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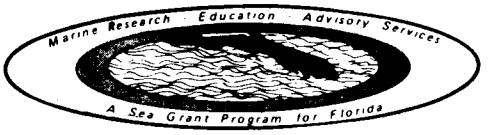

Florida Sea Grant

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INTRODUCTION

It is now well established that chemical stimuli control diverse types of behavior among marine organisms (Bardach, 1975). The chemical stimuli controlling feeding behavior are at least in part simple organic compounds such as amino acids and their derivatives. It follows that such compounds, once evaluated for their activity and appropriately packaged, could provide an effective, economical, and convenient artificial bait for economically important marine organisms. The present study evaluates the potential of simple organic compounds as attractants for the Florida spiny lobster, *Panulirus argus*.

Two issues introduce some question as to the potential role of simple organic compounds as feeding attractants. As noted by Carr et al. (1974), since most studies implicating simple organics as feeding stimulants have not systematically eliminated macromolecules as active feeding stimulants by "working down" from complete extracts of potential food organisms, small organic molecules may not entirely account for feeding competence in marine organisms. Secondly, "feeding" is a multicomponent behavior involving the more or less discrete stages of food-finding, food handling, biting, etc., each of which is potentially controlled by novel chemical (or non-chemical) cues. It remains, however, that relatively simple organic substances at least in part regulate feeding-associated activities in decapod crustaceans as the spiny lobster (McLeese, 1970; Kay, 1971; Shelton and Mackie, 1971; Mackie, 1973; Carr and Gurin, 1975; Hindley, 1975; Hamner and Hamner, 1977; Hartman and Hartman, 1977). In the one species of decapod subjected to systematic analysis of feeding behavior, the shrimp, *Palaemonetes pugio*, low molecular weight components account entirely for the stimulatory capacity of three food extracts out of six tested (Carr and Gurin, 1975).

Present research suggests chemosensitivity in decapod crustaceans is complex, minimally being partitioned between three sets of receptors, the antennules, the

mouthparts, and the periopod dactyls. The antennules are characteristically ascribed to low threshold chemoreception, the initial alerting and possibly orienting stages of chemically-elicited feeding (Maynard and Dingle, 1963; Hazlett, 1971). Certainly ablation of the lateral antennular filaments disrupts orientation towards sources of dissolved substances (McLeese, 1973; Ache, 1975), a phenomenon also demonstrated in *P. argus* (Reeder and Ache, In preparation). Physiological evidence verifies the presence of low threshold chemoreceptors in the antennular filaments of *P. argus*, receptors responsive to not only extracts of potential food organisms (Ache et al., 1976) but to solutions of single amino acids, as well (Laverack, 1964; Levandowsky and Hodgson, 1965; Johnson and Ache, 1978). While the evidence supporting distance chemosensitivity in the lobster antennule isn't conclusive, it suggests this appendage as a logical site for further analysis of such sensitivity.

Interest in the concept of defining artificial attractants or "baits" for commercially important marine organisms was facilitated by a Sea-Grant-sponsored symposium on potfishing and artificial baits (Jaeger, 1972). The basic concept was extended by Hancock (1974) who suggested repellants of predatory species, substances documented behaviorally, but yet to be characterized chemically, might enhance a particular fishery's catch-effort as much as attractant compounds. Previous workers have attempted to define artificial baits for commercially important decapod crustaceans with varying success. Trimethylamine solutions caught four times more American lobsters than unbaited traps (Moody, unpublished technical report, Bio-dynamics, Inc.). The amino acid glycine enhanced trapping the West coast dungeness crab, but not as well as natural baits (Allen et al., 1975). Studies in progress to define a chemical substance suitable for potfishing the western Australian rock lobster (*P. longipes*) are still inconclusive (R. Kage,

personal communication). A related finding is that specific amino acids attract 3 species of marine fish when dispensed in the natural habitat (Sutterlin, 1975). Field tests are confounded, however, by the additional requirement of an effective release vehicle, a vehicle that releases at suprathreshold concentration throughout the fishing interval. Suitable technology has yet to be applied on any large scale to releasing artificial attractants in aquatic environments, but exists in a number of forms of microencapsulation and slow release gels and polymers (e.g., Baker and Lonsdale, 1975). Allen et al. (1975) suggested and tested polyacrylamide gels as releasants for amino acid solutions and extracts of natural baits.

The rationale of the present project is to survey a relatively large number of chemical compounds for their ability to stimulate the antennular chemoreceptors of the spiny lobster. Included among the most stimulatory compounds should be those compounds, if any, that function as behavioral attractants. Their potential as attractants is then verified in an olfactometer designed to assay the chemotoxic component of feeding behavior. Those compounds most attractive in the olfactometer study are then field tested together with presently-used natural baits for subsequent comparison of catch (and cost) effectiveness. This latter step requires the definition of an effective releasant, which constitutes the last aspect of the project.

METHODS

Animals - Specimens of the lobster, *Panulirus argus*, were obtained locally from a commercial lobsterman and maintained (1) in 150 gal. recirculating seawater tanks in the laboratory for physiological analysis or (2) in running seawater holding facilities for behavioral analysis. Lobsters were fed frozen pink shrimp every third day unless noted otherwise. At no time during the capture, handling or holding were the animals exposed to air for periods exceeding 30 sec.

Physiological Assay - Antennular chemosensitivity was assayed using the distal 5-6 cm section of the lateral filament excised from adult lobsters and arranged for electrophysiological recording in a lucite recording chamber (Fig. 1) as described in earlier reports from our laboratory (e.g., Ache et al.,1976).

