12-1992

Early Behavior Formation in Larval Fathead Minnows, Pimephales promelas

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1992

EARLY BEHAVIOR FORMATION IN LARVAL FATHEAD MINNOWS, *Pimephales promelas.*

A Thesis

Presented to

the Faculty of the Department of Biology

Western Kentucky University

Bowling Green, Kentucky

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Shamali D. Salgado

December, 1992

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EARLY BEHAVIOR FORMATION IN LARVAL

FATHEAD MINNOWS, *Pimephales promelas*

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ACKNOWLEDGEMENTS

I would like to thank Dr. Robert D. Hoyt, my major professor, for the many hours he has spent on my behalf. Without his guidance, cooperation and patience this study would not have been possible.

I am grateful to my graduate committee members, Drs. Blaine R. Ferrell and Gary E. Dillard, for their continuous support and help throughout my graduate program. I would also like to thank Mr. Robert Cobb for his assistance with the statistical analysis of the data.

Appreciation is expressed to Western Kentucky University for the use of equipment and laboratory facilities.

This thesis is dedicated to my parents, Lakshman and Chintamani Salgado and to my "mom" Carol Barnes, whose love, encouragement and support made this work possible.

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Shamali D. Salgado December, 1992 39 pages
Directed by: R. Hoyt, B. Ferrell and G. Dillard
Department of Biology Western Kentucky University

This study was undertaken in order to identify the approximate times of recruitment of the senses of vision, mechanoreception and chemoreception into the feeding behavior of the fathead minnow, *Pimephales promelas*, during the first 15 days of life. Larvae were reared on diets of live or freshly killed brine shrimp nauplii and their feeding activity observed daily under light and dark conditions. On every third day during the feeding trials, the larvae were reverse-fed the type of food upon which they were reared. The first evidence of feeding occurred on Day 3, when larvae conditioned on dead food ingested live food in the dark. Larvae in all groups tested exhibited some degree of feeding on Day 4. Larvae tested under dark conditions exhibited maximum feeding capabilities earlier than their counterparts tested in the light. Larvae showed an earlier ability to locate and ingest live prey over dead prey in both light and dark conditions suggesting that the sense of mechanoreception was functional in the larvae at the time of commencement of feeding. Although the sense of vision is functional at or soon after hatching, there appeared to be a lack of coordination or integration between the eyes and the lateral line system. The sense of chemoreception appeared to be the last major sense to become fully functional. Locomotor activity increased with age under all test conditions in conjunction with the developing sense organs.

INTRODUCTION

The fathead minnow, *Pimephales promelas*, inhabits a wide range of habitats such as ponds, creeks and small lakes throughout most of the U.S.A., the Great Slave Lake region in Canada and Chihuahua, Mexico (Vandermeer, 1966; Klemm, 1985). Because of its widespread distribution, biological hardiness and ease of culture, it is an important species in the baitfish and aquaculture industry (Becker, 1983; Klemm, 1985). The fathead minnow also serves as the comprehensive vertebrate test organism used by the United States Environmental Protection Agency (Adelman and Smith, 1976).

The fathead minnow is behaviorally an egg guarder. The male fathead minnow guards and takes care of the fertilized eggs until they hatch (Klemm, 1985). However, upon hatching the young do not receive any type of parental care. They remain near the nest until the nutrients contained in the yolk sac are depleted, after which they commence exogenous feeding (Becker, 1983). Since the larvae are not cared for by the parents upon hatching, they are considered to be altricial. Altricial teleosts usually possess simple alimentary canals and begin feeding before their jaws and sensory systems are fully developed (Noakes and Godin, 1988).

The beginning of the larval stage during which exogenous feeding commences is a critical period in the life cycle of this species. Inability to locate and ingest sufficient food at this stage results in mortality (Noakes and Godin, 1988).

Upon depletion of the yolk sac reserves, teleost larvae use their eyes and

lateral line system to locate and ingest food (Jones and Jannsen, 1992). Larvae of most teleost species can detect motion soon after they hatch. This ability improves with age, as evidenced by several behavioral studies conducted on teleost fishes (Breck and Gitter, 1983; Li *et al.*, 1985). At the time of commencement of exogenous feeding, an increase in visual acuity would enable the larvae to detect both prey and predators from a greater distance, and thus enabling it to either capture food or escape from predators more successfully (Breck and Gitter, 1983). This would lead to an increase in the net energy gain of the larva.

The fathead minnow is an ostariophysian fish, meaning that the anterior end of the swimbladder is connected to the inner ear by a chain of small bones called Weberian ossicles (for review, see: Tavolga, 1971). Sound is conducted from the swimbladder to the ear via the Weberian ossicles thereby serving as an accessory mechanoreceptive organ along with the lateral line system. Most teleosts possess a lateral line system which is usually functional shortly after hatching (Blaxter, 1986). Therefore, post-embryonic fathead minnow larvae can utilize their eyes, the Weberian ossicles and the lateral line system to detect prey and avoid predators.

Most fishes are first aroused to the presence of food via their sense of chemoreception (Hara, 1986). The senses of mechanoreception and vision become involved in the procurement of food after the initial detection of the presence of food via olfaction. Under light conditions, the sense of chemoreception is also used to gather information about the environment. Natural chemical stimuli are identified and used by fish to avoid predators and pollutants as well as to locate food. Chemical stimuli also play a major role in homing and migration, reproduction and intraspecific communication (Kleerekoper, 1969). Therefore, the sense of chemo-

reception is very important to a fish's survival in nature.

The fathead minnow has been used for acute and chronic toxicity testing by the United States Environmental Protection Agency (Adelman and Smith, 1976). The majority of behavioral toxicology tests have been performed on adult *Pimephales promelas*. However, McKim (1977) reported that the embryo-larval and early juvenile life stages appeared to be the most sensitive phases of fishes subjected to chronic life-cycle toxicity tests. Leino *et al.* (1990) observed that larval fathead minnows reared in water at pH 6.0 exhibited abnormal swimbladder development, scanty glycogen reserves and delayed feeding. Hoyt and Abdul-Rahim (1991) reported that 18 to 24 day old juvenile fathead minnows exhibited decreased chemoreceptive abilities when subjected to low pH values. The results obtained from the above studies suggested that fathead minnow larvae inhabiting contaminated waters may be subjected to a high mortality rate. The impairment of the sense of chemoreception would result in a decreased ability to locate and ingest food. This in turn would lead to increased mortality of larvae, decreased growth of juveniles and reduced fecundity in adults. The final result would be the loss of fathead minnow populations from contaminated waters (Lemly and Smith, 1987).

Although there are several reports on the embryonic - developmental biology of the fathead minnow (Manner and Dewese, 1974; Buynak and Mohr, 1979), its early sense organ development has not been characterized. The time of recruitment of each sense organ during the normal growth and maturation period of fathead minnow larvae has to be identified before any type of behavioral toxicity studies can be performed on this species. Once the developmental patterns of larvae reared under normal environmental conditions are determined, these data can be used as a standard against

which the behavior of larvae exposed to toxicants can be compared.

The objectives of this study were to attempt to identify the role and time of recruitment of the major senses in feeding behavior during the first 15 days of life and to show how development and integration of the major senses altered locomotor activity, in response to feeding.

MATERIALS AND METHODS

Test Fish

Fathead minnows used in this study were hatched from brood stock obtained from the U.S. Environmental Protection Agency, Newtown, Ohio and maintained in the fish rearing facility in the Biology Department of Western Kentucky University. Three batches of fathead minnow eggs, each consisting of approximately 180-225 eggs, were hatched and reared during the study.

Fish Hatching

Fathead minnow eggs were incubated according to the method described by Klemm (1985). The eggs were obtained within two hours of fertilization while they were still attached to the spawning tile. The spawning tile containing the eggs was placed in an 800 ml beaker containing 600 ml of dechlorinated water plus 60 ml of 28 ppt salt water and was vigorously aerated with an air stone. The eggs were incubated at a temperature of 25±1.5 C and were subjected to a 16 L:8 D photoperiodic schedule. Hatching occurred between four to five days after the eggs were collected.

