 1985). First, the aggressing crayfish condition has two animals in the source aquarium rather than one. However, similar responses to waters were obtained in this study with one *O. virilis* when it is stressed (heat or predator). Secondly, the fact that self-water tests involved water from an aquarium without a rock shelter while the source tanks had rock shelters raises a problem of confounded differences in conditions. Yet the predator-stressed *O. propinquus* tests were run without any rock shelters in the source aquarium, and those tests yielded results similar to other stressed-crayfish conditions. In addition, five tests (not included in the Results section) were run with male *O. virilis* as observed crayfish in which I thought there was a male *O. virilis* under the rocks in the source aquarium. After observations were completed, I discovered the source tank was empty. In those five tests, the crayfish were in fully lowered postures for the entire 10 min, thus these served as a control (inadvertantly) both for source aquarium features unrelated to the crayfish present and for possible observer bias.

While these tests were obviously limited taxonomically to crayfish, it seems reasonable to expect disturbance semiochemicals in many species, especially aquatic organisms. Animal metabolisms are likely to shift slightly in some way (qualitatively or quantitively) when disturbed, and it would be to any individual's advantage to detect disturbances in their environment and learn to respond appropriately (Valenta and Rigby, 1968).

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