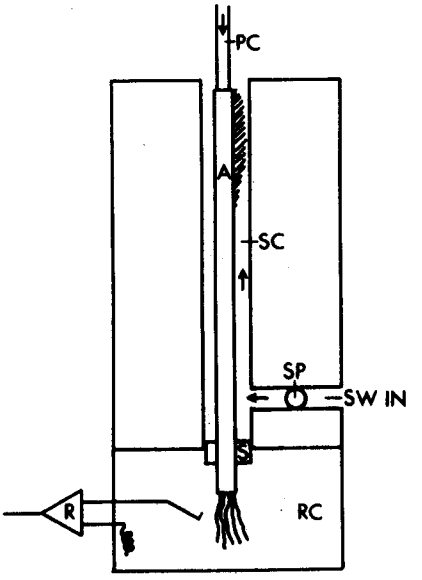

Fig. 1 - Preparation chamber for recording from excised antennules of *P. argus*. A, antennule; RC, recording compartment; SC, stimulating compartment; S, septum; PC, perfusion cannula; SW IN, seawater inlet; SP, stimulus port; R, recording electrode.

In this device, 50 µl volumes of potential stimulants injected into a 10 ml/min carrier flow of artificial seawater contact the preparation 0.4 sec post injection, rapidly peak to a maximum concentration approximately 10^{-1} times neat and subsequently tail off over the following 7 sec. All substances tested as potential stimulants were reagent grade purity. Solutions of test substances were prepared within 3 hr of use in reagent-grade artificial seawater (M.B.L. formula) and their pH adjusted to 7.6, the value of the seawater carrier flow that continously washed the preparation. Pure test chemicals were prepared for delivery at three concentrations, 10^{-3}, 10^{-4}, and 10^{-5} molar, unless noted otherwise. A few compounds of unknown molecular weight were prepared on a wt/volume basis as noted in the results section.

Nerve bundles containing 1-20 active chemosensory neurons were teased free of the main antennular nerve for recording. While recording neural activity (action potentials) from such a bundle, the preparation was stimulated sequentially with 50 µl volumes of standard shrimp extract (SSE)*, three test chemicals at a single concentration and a terminal replication of SSE. The sequence was then repeated with each of the remaining two concentrations of test chemicals. A 1.5 min wash period separated each test chemical application. A new nerve bundle was then teased free for recording and the stimulant series repeated. Each series of three test chemicals (at 3 concentrations) was evaluated on a minimum of 5 nerve bundles obtained from no less than two different antennular preparations.

The recorded action potential trains were passed through a window discriminator, adjusted to include all chemosensory activity and to discard any concomitant non-chemosensory activity, and inputed via a Schmitt trigger to an electric counter (Haer 7400 series) with the clock set at a bin width (0.5-5 HZ.) appropriate for the total number of spikes to be counted. The counter output was displayed as an instantaneous histogram on a storage oscilloscope and the activity recorded from a given bundle was calculated from the histogram's area within the time period the standard stimulant's activity remained above baseline in that bundle. Post stimulus activity of a given bundle was so quantified for each application of each of the three or, where noted, two, applied concentrations. To allow for across-bundle comparison, these values had to be normalized to some standard value, selected as a bundle's mean response to the initial and terminal

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*SSE consisted of 1 gm peeled pink shrimp (*Peneaus duorarum)* abdominal muscle homogenized in 2 ml artificial seawater, diluted 10 fold and subsequently centrifuged to obtain a clear solution.

applications of SSE. The resulting ratio is referred to herein as the "activity ratio". Activity ratios for all applications of a particular test chemical at all concentrations were then averaged (unweighted mean) to generate a "mean activity ratio" for that test chemical. Such "mean activity ratios" are reported herein as representative of a compound's ability to stimulate antennular chemoreceptors.

Behavioral Assay - Those test chemicals eliciting larger neural responses (higher mean activity ratios) were selected as candidate attractants and assayed behaviorally for their ability to elicit chemotaxis in intact organisms. Several assay devices, including two of "Y maze" design, were rigorously evaluated before selecting the device used as being most compatible with the "skittish" nature of *P. argus* and our interest to assay attractants per se, not necessarily generalized feeding stimulants. The system consisted of six, 1.5 meter diameter plastic pools arranged in a circle around a plastic head tank, all draining into a 4.6 meter diameter tank. (Fig. 2) A continuous flow of unfiltered natural seawater drained into the head tank, containing a standpipe to maintain uniform head pressure.