Larval Rearing

The larvae were reared under the same laboratory conditions that were used during the incubation period for the eggs, except that salt was not added to the rearing water. Upon hatching, the larvae were divided equally and placed in up to six aerated 2-liter glass finger bowls containing

dechlorinated water. Each finger bowl was cleaned and fresh water added every third day according to a rotating schedule.

Larval Feeding

All larvae used in the study were fed freshly hatched *Artemia*, brine shrimp nauplii, Salt Lake, Australia, variety. During the first phase of the study, all larvae were fed live brine shrimp twice daily at 0800 h and 1700 h. Approximately one-half of the larvae used in the second and third phases of the project were reared on live brine shrimp while the other one-half were reared on freshly killed brine shrimp. Brine shrimp were killed by placing them in an ultrasonic bath for 1 to 2 minutes. Each larva received approximately 250 brine shrimp per feeding. Unconsumed brine shrimp and detritus were siphoned from the rearing bowls prior to each morning and afternoon feeding.

Live and dead brine shrimp used in the feeding trials were counted while being aspirated into a 10 cc syringe with a large needle. One hundred live or dead brine shrimp were counted and injected into the test chambers containing the larvae by means of a food injection tube mounted on the side of the camera stand.

Behavior Recording

A video recording system utilizing infra-red illumination was used to record behavior (Batty, 1983). Since infra-red emitting diodes were used as the light source, it was possible to record activity in the light as well as in total darkness. All behavior trials were recorded on super 120 VCR tapes.

Light Feeding Trials

Larvae used in the light feeding trials were hatched on 22 February 1992. Upon hatching, they were distributed equally among six, 2-liter glass rearing-bowls and reared according to two feeding regimes. One-half of the fish were reared on live brine shrimp; the other one-half were reared on dead brine shrimp. Test fish were selected and placed in their test chambers at 1800 h the day before testing. Two sets of five fish from each of the feeding groups were randomly selected with a large mouthed pipette and transferred to four test chambers containing dechlorinated water. The larvae were not in the presence of food for at least 15 hours prior to the feeding trials. At 0845 h the following morning, one of the replicate test chambers containing fish reared on live food was placed under the camera and the other was placed on the camera stand. The larvae placed under the camera were allowed to acclimate for 15 minutes. Immediately following the acclimation period, the activity of the larvae in the absence of food was recorded for 5 minutes. At this time 100 live brine shrimp were introduced into the test chamber via the food injection tube and behavior in the presence of food was recorded for 10 minutes. Upon conclusion of the trial, the replicate group was tested in the same manner. The procedure used for the dead feeding trials was essentially the same as that described above, with one exception; the prey introduced during the trial were 100 freshly killed brine shrimp.

Immediately following a feeding trial, the fish were placed under a dissecting microscope in order to examine their digestive tracts for the presence of food. They were then returned to their respective rearing-bowls, unless they were used to obtain growth data.

As indicated earlier, the larvae used during the trials were reared in six bowls. Three of these bowls contained the larvae reared on live food and the other three contained those reared on dead food. Both sets of bowls were numbered 1 through 3 and the larvae in any two correspondingly numbered bowls were used for testing only once every three days. On every third day of testing, Day 3, Day 6, Day 9, Day 12 and Day 15, the feeding regimen was reversed during the trials. Therefore, the fish reared on live food were given dead food and vice-versa. All the trials were conducted in the light.

Dark Feeding Trials

The same procedure used for the light feeding trials was used for the dark feeding trials, with one exception; the lights were turned off 30 minutes prior to the time of recording. Therefore, the first test group was subjected to a 30 minute dark acclimation period on the camera stand. All trials were conducted in total darkness, and the lights were not turned on until the last test group had been removed from the camera stand.

Analysis of Behavior

The behavior of the larvae during the diel study and during the feeding trials was analysed using a frame by frame replay and counting the number of times each fish crossed any one of four quadrants outlined on the bottom of the test chamber. These data were quantified as mean movements per fish per minute.

Feeding activity during the light and dark feeding trials was recorded as frequency of occurrence of feeding and was determined by the number of fish that had food in their gut at the end of the 10 minute feeding interval.

Statistical Treatment

A two way ANOVA was performed using the Statistical Analysis System (SAS) computer package for all 15 day inter- and intra-treatments. The STATS PLUS computer package was used to perform a two way ANOVA for the intra-treatments between the different 3 day groups. The STATS PLUS package was also used to perform a Student's t - test on all the intra-treatments on the reversed feeding days.

The outcome of all statisitical tests was considered significant if the probability of exceeding the test statistic was less than or equal to 0.05.

DIEL ACTIVITY

The maximum daily activity period(s) of larval fathead minnows had to be determined before the feeding trials could be performed. Therefore, a series of diel activity studies was conducted. Larvae used for the diel studies were hatched on 9 October 1991. Three diel studies were conducted, on 15 October, 20 October and 26 October when the larvae were 6, 11, and 17 days old, respectively.

Prior to each diel trial, five fish were selected at 0730h, thirty minutes following the morning feeding, and placed in a 150 ml plexiglas test chamber. Following a 1.5 hour acclimation period on the camera stand, the activity of the fish was recorded for ten minutes on the hour at two hour intervals over a 24-hour period. The fish were not handled, disturbed or fed during the diel test period.

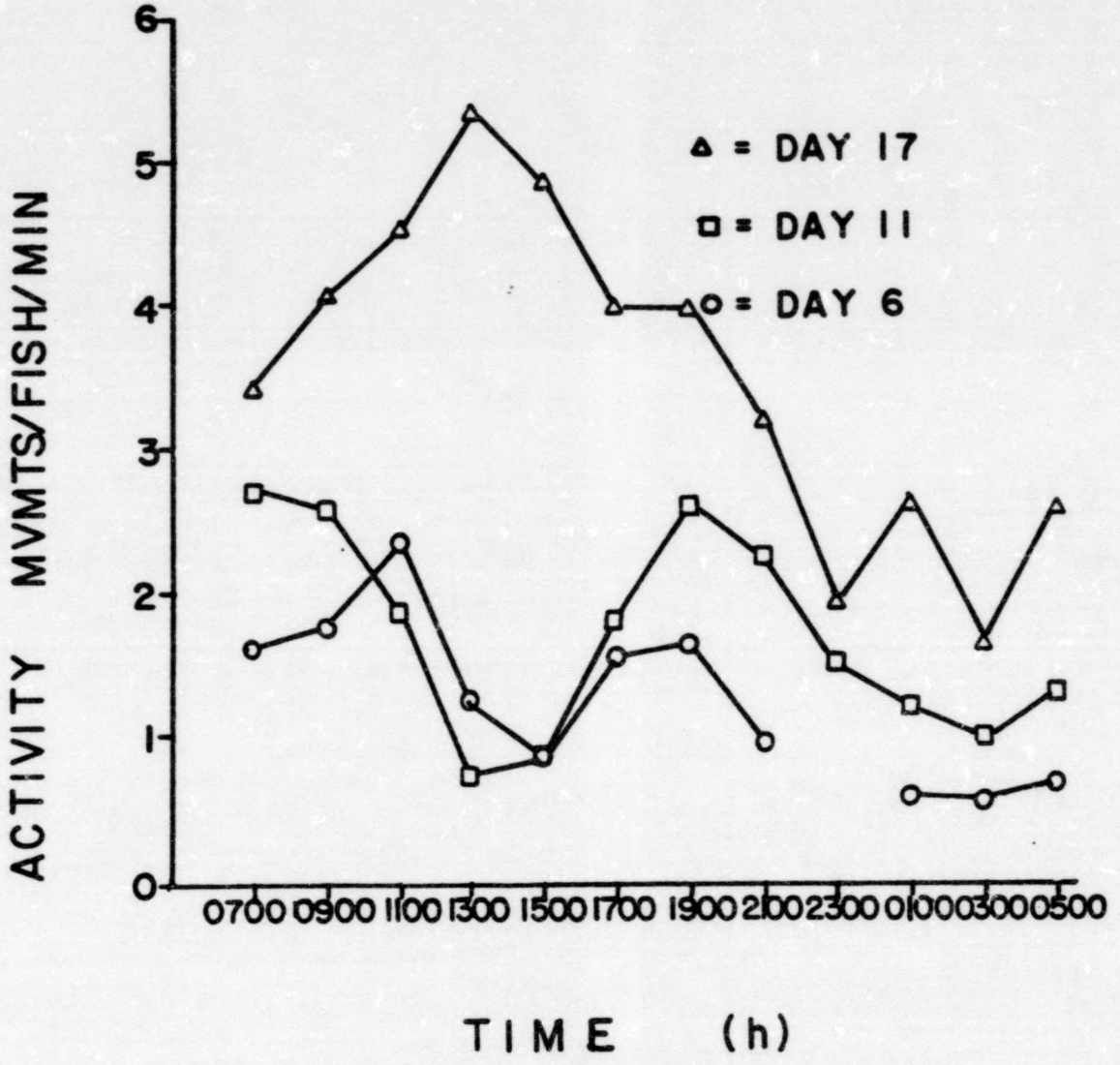
Diel activity patterns were markedly different among the larvae of different age groups. Six and 11 day old larvae exhibited two activity peaks daily while the 17-day old-larvae had one prolonged maximum activity period (Table 1; Figure 1). The six-day larvae were most active from 0700 - 0900h and 1700 -1900h (Table 1; Figure 1). Eleven-day larvae were most active from 0700h-0900h (Table 1; Figure 1). The 17-day larvae were most active from 1100h-1500h (Table 1 ; Figure 1).

