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SPECIES RECOGNITION AND REPRODUCTIVE ISOLATION IN TWO SPECIES OF SUNFISH (CENTRARCHIDAE)

by

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of the requirements for the degree of
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ABSTRACT

In the Middle Branch of the Thames River, 20 km east of London, Ontario, two species of sunfish are found: the pumpkinseed, Lepomis gibbosus L, and the northern longear, L. megalotis peltastes Cope. Male sunfish intermediate in some aspects of morphology and nesting behaviour and hence presumed to be hybrids, are present in relatively small numbers in the same area. Breeding season and habitat overlap in these two species suggesting that conspecific mate selection may be important in reducing the incidence of hybridization between the species. Mate selection could not be directly tested, as mate selection experiments depend on an adequate supply of ripe fish and these were available in only limited quantities. Consequently choice experiments were conducted in which the behaviour of fish (both sexes of both species) of a variety of ripenesses was recorded in response to two fish of the other sex.

Results from two types of choice apparatus were similar. However several simplifying assumptions were made in analysing the data from one choice apparatus. The tentative explanation offered for the results obtained with this analysis is that longear females, presented with nesting-sized longear males and either nesting-sized pumpkinseed or hybrid males (both types larger than longear males) directed more activity

toward conspecifics. This selection was influenced by male size as no choice was exercised when female longears were presented with male longears and equal-sized males of the other two types. Pumpkinseed females offered a choice of nesting-sized longear and pumpkinseed males directed more activity toward conspecifics. Male size was again implicated as female pumpkinseeds of various sizes directed more activity toward males closest to their own size when presented with unequal-sized male pumpkinseeds. Small punpkinseed females offered large hybrid males and smaller longear males chose the latter. Male pumpkinseeds chose conspecific females but male longears either did not choose or chose conspecifics when both were presented with unequal-sized pumpkinseed and longear females.

The clear conspecific choice shown by some of the fish in these experiments indicates their ability to distinguish between the two types of fish with which they were presented. This ability undoubtedly persists when the fish ripen and probably leads to the selection of a conspecific spawning partner as the alternatives (no choice or choice of a heterospecific spawning partner) would lead to more hybridization than appears to occur in the area.

The clear conspecific choice by both longear females and pumpkinseed males would therefore suggest hybridization is unlikely between them. However, reproductive isolation between longear males and small pumpkinseed females seems less complete. Such females seeking small spawning partners in the study area are unlikely to find conspecifics to breed with, since small male pumpkinseeds are not found nesting there. Sexually mature small pumpkinseed females are frequently found close to longear nesting colonies, and have been seen spawning with longear males. No such observations have been made of sexually mature large female pumpkinseeds. Hence, hybridization in this area most likely occurs between small female pumpkinseeds and male longears.

The role of hybrid sterility in reducing interbreeding between the species was also assessed by artificially fertilizing the eggs from females of the two species with milt from the three types of males. The hybrid cross produced a lower percent hatch of eggs than the intraspecific cross for eggs from both species of females. Selection against hybrids, operating through partial hybrid sterility, would tend to reinforce pre-existing isolating mechanisms between the two species except that the genotypes of those fish producing hybrids tend to be maintained in the population by their previous or subsequent conspecific matings. Under these circumstances hybridization between these two species of sunfish may continue for a long time.

ACKNOWLEDGEMENTS

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INTRODUCTION

Among vertebrates, it is usually the male that exhibits bright nuptial colours when sexual dimorphism exists. The suggested functions for this male colour are to warn other males they are invading his territory and to attract females (Mayr 1963, Bastock 1967). The male is usually the more active of a pair, not only in territorial defense, but also in courtship (Tinbergen 1953), with the result that male courtship behaviour is frequently more conspicuous than female courtship behaviour (Bastock 1967). However males often compete for territories, not for females, particularly when the males arrive first on the breeding grounds, and in such cases courtship begins only when females present themselves for courting. Hence the ultimate choice of a mate is often primarily determined by the female although her courtship role may be largely a passive one (Liley 1966).

Male sunfish arrive on the breeding grounds earlier than females, establish territories, build nests and begin courtship only when female sunfish arrive and enter their territories. Male sunfish courtship behaviour is distinctive and has been previously described (Breder 1936, Witt and Marzolf 1954, Miller 1963, Keenleyside 1967, 1971, 1972) but little has been said about the females' role other than as a passive accomplice in the courting and spawning process. Yet in an area where more than one species of sunfish breeds, female choice of a conspecific spawning partner might be extremely important in preventing hybridization.

Such an area is found in the middle branch of the Thames River just north of Thamesford, Oxford County, Ontario. The two species of sunfish nesting there are the pumkinseed sunfish, Lepomis gibbosus (L), and the northern longear sunfish, L. megalotis peltastes Cope, hereinafter referred to by their common names. The study area consisted of a short stretch of river, approximately 700 m long, in which there were two distinct habitat types: backwaters - weedy, mudbottomed areas where pumpkinseeds nested singly, and the river itself - relatively weed-free with silt over a sand and gravel bottom where longear males nested colonially (Appendix VI). Another type of male was found nesting in the study area in relatively small numbers, and shared nesting characteristics with males of the two species in that they nested in the river like longears and built large nests like pumpkinseeds; also they nested either colonially like longears or singly like pumpkinseeds. For these and other reasons (see description of fish below) these intermediate type fish were postulated to be hybrids and will henceforth be referred to as such.