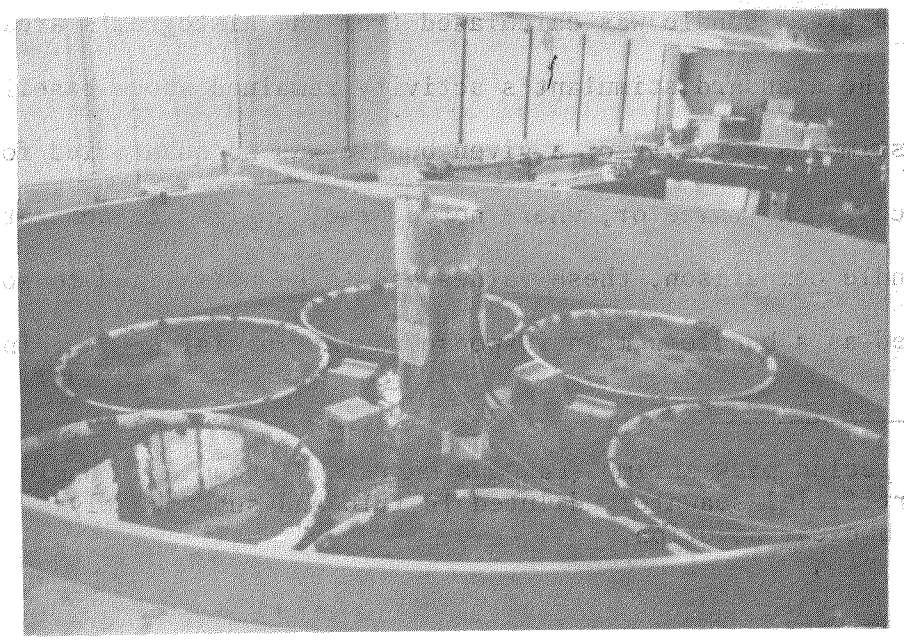

Fig. 2 - Circular olfactometers used to assay the behavioral attractiveness of selected test chemicals to *P. argus*. See text for details.

Water gravity-fed from the head tank to each test pool via tygon tubing in turn attached to a bent glass tube fixed to the side of the pool so as to direct flow of water (14.7 liter/min) parallel to the bottom. In each pool, one half of a cement block at the down stream end of the pool served as a habitat for the lobster. A potential attractant was delivered to each pool via a small diameter tygon tube inserting into each inlet tubing about 50 cm from the tube's end. This arrangement allowed for metered pumping (60 ml/min) of potential attractant solutions into the inlet flow of water where it was mixed by turbulance to a concentration 6×10^{-3} times the starting concentration before discharging into the tank. The flow established a chemical gradient across the pool such that the concentration reaching the cement block was 2×10^{-4} times the starting concentration, i.e., a 1.4×10^{-1} dilution across the diameter of the pool.

A test was preceded by introducing one test animal per pool, five days prior to testing and two days after being caught. During this "settling in" period, the animal was starved to heighten any response to potential attractants. All work was done between 1800-0400 hr under diffuse flourescent light. A potential attractant pumped into one pool reached the animal with a 10-12 sec latency as evidenced by dye observation. Lobsters which walked upstream to the immediate vicinity of the inlet tube within 4.0 min were scored as "responding". Those not meeting this criterion were scored as "not responding". Time to criterion was noted, as were the time to arousal and the time to initiate locomotion. Any unusual behavior was noted if it occurred. Repetition of this sequence in the five other olfactometers constituted one "run". A single potential attractant was tested on a single group of six "naive" lobsters over

two successive evenings. An evening's work started with two runs of shrimp extract* to provide a measure of that group's general level of responsiveness to chemical stimulation. A series of six runs were then made with the test substance at three concentrations, 10^{-2}, 10^{-3}, and 10^{-4} g/liter (where solubility allowed, otherwise as noted in results), in counterbalanced sequence so that the three concentrations occurred in every possible order. This protocol was repeated the second evening to provide a total of 72 presentations (to six organisms) of each potential attractant. A new group of six lobsters was used for each substance tested to eliminate any possible effects of conditioning introduced by repetitive testing and/or feeding, required for long term maintenance in the laboratory.

Data analysis is based on the responsiveness of each animal to the test substance. The number of responses (criteria met) of each animal to the test substance at one concentration (max possible = 4) is compared to that animal's number of responses to standard shrimp extract and expressed as a percentage. Animals not responding at all to standard shrimp extract are excluded from statistical analysis. Percent scores thus obtained are tested for significance in an analysis of variance (unequal group size, unweighted mean). The analysis indicated significant concentration-dependent effects, as expected; these were localized to the highest concentration used (10^{-2} gm/liter).

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*Shrimp extract was prepared from 50 gm wet weight headed, shelled *P. duorarum* homogenized in 200 ml artificial seawater. The resulting homogenate was centrifuged for 30 min at 12,000 G. The clear supernatant was decanted and frozen until use. For use, the supernatant was thawed and brought up to 1,000 ml volume with filtered natural seawater to provide a 50 gm/liter stock solution. One hundred ml aliquots of the stock solution were diluted to 1,000 ml to produce a 5 gm/liter standard shrimp extract concentration used in all behavioral assays. (This solution was diluted in the apparatus to a final concentration of 10^{-3} gm/liter at the animal.)

Rankings were therefore computed for responses to the 10^{-2} gm/liter runs only. Substances were ranked for relative attractiveness at this concentration by comparing the mean percentage of responses, obtained by summing the scores of all animals for a given substance and dividing the sum by the number of animals tested with that substance.

Releasant Assay - To evaluate the appropriateness of gels as dispensers for attractant compounds, the materials' leach rates were measured in a continuous-flow apparatus designed for this purpose. (Fig. 3) The retentiveness of samples