Based upon these data, feeding trials for all test larvae were conducted between 0900 and 1200h.

Table 1. Diel activity of fathead minnows on days 6, 11 and 17 post-hatching, expressed as mean number of movements/fish/minute.

	Mean number of movements/fish/minute		
Time	6 days post-hatch	11 days post-hatch	17 days post-hatch
0900 h	1.76	2.58	4.07
1100 h	2.34	1.87	4.51
1300 h	1.24	0.73	5.36
1500 h	0.84	0.84	4.88
1700 h	1.54	1.82	3.98
1900 h	1.62	2.60	3.98
2100 h	0.96	2.23	3.20
2300 h	---	1.51	1.93
0100 h	0.58	1.20	2.61
0300 h	0.54	0.975	1.63
0500 h	0.68	1.33	2.58
0700 h	1.62	2.69	3.40

Figure 1. Diel activity measured at two hour intervals, of larval fathead minnows maintained on a 16L: 8D photoperiodic schedule on days 6, 11 and 17 post-hatching, expressed as mean number of movements (MVMTS)/fish/ minute (MIN).


ACTIVITY MVMTS/FISH/MIN
0
1
2
3
4
5
6
△ = DAY 17
□ = DAY 11
○ = DAY 6
0700 0900 1100 1300 1500 1700 1900 2100 2300 0100 0300 0500
TIME (h)

LARVAL GROWTH

When more than one batch of larvae are used in a behavioral study it is important to ascertain whether all the larvae used in the study grow at the same rate and have similar physiological conditions (Usher and Bengtson, 1981). Therefore, growth in length as a measurement of physical fitness and physiological condition was determined for all the fish groups used in this study.

Two fish were selected every third day during the feeding trials and fixed in a 5% (V/V) formaldehyde solution. Upon death, each fish was placed under a dissecting microscope and its total length (TL) was measured with a ruler calibrated to 1.0 mm.

Fathead minnow larvae reared on live brine shrimp for light trial testing grew from an average total length (TL) of 5.25 mm on Day 1 to an average TL of 9.63 mm on Day 15 (Table 2). Larvae reared on dead brine shrimp for the light trials grew from an average TL of 5.25 mm on Day 1 to an average TL of 10.50 mm on Day 15 (Table 2). The average daily growth rate for the larvae used in the light trials was 0.44 mm/day. Larvae reared on live food for the dark trials grew from an average TL of 5.63 mm on Day 1 to an average TL of 13.25 mm on Day 15 (Table 3). The larvae reared on dead food for the dark trials grew from an average TL of 5.63 mm on Day 1 to an average TL of 11.0 mm on Day 15 (Table 3). The average daily growth rate for larvae used in the dark trials was 0.54 mm/day. Both groups of larvae used during the light trials and the group used during the dark dead feeding trials showed equal growth. The growth of the larvae reared on

Table 2. Fifteen day growth of fathead minnows reared on live and dead food for the light feeding trials as indicated by mean total length, (TL) in millimeters, total length range in millimeters and standard deviation, (SD).

Age (Days)	N	Mean TL mm		Range		SD	
		Live	Dead	Live	Dead	Live	Dead
3	2	5.25	5.25	5.0-5.5	5.0-5.5	0.354	0.354
6	2	5.50	5.75	5.0-5.0	5.5-6.0	0.000	0.354
9	2	7.75	6.757	8.0-9.0	6.5-7.0	0.354	0.354
12	2	8.25	8.75	8.0-8.5	8.5-9.0	0.345	0.345
15	2	9.63	10.50	9.3-10.0	10.0-11.0	0.530	0.707

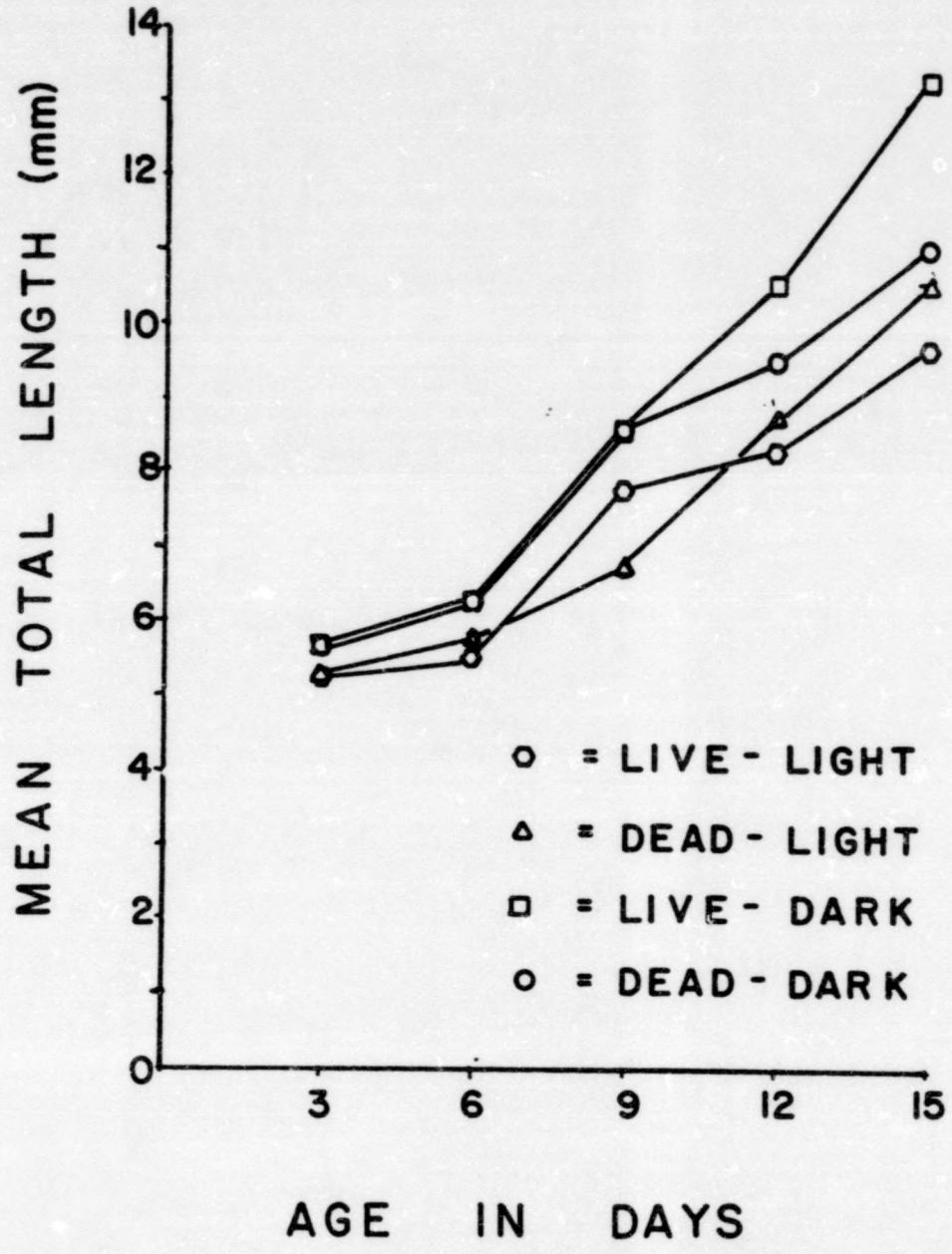
Table 3. Fifteen day growth of fathead minnows reared on live and dead food for the dark feeding trials as indicated by mean total length, (TL) in millimeters, total length range in millimeters and standard deviation, (SD).

