Effect of Prey Distribution and Density on the Searching and Feeding Behaviour of Larval Anchovy *Engraulis mordax* Girard

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INTRODUCTION

Laboratory estimates of the minimum concentration of food required for survival of marine fish larvae usually are much higher than the average concentrations of food in the sea (O'Connell and Raymond, 1970; Hunter, 1972). A common explanation for the fact that laboratory food requirements exceed natural food densities is that larvae are able to find and remain in patches of food in the sea which are considerably above the average food density estimated from plankton net catches. This explanation is supported in part by Ivlev (1961) who demonstrated with carp fry that an increase in the degree of aggregation of prey had the same effect on food consumed as an increase in the overall density of food material. A patchy distribution of larval food occurs under natural conditions. Thus, the effect of prey distribution on feeding behaviour of larval fish and the scale of "patchiness" of food items in the sea must be known to estimate the impact of food distribution on the feeding and searching behaviour of larval anchovy. This paper described some aspects of the effect of prey distribution and density on the feeding and searching behaviour of larval anchovy *Engraulis mordax* Girard.

The prey used in most of the experiments was the dinoflagellate, *Gymnodinium splendens*, which forms dense, stable, and easily-recognizable aggregations in rearing containers. *Gymnodinium* is readily cultured (Thomas et al., 1973), and promotes growth in larval anchovy equivalent to a wild plankton diet during the first week in the life of an anchovy larvae (Lasker et al., 1970). For comparative purposes we also used the rotifer, *Brachionus plicatilis*.

Larvae were reared from the egg in air conditioned rooms in static sea water which varied in temperature from 17° to 19°C. Anchovy eggs hatch in about 2 days when kept at these temperatures. At hatching the yolk-sac larvae have functional olfactory organs and naked neuromasts but they do not have a functional eye (O'Connell, pers. comm.). About 50 h after hatching the mouth is functional, the gut expanded, and occasionally food is present in the gut (D. Kramer, unpubl.), although we have never seen larvae actively feeding at this stage. About 72 h after hatching only a few granules of yolk remain (D. Kramer, unpubl.), the eye becomes functional and active feeding begins.

During the first few days after yolk absorption anchovy larvae have a higher food density requirement than at any other time in the larval stage (Hunter, 1972). The larvae have a low level of feeding success, capturing only 11% of the prey at which they strike, and the volume of water searched for prey is much less than that for older larvae. In addition, at this stage, larvae die of starvation if they do not find food within 1.5 days (Lasker et al., 1970). These laboratory findings suggested that effect of food distribution was most important during the first days of feeding. For this reason we restricted our observations to larvae of ages 1 to 8 days.

METHODS OF FORMING PATCHES OF *GYMNODINIUM*

We consider here our observations of the behaviour of *Gymnodinium* aggregations and our techniques for formation of the aggregations. These observations are important because the aggregations played a critical role in our experiments; if similar aggregations form under natural conditions, they could be an important factor in the survival of marine fish larvae. *Gymnodinium splendens* is an unarmored, marine, halophytic dinoflagellate about 53 μ in diameter. Reproduction appears to be sexual and occurs under natural conditions only at night (Sweeney, 1959).

In the laboratory distinct, visible aggregations or patches of *Gymnodinium* were produced under a variety of conditions. The patches were yellow-green, and occurred at the surface in daylight and in darkness. The aggregations had a dense central mass with striae of cells extending from it. Cells were most dense at the water surface; the average density for 8 aggregations measured at the water surface was 30,000 cells/ml, whereas 10 cm below the water surface the mean density was 250 cells/ml. The patches varied in diameter from a few mm to ones greater than 10 cm. Occasionally more than one patch formed in a larval-rearing container.

Our procedure for establishing an aggregation of *Gymnodinium* in a rearing container was simply to add an inoculum sufficient to bring the average density of the cells in the container to 100 to 150 cells/ml. The density of inoculum ranged from 1500 to 2000 cells/ml. On occasion less-dense cultures were used, but these caused greater variability in the frequency of occurrence of aggregations and in their longevity. The cells were cultured by William Thomas and staff (Univ. of Calif., San Diego); the technique is described by Thomas et al. (1973). After inoculating the container, we covered it with either a black opaque or a transparent top whereupon aggregations, 5 to 10 cm diameter, formed within 24 h. The container contained only filtered sea water, *Gymnodinium*, and the medium in which the cells were originally cultured. The number of cells in the initial inoculum had a direct effect on the time necessary for the formation of the aggregation and its size. Inoculations that brought the initial cell density in the container to 20 to 80 cells/ml required 24 to 72 h to form aggregations and the aggregations were small, 0.5 to 5.0 cm diameter. When the initial density in the container was only 10 cells/ml, no patches formed after 6 days.