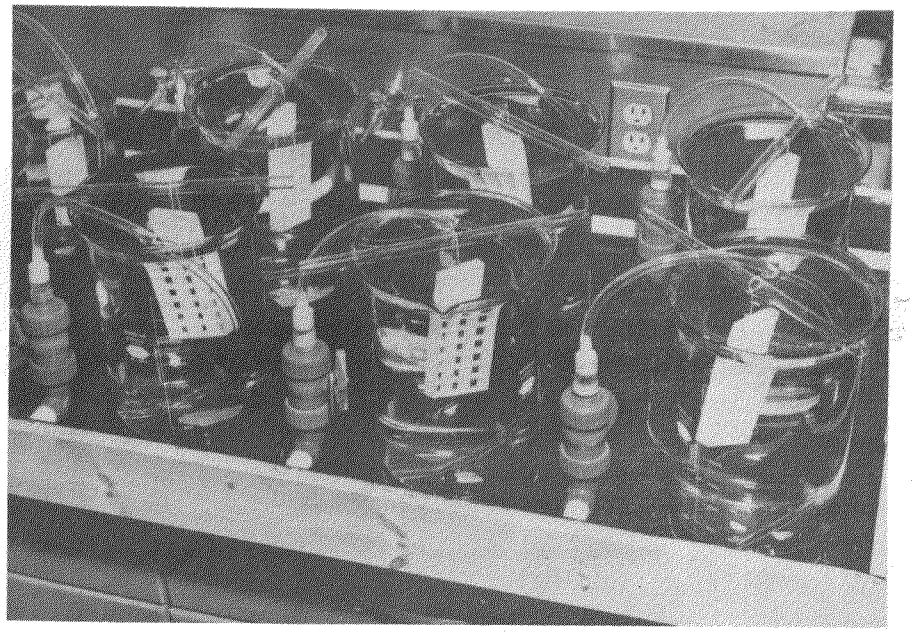

Fig. 3 - Apparatus used to measure leaching rates of various potential releasants. Flow rates into each test container can be regulated and balanced via flow-control valves.

for the water-soluble dye uranine (sodium fluorescein) was determined colorometrically by measuring dye concentration as a function of time in the effluent surrounding each sample. A continuous, regulated flow of tap water into each test container maintained maximum diffusion gradients around test samples. The gels were placed in perforated 9 x 9 cm diam, screw cap, polyethylene

wide-mouth jars (Scapro, Inc., Rockland, Maine 04841), in turn suspended in the test containers.

Laminated, controlled-release dispensers (Hercon brand, Herculite Protective Fabrics Corp., New York, NY 10010) were also tested as potential release vehicles. These represent a modified formulation of a plastic dispenser originally developed for insect attractants (Beroza et al, 1974, Hardee et al.,1975), in which a polymer matrix containing the dry chemical of interest is contained between two protective barrier layers to form a 20 x 28 cm sheet-type dispenser about 0.2 cm thick. (Fig. 4) Two forms of this dispenser were tested. One form

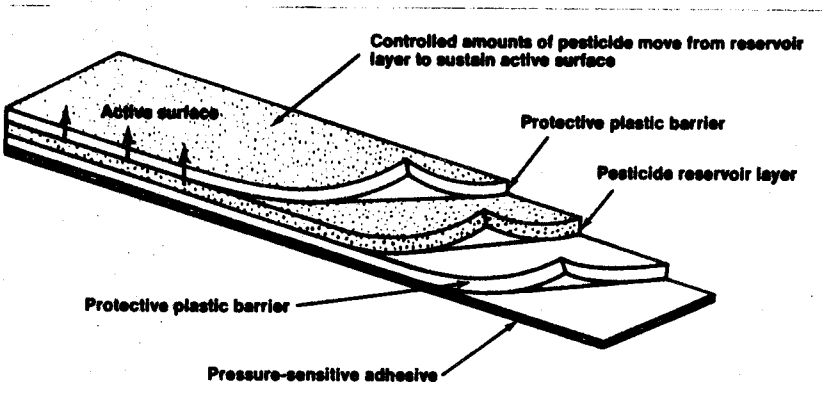

Fig. 4 - Diagramatic representation of the Hercon brand controlled-release dispensers used to release attractant compounds in field trials. Actual size, approximately 20 x 28 cm x 0.2 cm thick. The pressure-sensitive adhesive layer was eliminated on the dispensers used in the present study.

(Type A) used 5-mil polyvinyl chloride film as the barrier layers. The second form (Type B) used unbleached muslin as the barrier layer, essentially exposing the reservoir matrix directly to the environment. The amount of dry chemical contained in these dispensers varied between 1 and 10 gm and is noted appropriately in the Results section. Leach rates of these dispensers were laboratory assayed

by weight loss rather than colorometrically. Thirty, one inch squares were suspended in the test containers on a wire loop with space between samples to allow flushing. Weight loss was recorded daily and expressed as a percentage of the average weight of dry attractant in a one inch square of the laminate.

One attractant, citric acid, was also released in field studies from a natural source, dried citrus pulp (Life Guard Brand, Silver Springs Citrus Cooperative, Winter Garden, FL 32787). This material, available as a by-product of Florida's citrus industry is a mixture of dried seeds, small pieces of dried rind, and pelletized, dried pulp, commonly used as an agricultural feed supplement. It was not assayed prior to field testing; the actual rate of release of citrate from this material is unknown.

Field Tests - The most attractive test chemicals were field tested using the standard latch-trapping techniques common to the S. Florida fishery. Traps were set on a sand/patch reef bottom, 18-21 meters deep, just offshore from the Hillsborough Inlet, Broward County, Florida, in trawls of six traps (1976) or 12 traps (1978). Traps were placed at the reef edge, and returned as close to the same location as possible each time. The number of lobsters caught with a given test attractant was compared to that caught with a control bait, cowhide strips*. Baits were placed either in 25 x 12 x 2 cm wire mesh bait baskets (cowhide, laminate sheets) or in perforated polyethylene containers (agar, gels, dried citrus pulp). These in turn were secured to the top center support of the traps. Traps with test baits were alternated with control-baited

*Accurate quantification of cowhide "odor", required for rigorous control, was impossible since its fishing effectiveness appears correlated with its state of decay. Bait baskets were initially filled to capacity and kept filled by adding new material as needed each time the trap was pulled. Thus the cowhide complement of a trap included material in all states of decay for all but the first test.

traps within a trawl. The even number of traps/trawl assured both test and control traps were equidistant from the trawl ends, thus compensating possible end-effect bias. Forty-eight (24 test, 24 control) were fished in field experiments 1-10; 94 in experiments 11-14. Following an initial four day "soak", trawls were pulled every four days, weather allowing, the catch recorded (size, sex, molt condition, female reproductive state), and the lobsters returned to the habitat. Data were field-logged on magnetic tape and subsequently transcribed in the laboratory.