Age (Days)	N	Mean TL mm		Range		SD	
		Live	Dead	Live	Dead	Live	Dead
3	2	5.63	5.63	5.5-5.75	5.5-5.75	0.177	0.177
6	2	6.25	6.25	6.0-6.50	6.0-6.500	0.354	0.354
9	2	8.50	8.50	8.0-9.0	8.0-9.0	0.707	0.707
12	2	10.50	9.50	10.0-11.0	8.0-11.0	0.707	2.121
15	2	13.25	11.00	13.0-13.5	10.5-11.5	0.354	0.707

live food and used during the dark feeding trials paralleled that of the other larvae up to Day 9, after which they grew more rapidly than the other three groups (Figure 2).

The total length of these larvae upon hatching ($\bar{x}$ = 5.0 mm) was equal to that reported by Buynak and Mohr (1979). Since the growth data for test groups are similar to the results reported for the species by Hoyt (1990), it was concluded that these larvae were of average size and physical condition for obtaining valid behavioral responses. The average daily growth rates of larval fathead minnows used during the light and dark trials are similar to the daily growth rate of sheepshead minnow larvae (0.42 mm per day) reported by Usher and Bengston (1981).

Figure 2. Mean total length in millimeters of fathead minnows reared on live and dead food and used in the light and dark feeding trials, during the first 15 days of life.


MEAN TOTAL LENGTH (mm)
0
2
4
6
8
10
12
14
3
6
9
12
15
AGE IN DAYS
○ = LIVE - LIGHT
△ = DEAD - LIGHT
□ = LIVE - DARK
○ = DEAD - DARK

RESULTS

Feeding Activity

The first evidence of feeding occurred on Day 3 during a reversed feeding trial, when one-half of the fish conditioned on dead food consumed live food in the dark (Table 4). Representative larvae in all feeding groups consumed brine shrimp on Day 4.

Larvae fed live and dead brine shrimp in the dark exhibited different food preferences and maximum feeding capabilities sooner than larvae fed in the light. Dark-fed fish showed a distinct preference for live food over dead food, while light-fed fish, although favoring live food earlier (Days 4 and 5), showed a markedly higher feeding response to dead food from Day 7 to Day 10 (Table 4). From Day 3 through Day 9, larvae used in the dark feeding trials averaged 35% more fish feeding on both food types per day than their counterparts reared in the light (Table 4; Figure 3).

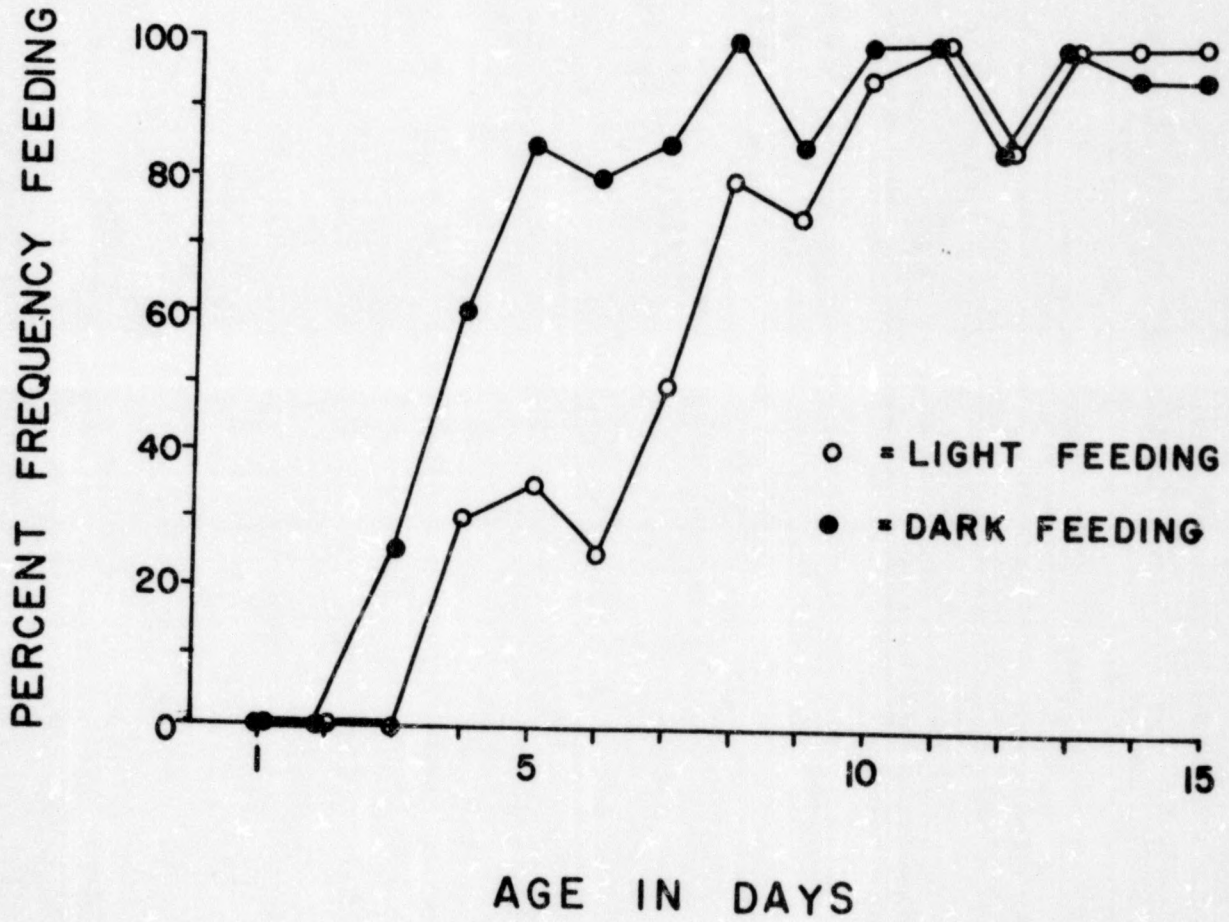
Fish reared and conditioned on live food in the dark exhibited 100% feeding by Day 4 and, with the exception of one fish on Day 14, fed maximally during the balance of the project. Fish reared and conditioned on dead food in the dark did not reach 100% feeding success until Day 8 (Table 4; Figure 3).

Larvae reared in the light learned to feed maximally on dead food sooner than live food. Larvae reared on dead food reached 100% feeding frequency on Day 10, while those reared on live food reached 100% feeding frequency on Day 11.