At the initial density in the container of 100 to 150 cells/ml patches formed equally well in constant dark (illumination $\leq 1 \times 10^{-6}$ ft-c) as they did under the fluorescent lamps located above the rearing containers. Distinct aggregations also formed in containers placed outdoors and exposed to direct sunlight. On occasion we used light to establish an aggregation of *Gymnodinium* in a particular position in a container. Once an aggregation had formed, however, it could not be moved to a different position in the container by illuminating a different section of the container. This was the case regardless of whether or not light had been used to establish the patch initially. The only way an established patch could be relocated was by dispersing the patch by stirring and allowing it to re-form. It would re-form in the light or in the dark and usually in a new region of the container. It could also be re-established in a particular region by illuminating one section of the tank. We occasionally used light to relocate an aggregation because we preferred to have the aggregations located near the center of the container. Patches were relocated by stirring the container, and placing an opaque cover over the container with a hole

cut in it so that light entered in only one section of the tank. Aggregations, 5 to 10 cm diameter, re-formed after stirring within 3 to 4 h. Relocation of the aggregation was successful 87% of the time (N = 31).

The longevity of *Gymnodinium* aggregations was not measured because the ending of a larval fish experiment required the removal of the patch. During a period of low availability of anchovy eggs, however, of 18 containers inoculated with *Gymnodinium*, all retained aggregations for 10 days and 6 for 16 days.

In summary, the most important characteristics of *Gymnodinium* aggregations were that they were stable, easily detected visually, and formed spontaneously independent of light. The only difficulty encountered was that wind-driven currents sometimes caused the patch to disperse temporarily. This problem was avoided by keeping covers on the containers and turning the air conditioner off before uncovering them.

ATTRACTION OF LARVAE TO PATCHES OF *GYMNODINIUM*

We noticed at the beginning of this study that yolk-sac larvae appeared to be aggregated in patches of *Gymnodinium*. We performed a series of tests to confirm this observation and determine the role that size and density of the patch and the density of cells outside the patch played in the behaviour.

Patches of *Gymnodinium* of various sizes and densities were established in 10 l rearing containers using the methods described in the previous section. We then placed 100 anchovy eggs in each container. The containers in this and all other experiments described in this paper were kept in air conditioned rooms at 17° to 19°C and under light cycles of 14 h light, 10 h dark. On the day the larvae reached the age of 1 day we measured the maximum diameter of the patch and sampled it by selecting an acrylic cylinder similar in size, lowering it over the patch and sealing it with an acrylic plate that had a circular groove cut to fit the cylinder. In such a sample and in control samples we counted the larvae, measured the volume of the water, and measured the density of *Gymnodinium* by counting the cells in ten 0.03, or 0.01 ml samples; the number of larvae remaining in the container were also counted.

Our estimate of the density of the patch was the mean number of cells/ml in the cylinder containing the patch. These estimates were less than the actual density because the patch was most concentrated at the surface and usually did not extend to the bottom of the container. The estimate of the background concentration of *Gymnodinium* was the average of the 4 mean densities taken from the 4 control samples.

Effect of the Volume and Density. We took 27 sets of samples of 1-day-old yolk-sac larvae in containers having patches of different density and volume. A comparison of the percent of larvae captured per unit volume of the sample of the patch to that of samples taken outside the patch showed a difference at $P < 0.00006$ Mann-Whitney U Test (Siegel, 1956). To evaluate the importance of the various characteristics of the patch we plotted the log of the number of larvae captured in the patch against the log of patch density (cells/ml), density outside the patch (cells/ml), patch volume (ml), and number of larvae in the

container. The number of 1-day-old larvae in the container varied among tests because of larval and egg mortality and infertile eggs. Consequently, N (the number of larvae present) had to be considered as a variable. Log transformations were used because a better fit was obtained with a multiplicative model than with an additive model.

Partial regression coefficients for number of larvae, patch density, and patch volume were significant ($P < 0.01$), whereas that for the cell density outside the patch was not. That the number of larvae present and patch volume are related to catch is obvious. On the other hand, these variables had to be considered in order to evaluate the effect of patch density. The final multiple regression of larvae caught (C) on the three independent variables - number of larvae present (N), volume of patch (V), and density of patch (D) - yielded the equation

$$C = N^{0.97} V^{0.51} D^{0.58} - 3.969$$

where coefficient of multiple correlation = 0.8493 and the standard error of the estimate = 0.1780. Since the coefficient for N was nearly unity and those for V and D were close to 0.5, the data were fitted to the general equation $C/N = K\sqrt{VD}$, using Marquardt's Algorithm for fitting nonlinear models (Conway, Glass and Wilcox, 1970) giving K = 0.000208 with 95% support plane confidence intervals for K of $0.000181 < K < 0.000235$ (Fig. 1). Thus, 1-day-old yolk-sac larvae aggregate in dense patches of *Gymnodinium* and the proportion of larvae attracted is correlated with the density and volume of the patch.