RESULTS

Physiological Assay - In all, 102 compounds were assayed physiologically. Eighty-four of these met criterion on final analysis for numbers of bundles and preparations tested. Mean activity ratios obtained for these chemicals are summarized in Tables 1 and 2. The compounds tested include those reported in the literature as (1) adequate stimulants for chemoreceptors of aquatic organisms or (2) components of blood, muscle and/or urine of common marine invertebrates and fishes. Some related analogs of these substances were also included. Taurine (Table 1) was the most stimulatory single chemical tested, with a mean activity ratio of 0.75 relative to that of standard shrimp extract. Of interest was that the 10 highest ranking chemicals represent different classes of compounds (e.g., fatty acids, amino acids, quatinary amines). Conversely, single classes of compounds included strongly, weakly and moderately stimulatory members (e.g., taurine - 0.75; L-asparagine - 0.16). No general correlation existed between molecular type and stimulatory capacity. Hydrolysates of proteins or protein mixtures proved moderately stimulatory at the concentrations tested (Table 2),although they are difficult to relate to the activities of single chemicals on a molar basis. On a wt/vol basis, the complex compounds

Table 1 - Mean activity ratios of various pure chemicals to *P. argus* lateral chemoreceptors.

Chemical	$\bar{X}$ activity ratio	Chemical	$\bar{X}$ activity ratio
Taurine	.75	L-glutamic acid	.33
L-ascorbic acid	.68	DL-ornithine	.33
Proprionic acid	.68	L-arginine	.32
n-Valeric acid	.65	Maltose	.32
L-glutamine	.61	D-fructose	.31
n-Butyric acid	.60	Acetylcholine chloride	.31
β-alanine	.57	Inosine	.31
Homarine	.57	Sucrose	.31
Creatine H_2O	.56	Citric acid	.31
Nicotinic acid	.54	D-glucose	.30
A M P	.54	DL-carnitine HCl	.29
NH_4Cl	.54	L-serine	.27
Histamine	.52	U M P	.27
Trimethylamine HCl	.51	A T P	.26
Betaine	.51	L-alanine	.26
L-proline	.51	N-acetyl-D-glucosamine	.24
Glycine	.50	Urea	.24
L-histidine	.50	Hypoxanthine	.23
L-isoleucine methyl ester HCl	.50	r-amino-n-butyric acid	.23
Fumaric acid	.49	L-cysteine	.22
Hydroxy-L-proline	.47	2-aminoethanol	.22
D-Galactose	.46	Levulinic acid	.22
L-lysine	.44	Sodium saccharin	.21
α-picolinic acid	.44	Succinic acid	.20
L-methionine	.43	Sarcosine	.20
D ribose	.43	Pyruvic acid	.19
L-valine	.42	Phosphoethanolamine	.19
L-tyrosine	.41	D-mannitol	.18
L-carnosine	.41	Adenosine	.17
Putrescine	.40	L-asparagine	.16
O-phospho-L-serine	.39	Coumarin	.16
DL-lactic acid	.39	I M P	.13
α-lactose	.39	D-sorbitol	.13
L-citrulline	.39	Adenine	.13
Glutathione	.38	5-hydroxytryptamine	.11
L-aspartic acid	.38		
i-inositol	.38		
Quinine	.37		
L-tryptophan	.36		
Guanidine HCl	.35		
L-leucine	.34		
L-malic acid	.34		
β-lactose	.34		

Table 2 - Mean activity ratios of various complex compounds to *P. argus* lateral antennular chemoreceptors.

Compound	Concentrations (gm/liter) tested	$\bar{X}$ activity ratio
Lactalbumin hydrolysate	0.046, 0.46, 4.6	.67
Bovine albumine	0.046, 0.46, 4.6	.57
Casein hydrolysate	0.038, 0.38, 3.8	.45
Ficoll	0.040, 0.40, 4.0	.39
Trypticase soy casein hydrolysate	0.046, 0.46, 4.6	.39
Hemoglobin (bovine)	0.068, 0.68, 6.8	.19
Taurine (from Table I)	0.013, 0.13, 1.3	.75

are 3-5 times more concentrated than, say, taurine, but have lower mean activity ratios, even the most active of the group, lactalbumin hydrolysate (0.67 vs 0.75 for taurine). Rigorous comparison between the two groups, however, is restricted by the possibility of anomolous dose/response relationships.

Behavioral Assay - Nineteen single stimulatory substances and three mixtures were assayed for their ability to elicit oriented locomotion in intact organisms. Single compounds assayed behaviorally included the most stimulatory compounds overall as well as the most stimulatory compound of each molecular type, unless excessive cost or limited solubility necessitated using another representative of a molecular type. Of the 19 single substances tested, 17 (proprionic acid and β-alanine runs were incomplete) were analyzed statistically. Mixtures assayed behaviorally included (1) natural shrimp extract* prepared on the same wt:vol basis as the dry single compounds, (2) a mixture of amino acids at

*Not to be confused with the single concentration of shrimp extract used as a reference stimulant for each of the behavioral assays as described in Methods.

their component concentrations in the shrimp extract (Mixture A - see Table 3) and (3) an equal part mixture of the four most attractive single compounds (Mixture B). The response to standard shrimp extract, 67.9% of all animals tested (72.6% of those moving in at least 1 trial), verified its non-saturating concentration, as estimated from preliminary experiments. Table 4 summarizes the behavioral response data. Analysis of variance indicated overall significance among chemicals tested, an overall concentration effect, and only a weak interaction effect (Table 5).