Table 4. Percent frequency of feeding of larval fathead minnows on normal and reversed feeding days during the light and dark trials.

Day		Light Feeding Trials			Dark Feeding trials	
		Live Food	Dead Food		Live Food	Dead Food
1		0%	0%		0%	0%
2		0%	0%		0%	0%
3	Reversed	0%	0%	Reversed	0%	50%
4		50%	10%		100%	20%
5		60%	10%		100%	70%
6	Reversed	0%	50%	Reversed	60%	100%
7		40%	60%		100%	70%
8		80%	80%		100%	100%
9	Reversed	50%	100%	Reversed	70%	100%
10		90%	100%		100%	100%
11		100%	100%		100%	100%
12	Reversed	70%	100%	Reversed	70%	100%
13		100%	100%		100%	100%
14		100%	100%		90%	100%
15	Reversed	100%	100%	Reversed	90%	100%

Figure 3. Percent frequency of feeding of larval fathead minnows on normal and reversed feeding days during the light and dark trials.


PERCENT FREQUENCY FEEDING
100
80
60
40
20
0
1
5
10
15
AGE IN DAYS
○ = LIGHT FEEDING
● = DARK FEEDING

Reversed feeding trials indicated that larvae reared on dead food fed considerably better when presented live food than did larvae reared on live food when given dead food. Dark-reared fish conditioned to dead food increased their feeding percentages when fed live food by 50% from Day 2 to 3 and by 30% from Day 5 to 6 (Table 4). In contrast, dark-reared fish conditioned to live food decreased their feeding percentages when fed dead food by 40% from Day 5 to 6, 30% from Day 8 to 9, and 30% from Day 11 to 12 (Table 4).

Light-reared fish showed similar trends to dark-reared fish. Light-reared fish conditioned to dead food increased their feeding percentages when fed live food by 40% from Day 5 to 6 and 20% from Day 8 to 9 (Table 4). Likewise, light-reared fish conditioned to live food decreased their feeding percentages when fed dead food by 60% from Day 5 to 6, 30% from Day 8 to 9 and 30% from Day 11 to 12 (Table 4).

Locomotory Behavior

Significantly different activity patterns were exhibited by the larvae during the various conditions tested before and during the feeding trials. Mean fish activity was greater during the dark than light ($P = 0.0001$) in the absence of food (Table 5; Figure 4). Likewise, activity increased significantly ($P = 0.0001$) with time as fish aged from Day 1 through Day 15 of the study for all conditions tested without food (Table 5). No consistent trends in activity were observed among intra-group comparisons in the absence of food (Table 5).

A similar pattern of greater activity in the dark was observed when the fish were in the presence of food, both live and dead (Table 6; Figures 5 and 6).

Table 5. Activity of larval fathead minnows in the absence of live and dead food under light and dark conditions during the first 15 days of life, expressed as mean number of movements/fish/minute.

Age (Days)	Group	Mean No. movements/fish/minute				Inter-treatment Probabilities			
		Light		Dark					
		A W/o Lv	B W/o Dd	C W/o Lv	D W/oDd	AxB	CxD	AxC	BxD
1		0.06	0.04	0.48	0.48	A>B	C>D	A<C	
2	I	0.44	0.44	0.14	0.20	P=0.045	0.043	0.0010	n.s.
3		1.70	0.64	3.22	1.64				
4		1.20	1.98	2.56	1.78				B<D
5	II	0.44	2.76	3.78	4.58	P =n.s	n.s	n.s	0.0030
6		1.54	0.56	3.30	4.14				
7		0.44	2.76	3.78	4.58				B>D
8	III	2.96	4.14	3.82	11.34	P=n.s	n.s	n.s	0.003
9		7.76	2.98	6.10	4.20				
10		5.56	6.64	4.92	12.88		C<D		B<D
11	IV	6.18	4.92	6.82	10.00	P=n.s	0.004	n.s	0.023
12		4.34	3.78	5.60	8.62				
13		4.02	5.36	8.80	5.72				
14	V	7.60	12.54	7.28	6.10	P= n.s	n.s	n.s	n.s
15		6.84	5.60	8.14	6.49				
Mean		3.4	3.52	4.43	5.45				

Inter-treatment probabilities for all 10 days:

	A<B	C<D	A<C	B<D
P=	0.0001	0.0001	0.0001	0.0001

Intra-treatment probabilities.

		A	B	C	D
Comparing all 15 days	P=	0.0001	0.0001	0.0001	0.0001
Group I vs. Group II	P=	n,s	0.0191	n.s	0.0030
Group II vs. Group III	P=	0.0010	0.0080	n.s	0.0050
Group III vs. Group IV	P=	0.0262	n.s	n.s	n.s
Group IV vs. Group V	P=	0.0147	n.s	0.0301	n.s
Group I vs. Group III	P=	0.0017	---	0.0060	---
Group II vs. Group IV	P=	---	n.s	0.0121	---
Group III vs. Group V	P=	---	n.s	---	n.s

Figure 4. Activity of larval fathead minnows in the absence of food during the light and dark trials, expressed as mean number of movements (MVMTS)/fish/minute (MIN).