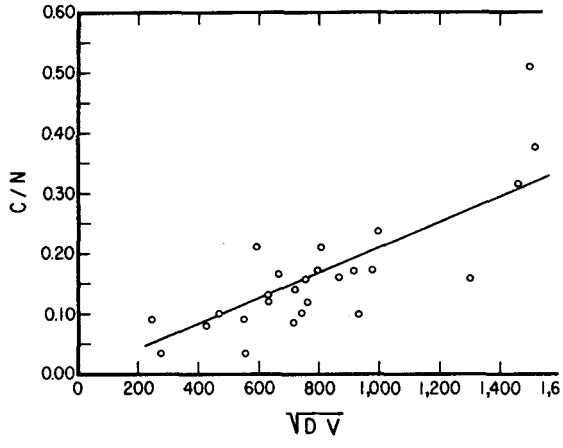

Fig. 1. Relationship between the proportion of 1-day-old anchovy larvae captured in a patch of *Gymnodinium spendens* and the square root of the volume and density of the patch; C/N is the number of larvae in the patch over the total larvae present in the tank; V is the volume of the patch in ml (range, 60 to 850 ml); and D is the density of the patch in cells/ml (range, 540 to 3240 cells/ml). Equation for line is, $C/N = 0.0002\sqrt{DV}$.

We also wished to establish that post-yolk-sac larvae that were actively feeding were also attracted to patches of *Gymnodinium*. For this purpose we made a short series of 11 tests of the same design on 4-day-old larvae. The results, summarized in Table 1, indicate that more 4-day-old larvae were present in the patch than in the first control sample ($P < 0.01$, Wilcoxon matched-pairs, signed-ranks test; Siebel, 1956).

Table 1. Percent of 4-day-old anchovy larvae captured in patch of *Gymnodinium splendens* and in the first of 4 control samples; samples taken during day

Percent of larvae in				
Patch of *Gymnodinium*	First control sample	Total larvae	Patch density cells/ml	Patch volume ml
6	8	47	2,085	630
10	6	68	375	580
12	8	72	1,314	710
15	6	52	1,620	680
15	8	52	1,600	610
21	8	77	2,000	600
23	7	87	1,200	750
26	3	92	1,950	600
27	6	67	1,500	600
40	1	183	3,300	800
43	7	56	900	1,000

Effect of Light. The object of this series of experiments was to determine if yolk-sac anchovy larvae continue to aggregate in patches of *Gymnodinium* in the dark and if they could find patches in the dark. In the first of three experiments, we reared larvae from eggs in 10 l containers in which a patch of *Gymnodinium* had been established and sampled the larvae in and out of the patch 6 h after the onset of darkness on the day they reached 2 days of age. In the second experiment, we reared larvae in 1000 ml beakers, added them to 10 l containers in which a patch of *Gymnodinium* was established at the onset of darkness on the day they reached 2 days of age, and sampled the container in the dark 6 h later. In the third experiment we used the same procedure as in the second, except the larvae were placed in the dark for 24 h before taking the samples. In the second and third experiments we added the larvae by submerging the beaker in the 10 l container, a procedure that always resulted in the dispersion of the patch of *Gymnodinium*. Previous tests had shown, however, that the patch would re-form within 3 to 4 h.

All containers were covered with black plastic lids and were kept in a darkened room. At the end of an experiment the lids were removed and samples in and outside the patch taken simultaneously with 9.5 cm diameter cylinders. All containers were stocked with 100 eggs; because hatching rates varied, the number of larvae sampled was expressed as a percentage.

When larvae were associated with the patch of *Gymnodinium* up to the time of capture on the night of age 2 days (experiment 1) more larvae were present in the patch in the dark than in the control sample taken in the dark ($P < 0.01$, Wilcoxon matched-pairs, signed-ranks test). On the average, 24% of the larvae in the container were captured in the patch sample whereas only 4% were taken in the control sample (Table 2). Thus, larvae remained aggregated in the patches of *Gymnodinium* in the dark.

Table 2. Percent of yolk-sac larvae (age 2 days) captured in dark in a sample of a patch of *Gymnodinium splendens* and in a control sample taken simultaneously outside the patch; when the patch was not dispersed, 6 h after dispersion of the patch; and 24 h after dispersion

Percent of larvae in			
Patch of *Gymnodinium*	Control sample	Total larvae	Patch density cells/ml
Patch not dispersed			
8	3	58	550
9	0	72	1,830
15	2	57	780
21	1	70	2,380
22	5	43	600
27	0	41	2,560
30	1	89	3,480
31	12	68	2,020
37	10	93	1,630
42	2	103	910
52	8	63	740
6 h after dispersion			
3	7	59	900
3	6	67	450
4	1	103	1,000
5	5	125	1,240
6	1	151	1,000
10	4	67	1,380
16	12	69	1,200
17	3	66	450
19	2	63	1,820
21	12	65	2,280
24 h after dispersion			
13	1	78	1,350
23	8	78	1,110
24	1	95	1,500
26	0	92	1,170
27	3	89	1,410
27	1	85	600
27	0	93	1,770
28	2	82	1,440
28	3	79	1,350
29	4	78	1,620
29	1	106	2,050
30	2	91	1,830
37	2	86	2,220