Of the single compounds, citric acid attracted the greatest percentage of lobsters relative to standard shrimp extract at the high and medium concentrations. Overall significant differences occurred only at the highest concentration, however. It was selected to rank order at this concentration since the outcome would be little affected by addition of medium and low values in view of the significant interaction effect. Visual inspection of Table 4 verifies this. Other attractive single substances are L-ascorbic acid (112.5%), succinic acid (108.3%), glycine (98.5%), and trimethylamine HCl (90.3%). Limited solubility of three compounds (valeric acid, egg albumin, nicotinic acid) prevented testing them at the same concentrations as other substances, thus negating direct comparison of their attractiveness. Overall, however, they appear less attractive than the highest ranking compounds. No stimulatory single substance elicited repulsion in the behavioral assay, although since the apparatus was not designed to test the repulsion per se, a subtle repulsive effect could have gone unnoticed. It is concluded from these studies that citric acid is the most attractive single chemical of the group assayed, and therefore is the most likely substance for field testing.

Table 3 - Amino acids comprising abdominal tissue of *Penaeus duorarum*[a]

A.A.	µM/ml	A.A.	µM/ml
Glycine	15.62	Isoleucine	0.176
Taurine	5.5	Methionine	0.173
Alanine	3.54	Asparagine	0.167
Proline	1.904	Tyrosine	0.153
Arginine	1.473	Aspartic Acid	0.138
Glutamine	1.053	Threonine	0.106
Valine	0.384	Phenylalanine	0.090
Leucine	0.317	Lysine	0.081
Glutamic Acid	0.275	Histidine	0.054
Serine	0.334		

[a]Formulation supplied by Dr. Kenneth Blumenthal, University of Florida.

Table 4 - Response (oriented locomotion towards a source of ...) of intact *P. argus* to selected stimulatory substances at three concentrations, expressed as a percentage of response to standard shrimp extract.

Compound	% response at concentrations of:		
	10^{-2} gm/liter	10^{-3} gm/liter	10^{-4} gm/liter
Shrimp extract	200.0	100.0	66.8
Citric acid	161.0	108.3	44.5
Mixture B	115.2	86.2	84.7
L-ascorbic acid	112.5	54.2	27.8
Succinic acid	108.3	45.8	33.2
Glycine	98.5	52.8	32.0
Trimethylamine HCl	90.3	41.7	41.7
Betaine	87.5	70.8	16.7
Mixture A	87.5	62.5	112.5
Taurine	83.3	63.8	59.7
L-glutamic acid	77.8	63.8	30.5
2-Aminoethanol	65.0	31.6	10.0
L-lysine	54.2	20.8	33.3
L-aspartic acid	54.2	33.3	25.0
Casein hydrolysate	45.8	20.8	25.0
Sucrose	45.0	30.0	50.0
α-lactose	40.3	33.3	32.0
Valeric acid*	40.2	5.5	5.5
Egg albumin*	33.2	40.0	51.6
Nicotinic acid*	20.0	5.0	5.0

*See p. 17, top

*Solubility limited the concentrations tested of these chemicals to:

Nicotinic acid	-	3×10^{-3}	3×10^{-4}	3×10^{-5}
Egg albumin	-	6×10^{-4}	6×10^{-5}	6×10^{-6}
Valeric acid	-	6×10^{-3}	6×10^{-4}	6×10^{-5}

Table 5 - Analysis of variance of the results presented in Table 4*.

Source of variance....	Calculated F score	F score required for significance ($p \leq .01$)
1. among substances, overall	3.01	$2.35_{F_{.01}}$ (15,60)
2. among concentrations of single substances	33.23	$4.61_{F_{.01}}$ $(2,\infty)$
3. interaction of 1. & 2.	1.62	$1.46_{F_{.05}}$ $(30,\infty)$
4. among substances, high concentration	2.51	2.35
5. among substances, medium concentration	0.97	2.35 (N.S.)
6. among substances, low concentration	0.17	2.35 (N.S.)

*Excluding nicotinic acid, egg albumin, and valeric acid due to their limited solubility.

Of the mixtures assayed behaviorally, the complete shrimp extract attracted the greatest percentage of lobsters (at the high test concentrations) relative to the standard shrimp extract. It also attracted more lobsters than did the most attractive single compound, citric acid (200% vs 161%). The amino acid component of the shrimp extract (Mixture A) was less attractive than the complete extract (87.5% vs 200%). Combining the four most attractive single compounds - citric acid, ascorbic acid, succinic acid and glycine - into Mixture B attracted fewer lobsters than did the most attractive single substance by itself (115.2% vs 161% for citirc acid). Mixture B elicited a response (115.2%) about the same as the mean of its four component substances tested

individually (120%).