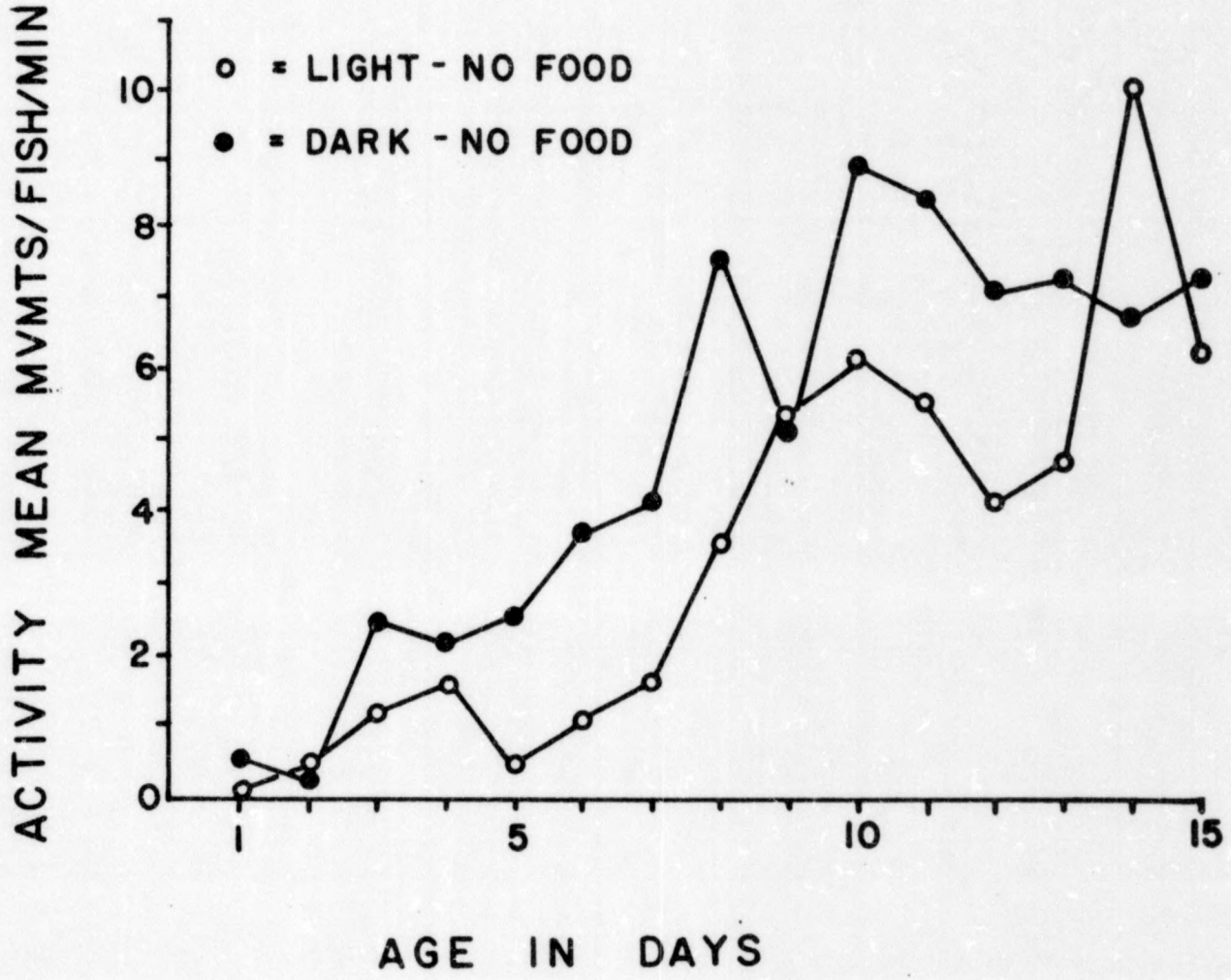

o = LIGHT - NO FOOD
● = DARK - NO FOOD
ACTIVITY MEAN MVMTS/FISH/MIN
0
2
4
6
8
10
1
5
10
15
AGE IN DAYS

Table 6. Activity of larval fathead minnows in the presence of live and dead food under light and dark conditions on the normal feeding days, expressed as mean number of movements/fish/minute.

Age (Days)	Group	Mean No. movements/fish/minute Light E Live	Light F Dead	Dark G Live	Dark H Dead	Inter-treatment Probabilities ExF	GxH	ExG	FxH
1		1.27	1.58	3.85	2.30				
2	I	1.95	0.69	2.53	1.81	n.s	n.s	n.s	n.s
4		1.07	0.88	2.38	2.18			E<G	
5	II	0.78	0.82	2.94	2.17	P= n.s	n.s	0.036	n.s
7		1.01	2.73	3.16	4.81				
8	III	2.35	1.49	2.82	6.30	n.s	n.s	n.s	n.s
10		4.52	4.76	4.01	5.09				
11	IV	6.28	5.01	6.16	7.36	n.s	n.s	n.s	n.s
13		4.15	5.07	8.24	5.02			E<G	
14	V	5.30	9.74	5.96	4.86	P= n.s	n.s	0.030	n.s
Mean		2.88	3.28	4.20	4.19				

Inter-treatment probabilities for all 10 days:

	ExF	GxH	E<G	FxH
P=	n.s	n.s	0.002	n.s

Intra-treatment probabilities.

	E	F	G	H
Comparing all 10 days	P=0.0001	0.0001	0.0001	0.0001
Group I vs. Group II	P=n.s	n.s	n.s	n.s
Group II vs. Group III	P=n.s	n.s	n.s	n.s
Group III vs. Group IV	P=0.001	0.033	n.s	n.s
Group IV vs. Group V	P=n.s	n.s	n.s	n.s
Group I vs. Group III	P=n.s	n.s	n.s	n.s
Group II vs. Group IV	P=---	---	n.s	n.s
Group III vs. Group	P=0.013	0.045	0.008	n.s

Figure 5. Activity of larval fathead minnows in the presence of live food during the light and dark trials, expressed as mean number of movements (MVMTS)/fish/minute (MIN).

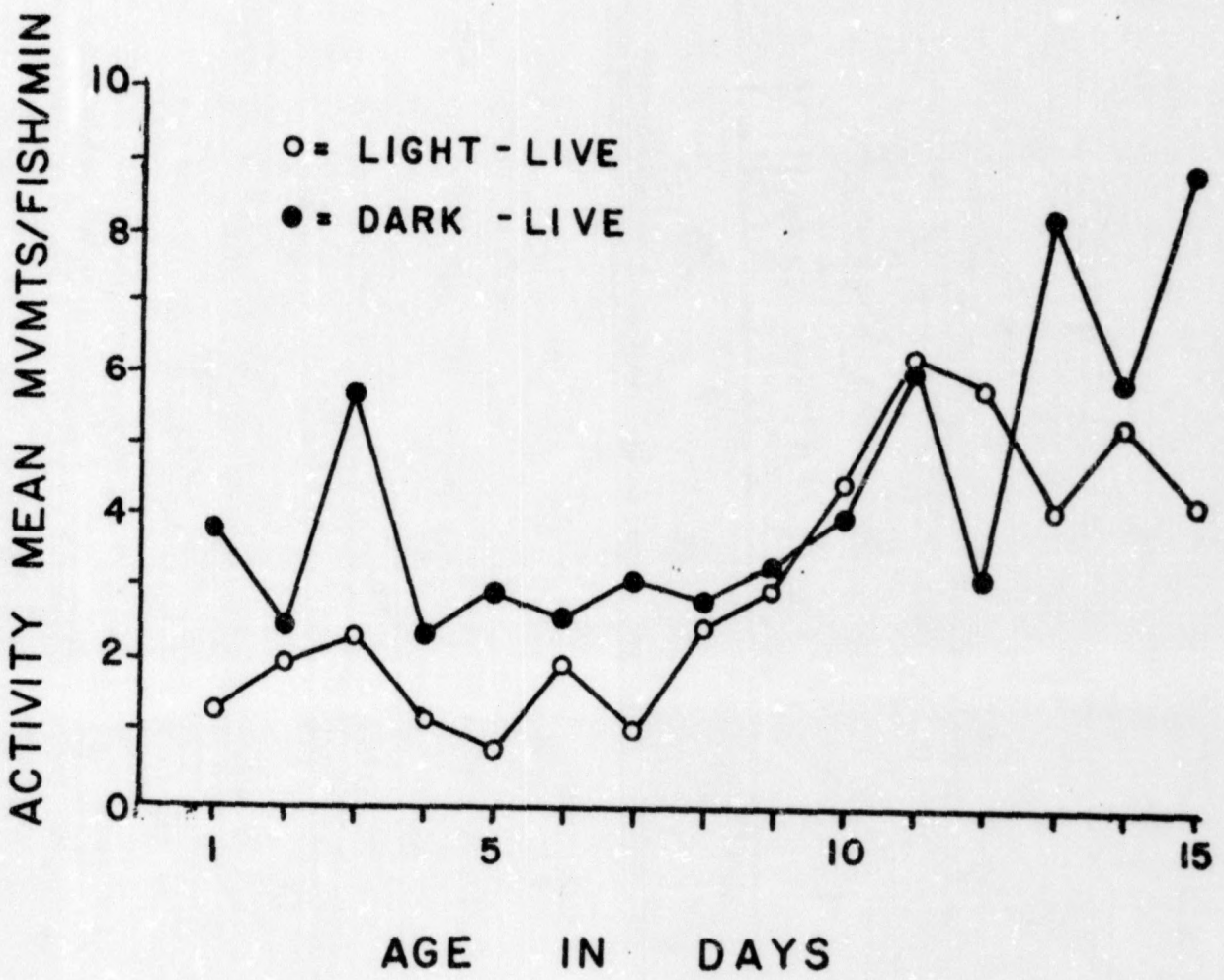
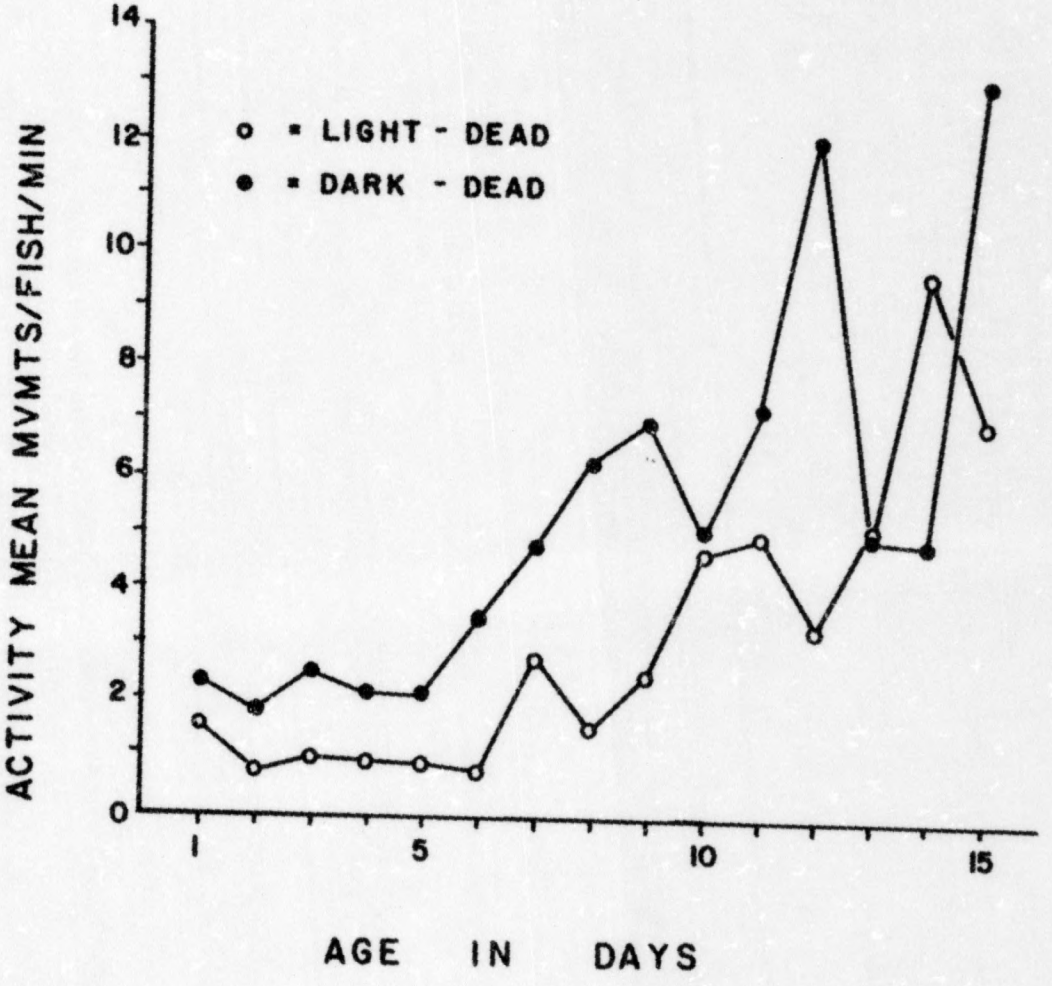