In the two experiments in which larvae were added at the onset of dark, more larvae were taken in the sample from the newly re-formed patch of *Gymnodinium* than were taken in the control samples ($P < 0.05$ for experiment 2 and $P < 0.01$ for experiment 3 (Wilcoxon matched-pairs, signed-rank test; Siegel, 1956). After 24 h in the dark the percent of larvae captured in the patch was greater than after 6 h in the dark and about the same as in the first experiment. Presence of fewer larvae in the patch after 6 h might be caused by the shorter recruitment period, or because the patches were less dense on the average after 6 h than after 24 h. On the other hand, a long period may be required for the larvae to recover from the disturbance caused by being introduced

into the container. It appears that search for concentrations of *Gymnodinium* can proceed on a 24-h basis regardless of the daily changes in light level.

These experiments also show that visual stimuli are not required for finding patches of food. One-day-old yolk-sac larvae have well-developed olfactory organs, and naked neuromasts; but the eyes are not developed. Thus, chemical, acoustic or tactile stimuli could be used by yolk-sac larvae to find concentrations of *Gymnodinium*. A chemical stimulus seems the most likely because algae can produce considerable quantities of extracellular substances (Fogg, 1962; Hellbust, 1965). On the other hand, in post-yolk-sac larvae the search pattern for food could also bring about an aggregation of larvae in areas of high food concentration.

STRUCTURE OF SEARCH PATTERNS

The object of these experiments was to determine how food type and distribution affected the structure of the search pattern of larval anchovy. For this study a large, circular, black fiberglass tank of 122 cm diameter with a 1 cm grid inscribed on the entire bottom was used. We filled the tank to a depth of 18 cm and inoculated it with a prey organism. When the desired food density and distribution was obtained we added eggs or larvae.

We recorded on a key board each time a larva completed a feeding act, when it swam in and out of a patch of *Gymnodinium*, and when it crossed a grid line. A different key was used for each of the four grid directions. If a larva did not move, no event was recorded. Each time a key was depressed the data and the elapsed time to the nearest 0.1 sec were entered on a 8 channel paper tape. The computer output included a plot of movements, swimming speed, frequency of feeding and directional probabilities. Directional probabilities were the proportion of grid intercepts made by a larva that were ahead, to the right, to the left, and backward (a 180^{o} change in direction). Swimming speed was the sum of grid intercepts made by a larva divided by the observation period which was 5 min. It was expressed in cm/sec because the distance between grid lines was 1 cm.

To evaluate how differences in directional probabilities (the proportion of movements made in each grid direction) could affect the area covered by a larva during search we used a computer model of bounded random walks used by Cody (1971) to analyze the movements of bird flocks. The bounds of the walk are boundaries of an 11 X 11 unit grid. The larva is started in the centre and moves only along the sides of 1 X 1 unit squares, one side per step. Directional probabilities were fixed relative to the axis of motion of the larva. For each set of directional probabilities (data from one larva), 100 walks of 200 steps each were made. The results of the simulation included the percentage of possible points which had been visited, the proportion that were visited zero, once, twice, up to 10-plus visits.

Of the statistics generated by the random walk program we used only the percent area covered, the percentage of possible points visited one or more times during the walk for each larva. The percent area covered was considered to be a relative measure of the effect of the directional probabilities on the area searched by a larva. Some may consider its procedure too artificial; thus, in some cases we also

compared the directional probabilities of groups directly. This was done by summing the frequencies each larva moved, ahead, right, left, and behind; the sum of the frequencies for each direction for each group of larvae were entered in a contingency table and the Chi-square test was used to determine differences between groups. This procedure inflated the number of observations because the total for each group was the total number of directional movements for the group rather than the total number of larvae observed. We preferred the values from the random walk program because the observations were independent and it indicated the possible effect of directional probabilities on the area searched.

We recorded search patterns and feeding rates of anchovy larvae in patches of *Gymnodinium*, out of patches, and at low densities of *Gymnodinium* where no patches were present. For comparative purposes, search patterns for larvae feeding on the rotifer *Brachionus plicatilis* over more limited density ranges were also studied. In all experiments larvae ranged in age from 4 to 8 days. No difference in behaviour associated with age was detected, so we combined the data for all ages.