Releasant Assay - Gelatin redissolved in seawater within a few hours of solidifying, thereby proving it ineffective as a releasant. Polyacrylamide gels, prepared as described by Allen et al. (1975), would not set when combined with our attractant solutions. In light of the neurotoxic effects of the acrylamide monomer, this alternative was not pursued further in the present study. Agar gels (Difco bacteriological agar, technical grade) remained stable in seawater and, contained in polyethylene bait containers, resisted damage from handling. Dye concentration from 10 cm diam X 3 cm thick discs of 1.5% agar decremented 2 orders of magnitude over 3 days. Increasing agar concentration to 5%, increased dye retention slightly, with dye concentrations decrementing 1 1/2 orders of magnitude over 3 days. Containing 1.5% agar discs in plastic mesh bags instead of the polyethylene bait containers did not alter release rates, indicating the container itself was not rate limiting. Stronger acidic attractants (e.g., citric acid) prevented hardening of the agar discs when prepared by dissolving powdered agar in the attractant solution. This problem was overcome by buffering attractant solutions ($NaHCO_3$) to pH 7.5. Two formulations of the plastic laminate were tested. One (Type A), prepared with 18-20% citric acid by weight, lost 30% of its contained dry attractant over 5 days. The second (Type B), prepared with 17% citric acid by weight, lost 30% of its contained dry attractant over 5 days.

Field Assay - An initial series of 8 trawls placed varying distances offshore localized the greatest catches (1976) to the 18-21 meter depth zone subsequently used in the present study. Table 6 summarizes the results of the field trials. Experiments 1-3 test the effectiveness of two concentrations of citric acid as an attractant when released from agar discs. The low

catches with citric acid indicate this system is ineffective. Experiment 4, identical to experiments 2 and 3 except that betaine (as the hydrochloride) is substituted for citric acid as the potential attractant, tests the possibility that the agar discs may provide an effective release vehicle if used with another type of compound. The relatively higher catch (33% vs 2-8% of expts. 2,3) with betaine suggests that citrate may be limiting in the first experiments, but this difference must be considered re inter-trial variability. Experiments 5-7 show that within the test design, unbaited traps can fish as well (expts. 5,7) or much better (expt. 6) than the artificially baited traps, thereby invalidating the significance of the differences obtained in experiments 1-4.

Technology exists to produce more controlled release than that possible using agar blocks by dispersing the attractant in various types of polymeric matrices. Experiments 8-12 test the effectiveness of citric acid as an attractant in one form of polymeric release system, a laminated sheet. Two formulations of the polymer were tested (see Methods). While laminated dispensers of the first formulation (Type A) containing 1 gm (expt.8) and 10 gm (expts. 9, 10) citric acid caught more lobsters than did the citric acid/agar disc system, the number of lobsters caught was less than that caught with cowhide. Again, the percentages caught overall in experiments 8-10 did not exceed the percentages caught overall in unbaited traps (expts. 5-7), indicating that variations in the catch effect were likely due to parameters other than those controlled in the present experiments. This idea is supported by the lower catch with 10 gm of citric acid (expt. 9) than with 1 gm of the attractant (expt. 8).

Experiments 11 and 12 test the effectiveness of the second formulation (Type B) of the citric acid/polymeric sheet release system. These experiments (see Methods) fished about twice as many traps as previous experiments in an attempt to offset

the large variations in catch effort characteristic of experiments 1-10. In both experiments the number of losters caught was less than that caught with cowhide. In these trials, however, the total catch was too low for meaningful results. The limited supply of laminated dispensers available precluded further testing of the citric acid/polymer system.

Experiments 13 and 14 test the effectiveness of a natural source of citric acid available as a by-product of Florida's citrus industry, dried citrus pulp. While the number of lobsters caught with citrus pulp is not significantly lower than that caught with cowhide, the small size of the total catch again precludes drawing any meaningful conclusion from these results. Further testing of the dried citrus pulp was not attempted.

Table 6 - Results of field trials with selected chemical attractants for *P. argus*.

Expt. No.	Date	Attractant Tested	Attractant Concentration*	Releasant	No. Caught Test	No. Caught Control	Catch as % of Control
1	5/76	Citric acid	2 gm	Agar	9	77	11.7%
2	5/76	Citric acid	20 gm	"	1	60	1.7%
3	5/76	Citric acid	20 gm	"	3	38	7.9%
4	6/76	Betaine HCl	20 gm	"	14	42	33.3%
5	6/76	none(empty)	-	-	18	109	16.5%
6	6/76	none(empty)	-	-	36	41	87.8%
7	6/76	none(empty)	-	-	23	69	33.3%
8	6/76	Citric acid	1 gm	laminate A	25	63	39.7%
9	6/76	Citric acid	10 gm	"	10	36	27.8%
10	7/76	Citric acid	10 gm	" **	16	66	24.2%
11	5/78	Citric acid	7.5 gm	laminate B	1	7	14.3%
12	5/78	Citric acid	7.5 gm	"	3	26	11.5%
13	6/78	Dried citrus pulp	-	-	5	9	55.6%
14	6/78	Dried citrus pulp	-	-	4	7	57.1%

*per trap
**Laminate A also included a surfactant (Triton X-100) in this expt.

DISCUSSION

The discussion will interpret the results relative to the development of an artificial attractant for the spiny lobster fishery. Implications of the results towards understanding basic chemosensory organization in lobsters has appeared elsewhere (Johnson and Ache, 1978) and will be considered further in a forthcoming publication (Clark and Ache, In preparation).