ACTIVITY MEAN MVMTS/FISH/MIN
10
8
6
4
2
0
○= LIGHT - LIVE
●= DARK - LIVE
1
5
10
15
AGE IN DAYS

Figure 6. Activity of larval fathead minnows in the presence of dead food during the light and dark trials, expressed as mean number of movements (MVMTS)/fish/minute(MIN).


14
12
10
8
6
4
2
0
o = LIGHT - DEAD
● = DARK - DEAD
ACTIVITY MEAN MVMTS/FISH/MIN
1
5
10
15
AGE IN DAYS

However, significance was obtained (P = 0.002) only between activity in the presence of live food in the dark and live food in the light. No significance was observed between activity in the presence of dead food in the light and dark, although absolute values were greater in the dark (Table 6). No trends and few significant differences were observed among intra-group and intra-day comparisons.

No differences were observed between activity in the presence or absence of live or dead food in the light (Table 7) or dark (Table 8). Activity during the reverse feeding trials exhibited the same quantitative trend, only significantly, as that during the normal feeding trials; dark activity was greater than light activity for both live and dead food (Table 9).

Table 7. Activity of larval fat head minnows, in the absence and presence of live and dead food, under light conditions on normal feeding days, expressed as mean number of movements/fish/minute.

Age (Days)	Group	Mean No. movements/fish/minute A W/o Live	B W/Live	C W/o Dead	D W/Dead	Inter-treatment Probabilities AxB	CxD
1		0.06	1.27	0.04	1.58	P= n.s	C<D 0.0185
2	I	0.44	1.95	0.44	0.69		
4		1.20	1.07	1.98	0.88	n.s	n.s
5	II	0.44	0.78	0.38	0.82		
7		0.44	1.01	2.76	2.73	n.s	n.s
8	III	2.96	2.53	4.14	1.49		
10		5.56	4.52	6.64	4.76	n.s	n.s
11	IV	6.18	6.28	4.92	5.01		
13		4.02	4.15	5.36	5.07	n.s	n.s
14	V	7.60	5.3-	12.54	9.74		

Inter- treatment probabilities for all 10 days:	AxB	CxD
	n.s	n.s

Table 8. Activity of larval fathead minnows in the presence of live and dead food, under light and dark conditions on the reversed feeding days, expressed as mean number of movements/fish/minute.

Age (Days)	Mean No. Movements/fish/minute Light		Dark		Inter-treatment Probabilities			
	I Live	J Dead	K Live	L Dead	IxJ	KxL	IxK	JxL
3	2.36	0.98	5.71	2.51	P=n.s	n.s	I<K 0.038	n.s
6	1.95	0.75	2.61	3.55	n.s	n.s	n.s	n.s
9	3.03	2.47	3.37	6.96	P=n.s	K<l 0.026	n.s	J<L n.s
12	5.82	3.38	3.18	12.07	I<J P=0.040	n.s	n.s	n.s
15	4.20	7.04	8.94	13.13	n.s	n.s	n.s	n.s
Mean	3.47	2.92	4.76	7.64				

Inter-treatment probabilities for all 5 days:	IxJ	K<L	I<K	J<L
	P= n.s	0.003	0.021	0.001

Intra-teatment Probabilities.

Comparing all 5 Days:	I	J	K	L
	P= n.s	0.012	0.001	0.018
Day 3 vs. Day 6	P= n.s	n.s	n.s	n.s
Day 6 vs. Day 9	P= n.s	0.045	n.s	0.005
Day 9 vs. Day 12	P= n.s	n.s	n.s	n.s
Day 12 vs. Day 15	P= n.s	n.s	0.004	n.s
Day 3 vs. Day 9	P= n.s	---	0.007	---
Day 6 vs.Day 12	P= ---	n.s	---	n.s
Day 9 vs. Day 15	P= n.s	0.027	---	0.029

Table 9.Activity of larval fathead minnows in the absence and presence of live and dead food under light conditions on reversed feeding days, expressed as mean number of movements/fish/minute.

Age	Mean No. of movements/fish/minute				Inter-treatment Probabilities	
(Days)	A W/o Live	B W/ Live	C W/o Dead	D W/ Dead	AxB	CxD
3	1.70	2.36	0.64	0.98	P= n.s	n.s
6	1.54	1.95	0.56	0.75	P= n.s	n.s
9	7.76	3.03	2.98	2.47	P= n.s	n.s
12	4.34	5.82	5.60	7.04	P= n.s	n.s
15	6.84	4.20	5.60	7.04	A<B P=0.037	n.s
Inter-treatment comparisons for all 5 days:				A<B P= 0.030		CxD n.s

DISCUSSION

Sense organ development among fishes is progressive and is closely associated with early changes in behavior. As young fish grow, new behavior patterns emerge as the various sensory organs develop (Kawamura and Ishida, 1985; Noakes and Godin, 1988). These events represent adaptation of these fishes for survival (Kawamura and Washiyama, 1989) and are species specific. Complex behavior patterns, such as feeding, often require the integration of more than one sense, for example, vision for locating food, chemoreception for detecting food and taste in selecting or rejecting food within the oral cavity (Baerends, 1971). Although there is a large volume of information on each individual sense organ, little information is available on the singular role of each sense in the feeding process (Wanzenbock and Schiemer, 1989; Jones and Janssen, 1992) or the time of recruitment of each sensory system into fully integrated behavioral patterns.