Searching Behaviour of Larvae Fed on *Gymnodinium*. Anchovy larvae swam more slowly in patches of *Gymnodinium* ($P < 0.0006$) and fed more frequently than when not in such a patch ($P < 0.0006$ Mann-Whitney U test, Siegel, 1956). Larvae inside a patch swam less frequently directly ahead and more frequently reversed their direction than did ones outside a patch ($P < 0.001$ Chi-square test for two independent samples). The percent area covered, computed by the random walk program from directional probabilities was less for larvae inside a patch than for ones outside ($P < 0.00006$ Mann-Whitney U test). Thus, on the basis of the directional characteristics of the search pattern alone, larvae inside patches of *Gymnodinium* would be expected to cover less area per unit time than ones outside of a patch. The combined effect of the reduction in speed and change in directional probability for larvae in a patch was that ones inside remained within a small area whereas those outside swam over a much greater area.

The change in speed of larvae when they entered or left patches of *Gymnodinium* was abrupt. In 7 observations, the larvae swam in and out of a patch during the 5-min observation period. In all 7, the speed of the larva was higher (median 0.30 cm/sec) when it was outside the patch than when it was inside (median 0.15 cm/sec). Directional probabilities and feeding rates followed the trends described above, but no statistical differences existed - probably because of the small sample size.

The behaviour of larvae in patches of *Gymnodinium* could be a discrete pattern that occurs only when food is at a very high density and sharp boundaries exist; on the other hand, larval searching behaviour may be modified continuously with changes in density and food distribution.

To determine which alternative was the more likely, we divided the data into 3 density classes: 1 to 21 cells/ml; 24 to 260 cells/ml and patch, $\geq$ 1,000 cells/ml. A plot of the data segregated into the three classes indicated that the speeds and percent area covered for larvae in the 24 to 260 cells/ml class fell between those for larvae in the other two density classes (Fig. 2). This trend is also apparent in the medians (Table 3). The 95% confidence ellipse for these distributions plotted on a log scale also suggest a continuous change in characteristics of the search pattern (Fig. 3). A log transformation was required to normalize the distributions because speed distribution

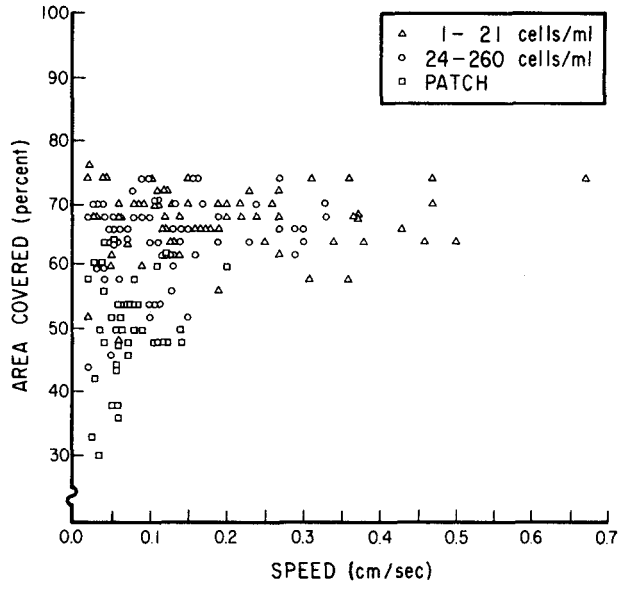

Fig. 2. Percent area covered from random walk program and speed for anchovy larvae 4 to 8 days old fed on *Gymnodinium splendens* at different densities

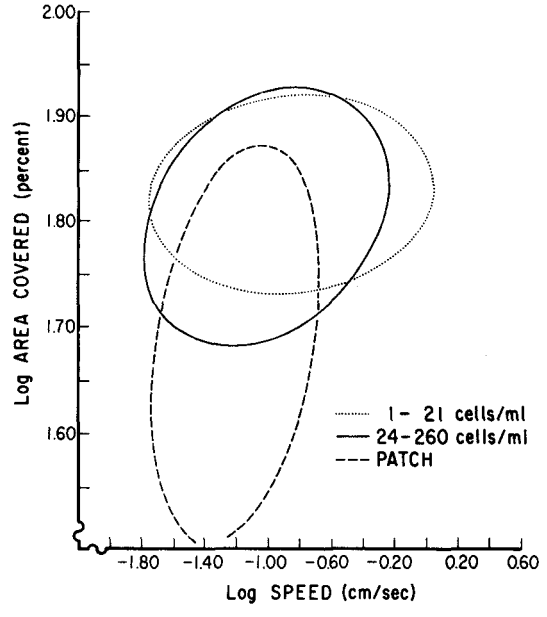

Fig. 3. 95% confidence ellipses for distribution of log of percent area visited on log of speed for larvae fed on *Gymnodinium splendens* at different densities

was strongly skewed. Comparisons of the speed, percent area covered, and feeding rates among the three classes of food density indicated that the distribution of each of these variables in each class was different from that in every other ($P < 0.05$ Mann-Whitney U test). We also compared the directional frequencies directly using the Chi-square test. These tests indicated that the frequency larvae moved in each grid direction differed among the three density classes ($P < 0.001$). From the above evidence we conclude that anchovy larvae responded to the density and distribution of *Gymnodinium* by continual modification