The physiological assay, in retrospect, provided little additional definition of potentially attractive compounds than did the initial selection of compounds to be assayed physiologically from the published literature. The 10 most attractive compounds in the behavioral assay (Table 4) spanned 80% of the range of physiological activity (Table 1). More formally, calculation of the variance between the rank order of compounds based on attractiveness (Table 4) and the rank order of these same compounds based on stimulatory ability for antennular chemoreceptors (Table 1) shows no correlation in the paired rank orderings at the 0.05 confidence level. A likely reason for the apparent lack of power of the physiological assay is that any one substance was only tested on too small a percentage of the receptor population. It is known that *P. argus* lateral antennular filaments contain over 100,000 aesthetasc-type chemoreceptors per filament (Laverack and Ardill, 1965). It also now appears the receptor population is not homogeneous relative to the response spectra of individual receptors (Fuzessery et al., 1978). This problem would be minimized by using a whole-organ assay as the electroantennagram, but this technique has not been applied successfully to marine organisms in our laboratory to date. It is also possible that resolution among chemostimulants, particularly those of similar composition, is a higher-order function of lobsters requiring at least partial integration of the primary sensory information. If this is true, a higher-order

physiological assay, e.g. heart rate or some other parameter associated with arousal, would be more appropriate as an attractant screen than assaying primary receptor activity. Physiological assays have the potential to efficiently screen behaviorally-active chemical stimuli, but further work is in order to define a system with stronger behavioral correlation than the one used in the present study.

Some of the single compounds that were maximally attractive to the spiny lobster also ranked high in other behavioral studies rank ordering stimulatory compounds for decapod crustaceans and, as such, may be suitable attractants for decapods other than the spiny lobster. Table 7 summarizes the appropriate data. One other study ranked citric acid among the more stimulatory chemicals; glycine was ranked among the more stimulatory chemicals for four other decapod species. Caution is in order, however, for these studies, except Carr and Gurin (1975), initially select substances for testing based on the same sources, i.e., the published literature. They do not "work down" from a complete, complex food source to identify all the stimulatory compounds. For example, in *Palaemonetes pugio*, glycine, taurine and glutamic acid are the most stimulatory "off the shelf"-type components of human serum that elicit feeding behavior, but other compounds, higher molecular weight proteins, are more potent stimulants of feeding behavior (Carr and Gurin, 1975). It appears each species should be assayed independently to determine its maximum sensitivity until further knowledge of crustacean chemostimulants allows more rigorous generalization.

The above discussion leads to the question of mixtures. It may be limiting to focus on the "best" single compound if mixtures of substances are far more attractive than any single substance. The idea of mixtures being more potent stimulants than single compounds is supported by studies on *Carcinus maenas*

Table 7 - Comparison of single compounds found maximally stimulatory in eliciting food-finding (*) or feeding (**) behavior in decapod crustaceans. Each study tested a different number and array of compounds from which those listed were scored as most stimulatory.

	Panulirus[1]	*Palaemonetes*[2]	*Petrolisthes*[3]	*Penaeus*[4]	*Homarus*[5]	*Cancer*[6]
Citric acid	X					X
Ascorbic acid	X					
Succinic acid	X				X	
Glycine	X	X	X	X		X
TMA	X					
Betaine	X					X
Taurine	X	X				
Glutamic acid	X	X	X		X	

[1] Table 4
[2] Carr and Gurin, 1975**
[3] Hartman and Hartman, 1977**
[4] Hindley, 1975*
[5] Mcleese, 1970*
[6] Allen, et al., 1975*

(Shelton and Mackie, 1971) and *Homarus gammarus* (Mackie, 1973) that show none of the components of synthetic mixtures of chemicals based on the composition of natural foods are as attractive as the complete mixtures. Similarly, stimulation of feeding in *Palaemonetes pugio* by human serum can only be fully accounted for by the combined action of 6 types of serum proteins and 37 low molecular wieght constituents (Carr and Gurin, 1975). The results of the present study, however, show that selected single compounds, i.e., citric acid, approach the attractiveness of complex mixtures, i.e., shrimp extract, when compared on an equal weight per unit volume basis. They further show that a single compound, citric acid, can be more attractive than a mixture of four individually attractive compounds, Mixture B, again when compared on an equal weight per unit volume basis. One can conclude for the spiny lobster, at least, that single compounds can effectively substitute for mixtures as artificial attractants. Assuming a positive dose/response

curve characterizes citric acid over its field-effective concentration range as it does over the concentration range tested herein, rate-of-release might be a suitable variable with which to even increase the catch effect of a single substance as citric acid.

None of the substances tested was a "super-attractant", i.e., none greatly exceeded the potency of a natural stimulus as shrimp extract. It remains to be tested if highly stimulatory single compounds as citric acid could potentiate or enhance the potency of more complex natural stimulants as shrimp extract when combined with the latter and appropriately released.

The field trials were inconclusive, as noted in Results, which precludes a meaningful economic analysis of the use of artificial attractants and its potential impact on the fishery. The field trials do provide a useful base on which to design future field tests by indicating lower limits of the number of trap-efforts required to offset the large variability inherent in these trials. The use of natural sources of "artificial" attractants rather than commercially prepared controlled-release vehicles is worthy of immediate further evaluation for, if successful, it could be incorporated into the fishery directly without the relatively large (estimated $15,000) initial cost to develop and pilot manufacture a sufficient number of controlled-release devices for adequate field testing.

CONCLUSION

Overall, the results indicate that simple organic compounds are effective attractants for the spiny lobster. Of particular interest to the S. Florida lobster fishery is that the strongest attractant defined by this study, citric acid, is readily available in the form of dried citrus pulp as a byproduct of the Florida citrus industry and, as such, must be considered a potentially

useful source of trap bait for the fishery. Deployment of artificial attractants as citric acid using controlled-release methodology remains an interesting concept, but requires further testing to evaluate its potential application to the marine environment.

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