Feeding challenges in this study were designed to limit the initiation and completion of the feeding process to individual senses or selected pairs of sense organs. This strategy was adopted so that the role of each sense organ in feeding and the approximate time of recruitment of each sense organ into the fish's feeding behavior could be determined. The earliest evidence of larval fathead minnow feeding occurred on Day 3, when some of the larvae ingested live food in the dark. This observation generally agreed with that of Hoyt (1990) who reported that fathead minnow larvae commenced feeding on Day 2 also on live food in the dark. These observations were important in conclusively identifying the role of

mechanoreceptors and/or chemoreceptors in detecting live prey in the dark very early in larval development. By Day 4, 50% of the fish in the light and 100% of those in the dark fed on live food. These observations suggested that although the fish could both see and "feel" live food in the light, a lack of coordination between the senses of vision and mechanoreception, or the failure to fully integrate these two senses inhibited maximum participation in feeding. In fact, this confusion between the senses of vision and mechanoreception existed until Day 11, the first day that 100% of the larvae given live food in the light fed successfully. According to Jones and Janssen (1992), vision and mechanoreception are the first senses to be used by larval teleosts once the yolk sac reserves are depleted and exogenous feeding is initiated. Their observation is generally consistent with the results obtained in the present study.

In contrast to the "sensory confusion or inhibition" demonstrated by the larvae in the light, those presented with live food in the dark, which could only "feel" the prey, exhibited maximum (100% frequency) feeding on Day 4. The only senses which could have been utilized under these feeding conditions would have been mechanoreception and/or chemoreception (Enger *et al.*, 1989; Montgomery, 1989).

Larval minnows fed dead food in the light and dark also exhibited first feeding on Day 4, but at a much lower frequency than that of larvae fed live food. Under these conditions, the larvae could use their senses of vision and chemoreception in the light and chemoreception alone in the dark (Kleerekoper, 1962). The delayed onset of maximum feeding response to Day 8 in the dark and Day 10 in the light suggested that chemoreception is functionally integrated into patterns of feeding behavior considerably later than mechanoreception. This observation suggests that chemoreception

might represent a learned faculty. The time lag between maximal feeding responses under dark and light conditions again reinforced the fact that some degree of "confusion" or "inhibition" may have existed between the early integration of the senses of vision and chemoreception in the light. In contrast, the single sense of chemoreception, free of secondary background stimuli, provided a simple, direct, successful behavioral response in the dark. This strongly suggested that at an early stage in the development of complex behavior patterns, such as feeding, one major sensory system presented with an *a priori* stimulus will, in the absence of background secondary stimuli, initiate a simple, direct, successful behavioral response.

The conclusion that chemoreception did not appear to become fully functional until Day 8 suggested that mechanoreception acted alone in detecting live food in the dark at Day 3, in the absence of strong chemoreceptive input. Therefore, it appeared that mechanoreception does play an important independent sensory role in early feeding behavior formation. The authenticity of mechanoreceptive involvement in detecting moving prey has been questioned (Enger *et al.*, 1989). However, the observations made by Jones and Janssen (1992) that post-embryonic mottled sculpin, *Cottus bairdi* , utilize their lateral line system to detect live prey and the reports of Dijkgraaf (1962), Enger *et al.*, (1989) and Montgomery (1989), as well as the observations from the present study support the role of mechanoreceptors in early feeding behavior.

The results obtained from this study indicate that larval fathead minnows are not capable of successfully integrating stimuli received from different sensory organs until they are 9 to 10 days old. This conclusion was also substantiated by the fact that from Day 3 through Day 9 larvae used in the dark feeding trials, where sense organs employed were limited to

mechanoreception and/or limited chemoreception, averaged 35% more larvae feeding on both food types per day than those larvae in light conditions where multiple senses could be employed.

Results of the reverse feeding trials supported the suspected roles and time of employment of the individual senses in feeding under the different feeding conditions and identified a mechanoreceptive-chemoreceptive bias in sensory involvement in feeding as a result of learning. Croy and Hughes (1991) reported that fifteen-spined sticklebacks, *Spinachia spinachia* exhibited decreased feeding success when they were presented with two types of prey on alternate days than when they were given only one type of prey daily. The results of their study provided further evidence that fish "learn" to look for a particular type of prey if they are exposed to it frequently. In the dark, fish reared on dead food increased their feeding frequency dramatically from Day 5 to Day 6 when reverse-fed with live food, suggesting that their sense of mechanoreception was fully capable of functioning, in addition to chemoreception, although the lateral line had not been stimulated in normal feeding. Chemoreception, as mentioned earlier, functioned effectively in those fish that had experienced only dead food with 100% feeding occurring on Day 9. However, when dark-reared fish that had been fed only live food were reverse-fed with dead food, their feeding success dropped dramatically below that of the previous day's feeding percentage with each new challenge. By Day 15 they were still unable to feed maximally on dead food. These observations suggested that chemoreception may become fully functional when the stimulus picture in the environment challenges the sense daily but has a delayed integration into feeding behavior if another, perhaps dominant, stimulus replaces it. Atema *et al* (1980) and Beukema (1968) suggested that as predators, fishes

gain experience by repeatedly locating and ingesting a particular type of prey and ultimately form an active search image for the prey by using its olfactory system. Therefore, it can be inferred that larvae reared on dead food in this study learned to search for dead brine shrimp as a result of being conditioned to that type of food, whereas those reared on live brine shrimp could not do so because they did not receive adequate reinforcement.

The reverse feeding data supported the conclusion that mechanoreception was fully developed and functional by Day 3, whereas chemoreception was not fully integrated into the sensory pathway for feeding behavior until Day 8 - 10. Larvae reared in the light, when tested with the opposite type of food on the reverse feeding days, exhibited the same response as those in the dark, reinforcing the conclusions drawn from the dark feeding trials.

Locomotory Behavior

Swimming activity has been described as the most basic and easily measured behavioral response of fishes (Little, *et al.*, 1990), being highly sensitive, appropriate to most species and relevant to survival (Little and Finger, 1990). As fish age and grow, their activity increases consistent with ontogenetic development of various sense organs thereby contributing to the formation of various new behavioral patterns as well as reinforcement of earlier, more simple expressions (Kawamura and Ishida, 1985; Noakes and Godin, 1988). Blaxter (1986) also reported that spontaneous activity as well as major locomotor activities, such as feeding, increased with age in teleost larvae.

The activity patterns of the fathead minnow larvae observed in this study agreed with those reported in the literature. Mean fish activity increased

significantly from Day 1 through Day 15 for all conditions tested. Hoyt (1990) reported similar findings for the fathead minnow tested through the first 28 days of life. However, several differences in activity patterns were noted during this study compared to those reported on by Hoyt (1990). Most notably, following Day 3, Hoyt (1990) observed greater activity in the light than the dark, which was opposite to the observations made in the present study. The dark-tested larvae may have been more active in the presence of food because they consistently exhibited a greater feeding frequency than their counterparts in the light trials.

In summary, the results obtained from this study indicate that the time of recruitment of the senses of vision, mechanoreception and chemoreception into the feeding behavior of larval fathead minnows was staggered with mechanoreception and/or vision being the first sense(s) to be employed, and chemoreception the last to be recruited. It was also apparent that there was a lack of coordination among or integration of these three sensory systems into feeding behavior during the first few days of exogenous feeding. The results obtained from the reversed feeding trials suggested that the larvae formed a search image of the prey after being exposed to it for several consecutive days, enabling them to forage more efficiently.

The activity of the larvae increased with age, which demonstrated the fact that their sense organs were developing, and that they were adding new behaviors to their repertoire. However, the activity patterns in the presence and absence of food did not show a definite trend. The results obtained from this study could be compared only with those of Hoyt (1990). This was due to the fact that very little work has been done in this field. This study has provided evidence for the approximate times of recruitment of the major senses into the feeding behavior of larval fathead minnows.

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