Table 3. Characteristics of search pattern of larval anchovy 4 to 8 days old in different concentrations of *Gymnodinium splendens*, and *Brachionus plicatilis*

Food type	Density range no/ml	N	Median cm/sec	Median feeding strikes/min	Median percent area covered	Mean directional probabilities				Total moves
						Ahead	Right	Left	Behind	
Gymnodinium splendens	1-21	72	0.151	0.00	68.5	0.355	0.311	0.280	0.054	3,821
"	24-260	65	0.105	0.21	65.0	0.319	0.304	0.306	0.071	2,416
"	patch	41	0.058	0.79	49.7	0.161	0.282	0.323	0.234	888
Brachionus plicatilis	10-15	38	0.212	0.86	68.3	0.341	0.305	0.315	0.039	2,465
"	42-72	23	0.162	0.79	65.3	0.324	0.305	0.302	0.068	1,247

of the speed and directional components of their searching behaviour. The result of these modifications was an expansion of searching area at low density and a reduction of the area searched at higher food densities.

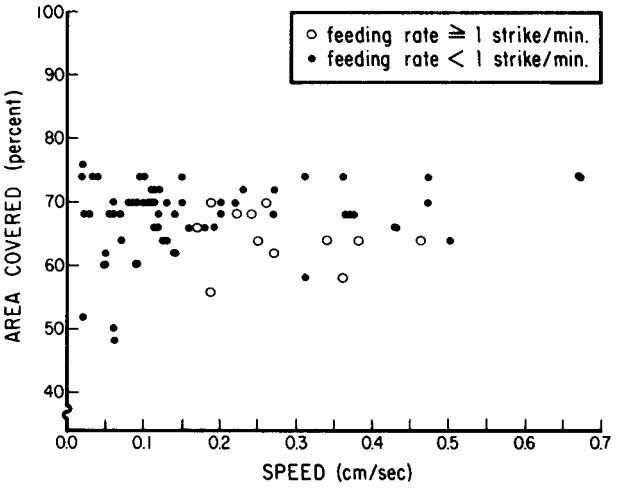

Fig. 4. Feeding rate, speed, and percent area covered for anchovy larvae 4- to 8-days-old fed on 1 to 21 *Gymnodinium splendens*/ml

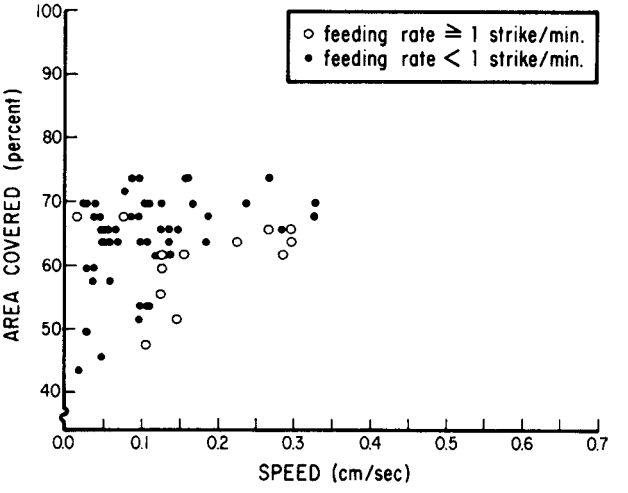

Fig. 5. Feeding rate, speed, and percent area covered for anchovy larvae 4- to 8-days-old, fed on 24 to 260 *Gymnodinium splendens*/ml

Within these classes a relationship existed between speed and feeding rate. Larvae that fed at higher rates tended to swim faster than those that fed less often or did not feed (Fig. 4 and 5). We measured the degree of correlation between feeding rate (completed feeding acts/min) and swimming speed by calculation of Spearman r_s and Students t associated with that value (Siegel, 1956). In each of the groups a strong positive correlation existed between feeding rate and swimming speed ($P < 0.005$). Thus, within each level the larvae that swam the fastest tended to feed more often, but larvae at lower densities swam faster and fed less often than ones at higher densities.

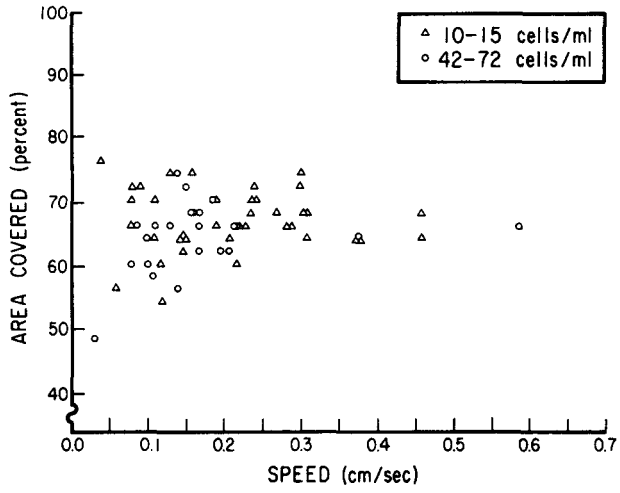

Fig. 6. Percent area covered calculated from random walk program and speed, cm/sec for anchovy larvae 4- to 8-days-old, fed on *Brachionus plicatilis* at different densities

Searching Behaviour of Larvae Fed on *Brachionus*. We used the same techniques and procedures to study the searching behaviour of larvae fed on the rotifer, *Brachionus plicatilis*, as we did for those fed on *Gymnodinium*; however, *Brachionus* was studied over a more limited density range. The data were divided into two density classes, 10 to 15 *Brachionus*/ml and 42 to 72/ml, to determine if density affected any characteristics of the search pattern. The distribution of speeds and percent area covered resembled those for *Gymnodinium* at comparable density levels; the medians were also similar (Fig. 6, Table 3). Comparisons of speed, percent area covered, and feeding rate between the

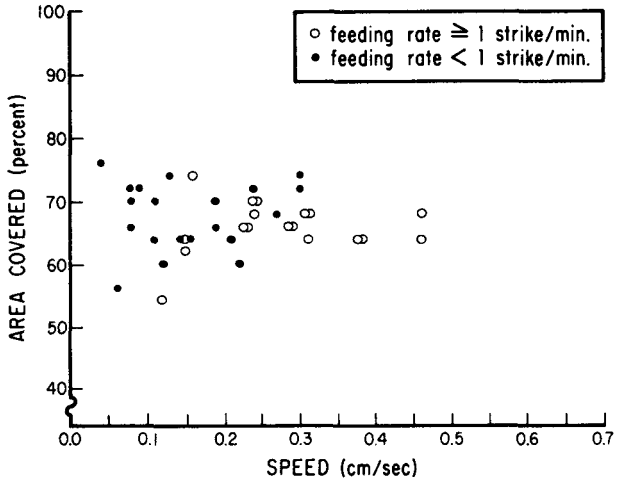

Fig. 7. Feeding rate, percent area covered and speed for anchovy larvae 4- to 8-days-old, fed on 10 to 15 *Brachionus plicatilis*/ml

two density classes of *Brachionus* showed a significant difference only in the case of percent area covered ($P < 0.05$ Mann-Whitney U test). The directional frequencies also differed between the two density classes ($P < 0.01$, Chi-square test).

A tendency existed in both density classes for the larvae that fed at higher rates to swim faster than those that fed at lower rates as was the case for *Gymnodinium* (Fig. 7 and 8). In both density classes of *Brachionus* the association was significant at $P < 0.005$ (Spearman rank correlation, Siegel, 1956).

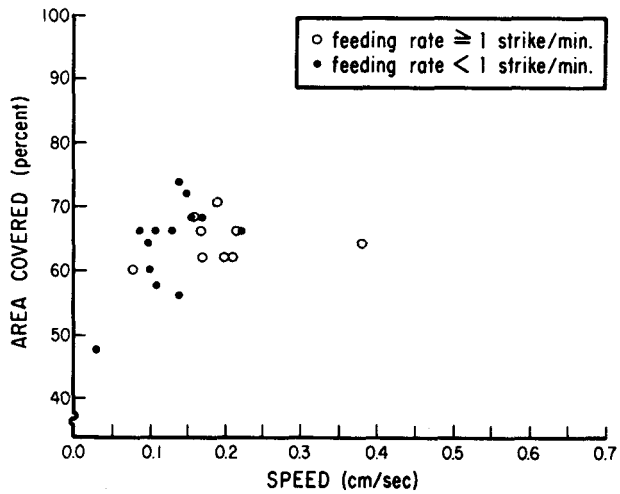

Fig. 8. Feeding rate, percent area covered and speed for anchovy larvae 4- to 8-days-old, fed on 42 to 72 *Brachionus plicatilis*/ml

DISCUSSION

The "non-randomness" of a search pattern of anchovy larvae is in agreement with other findings. Kleerekoper has shown that fish do not move at random in experimental environments that are void of directional cues (Kleerekoper, 1967; Kleerekoper et al., 1970). He also found that the presence of an odor without directional cues caused drastic changes in directional parameters (Kleerekoper, 1967). Beukema (1968) showed that sticklebacks found food in a maze about two times more efficiently than predicted from a random model. The food in the maze was at a lower density than would be expected under natural conditions and the fish improved their efficiency by making fewer reversals of direction than would be expected from the random model. The chance of an immediate occurrence of a reversal in direction was more than doubled when a prey was found. He also pointed out the adaptive advantage of this behaviour for a highly aggregated prey. Wyatt (1972) showed that the time plaice larvae spent swimming increased with a decrease in food density. In our experiments with *Gymnodinium* speed also increased with a decrease in food density and the probability of reversals in direction increased with density when either *Gymnodinium* or *Brachionus* was the prey. The increase in probability of direction reversals, 180° turns, was the principle reason for the decrease in area covered in

the random walk at high food densities. We were unable to establish a direct link between reversals of direction and frequency of feeding; but since feeding and reversals of direction both increased with an increase in density they may be related.

The non-randomness of larval anchovy search patterns and the ability of yolk-sac and older larvae to find and remain in concentrations of the dinoflagellate, *Gymnodinium*, are adaptations that should equip them to take advantage of the contagiousness of the distribution of food in the sea. This could be of considerable adaptive significance, depending upon the extent of scale of patchiness of food in the sea. It could explain, for example, why laboratory estimates of food-density requirements of larval anchovy appear to be consistently higher than the average concentrations of food in the sea (O'Connell and Raymond, 1970; Hunter, 1972); and why no difference in mortality of anchovy larvae exists at the onset of feeding in the sea, although their food-density requirement determined in the laboratory is much higher at this time (Lenarz, 1972; Hunter, 1972). Laboratory estimates of food requirements for first-feeding larvae are higher because of their low level of feeding success, and they search a much smaller volume per unit time owing to their small size. If larvae can search for food and remain in concentrations while in the yolk-sac stage, the chance of finding a significant concentration of food before they starve is increased. Anchovy larvae can survive without food for 1.5 days after yolk absorption at temperatures of 22° to 15°C; but if the time to starvation is calculated from hatching, larvae can survive from 3 days at 22°C to 5.5 days at 15°C (Lasker et al., 1970). The variation in times is caused by temperature-dependent differences in the rate of yolk absorption. Since most anchovy eggs are spawned at 13° to 14°C, the time available to find food before starvation is increased 3 to 4 times and this conceivably could counteract the effect of a higher food density requirement.

Also pertinent is the extent that phytoplankton is eaten by larval anchovy under natural conditions. The importance of phytoplankton in the diet of anchovy larvae depends on the availability of patches and on whether or not larvae feed on these organisms in the sea. Phytoplankton form dense patches in the sea. A remarkable example of this is a very extensive bloom observed in the Weddell Sea by El-Sayed (1971).

He recorded densities of phytoplankton of over 2000 organisms/ml for combined species and densities as high as 1700/ml for individual species. These densities are somewhat higher than the ones we typically measured in patches in our containers. Not all species of phytoplankton will sustain life in anchovy larvae, however. To survive, anchovy larvae appear to require that the cells be unarmoured and larger than 30 μ (Lasker et al., 1970). The abundance of phytoplanktonic organisms in the California Current meeting these requirements at the time of spawning of the anchovy is not known. On the other hand, stomach contents of anchovy larvae analyzed by Arthur (1956) indicate that phytoplankton may be of considerable importance in the diet of first-feeding anchovy. 11% of the items he identified in the guts of anchovy larvae 4.5 mm and smaller were phytoplanktonic organisms. If a group Arthur classed as unidentified spheres, presumably unicellular algae 20 μ in diameter is included, then phytoplankton comprised 32% of the diet. We consider 32% a conservative estimate because all organisms Arthur identified had hard parts that could withstand digestion; whereas the cells of a preferred phytoplankter, such as *Gymnodinium*, are broken down very rapidly in the gut and leave no identifiable elements. It should be noted, however, that phytoplankton is probably important for only about the first week of feeding. Lasker

et al. (1970) showed that growth of larvae fed on *Gymnodinium* became retarded after about 1 week; Arthur (1956) showed a decline in the percent of phytoplankton in the guts of anchovy larvae with an increase in size. Only 10% of the items in guts of larvae 5.0 to 6.5 mm were phytoplankton and only copepods were present in the guts of larvae larger than 6.5 mm (Arthur, 1956).

SUMMARY

Searching and feeding behaviour of larval anchovy was studied when prey were highly aggregated and at various densities when prey were not highly aggregated. The prey used in most experiments was the dinoflagellate, *Gymnodinium splendens*; the rotifer, *Brachionus plicatilis*, was used in comparative studies.

Yolk-sac and post-yolk-sac larvae were found and remained in patches of *Gymnodinium* in light and in darkness. The number of larvae attracted to a patch depended upon the density and volume of the patch. The structure of searching behaviour at various prey densities was analyzed by recording movements of larvae as they swam over a grid. Speed and direction of search patterns of larval anchovy were density-dependent: larvae swam faster at low prey densities than they did at higher ones; and the expected area covered by a larva on the basis of directional components alone was greater at low prey densities than at higher ones.

ACKNOWLEDGEMENTS

We thank William Rand, Massachusetts Institute of Technology, for permission to use his computer model of bounded random walks, and Martin Cody, University of California at Los Angeles, for loaning a copy of the program deck. Alan Good wrote the program for processing the paper-tape data and James Zweifel fit various models to the data. Ms. Gail Theilacker developed the cylindrical sampler.

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