Suggested factors leading to sunfish hybridization include recent sympatry, habitat disruption, crowding spawning areas and a disproportionate ratio of the two species (Bailey and Lagler 1938, Hubbs 1955, 1961). Pumpkinseed sunfish are distributed throughout the lakes and streams of southern Ontario extending into northern Ontario; longear sunfish are commonly found only in streams of the Lake Huron and Erie drainages of southwestern Ontario (Scott and Crossman 1973), except for an apparently isolated population in the Rainy River district of northwestern Ontario (Gruchy and Scott 1966). Both species were reported in the Thames River system in the 1920's (Hubbs and Brown 1929) but their earlier history in that river is unknown. Consequently, whether their present sympatry is recent or longstanding is also unknown.

On the other hand there is no doubt that the original habitat of this portion of the Thames River has been disrupted by man. The whole area was cleared for farming in pioneer days during the early 19th century. The removal of numerous shade trees at that time likely increased the river temperature producing a more favourable sunfish habitat and may also have permitted more topsoil runoff producing silting in the river. Certainly the river is presently warm in the summer, reaching maximums of 30-35C and the river bottom is covered with a thin layer of fine silt. The course of the river through an extensively farmed and fertilized area, coupled with the pasturing of cows in the study area has undoubtedly contributed to the eutrophication of the river which currently supports a rich flora, particularly in the backwaters. Downstream several hundred meters from the study area is a large concrete dam probably erected in 1913 or 1914 to replace a preexisting wooden dam (A. MacKay, pers. comm.). These dams have undoubtedly hampered the free upstream movement of fish for many fish generations. In short, the study area habitat is now very different from what it was 150 years ago.

Crowding of breeding fish could be caused either by silting of some of the gravel substrate on which sunfish normally spawn, or by vegetation occupying a substantial portion of the spawning area. This might foster the occurrence of heterospecific nesting so close together that the sperm from one male might accidentally wash into the other nest and fertilize heterospecific eggs. While both silting and abundant vegetation were found in some areas of the river there did not appear to be any shortage of suitable nesting substrate. Longear colonies were spread unevenly along the river bank with no distinguishable difference between nesting and non-nesting areas. The average distance to the nearest nest was 1 m for 81 pumpkinseed nests in the study area (Keenleyside unpublished) while the average distance to the nearest nest ranged from about a quarter to a half a meter for 173 uncrowded pumpkinseed nests in Lake Opinicon, Ontario (Colgan and Ealey 1973). Thus pumpkinseed nests in the study area were not crowded. Again there was no distinguishable difference between occupied and potential pumpkinseed nest sites. Hence crowding of nest sites was probably not the cause of hybridization in the study area.

Hubbs (1955, 1961) suggested than an abundance of one species coupled with a scarcity of the other could also lead to hybridization as individuals of the scarce species might have difficulty in locating a conspecific mate. While the population of longears is greater than that of pumpkinseeds in the study area (Cresswell unpublished), the effect of this difference on conspecific mate location is unknown.

In short, the presence of hybrids in the study area suggested interspecific breeding had occurred, but the factors causing this hybridization were not readily apparent. Certainly the river habitat has been radically altered over the years and this alteration may well account for the interspecific matings, perhaps by recently permitting these two species to occupy the same area of the river for the first time, and, as previously mentioned, such recent sympatry has been suggested as one factor promoting breeding between species (Bailey and Lagler 1938, Hubbs 1955, 1961). Nevertheless, hybrids constituted only about 6% of the total sunfish population in this area

(Keenleyside unpublished), suggesting that some mechanism was preventing random mating between the two species.

Mayr (1963) suggested a hierarchial classification of such reproductive isolating mechanisms; premating mechanisms (those which act before gametes are shed), and postmating mechanisms (those which act after gametes are shed). Premating mechanisms include: 1) Seasonal isolation. While pumpkinseeds began nesting earlier in the spring than longears, the two breeding seasons were out of phase by only a week or two, overlapping broadly throughout most of the season of two to two and one half months, so seasonal isolation is not likely an important barrier to hybridization. 2) Habitat isolation. Male pumpkinseeds nested exclusively in the backwaters and male longears nested chiefly in the river, but were also observed nesting in the backwaters. For habitat isolation to be affective, females should have demonstrated the same habitat preference as males. However, ripe female longears were seined from the backwaters, while small ripe female pumpkinseeds were frequently seined from the edges of longear colonies in the river. Thus if habitat isolation was operating at all, it operated at less than full efficiency. 3) Mechanical isolation. This premating mechanism is not applicable to sunfish as their fertilization is external. 4) Ethological isolation. Reproductive behaviour is similar in the two species (Miller 1963). During the spring the males move into shallow water of either the backwaters (pumpkinseeds) or the river (longears), establish territories, dig nests and court females. Ripe females enter these nests and spawning occurs. Subsequently the male drives the female out of the nest and guards the eggs until they hatch and become free-swimming fry.

Ethological barriers preventing hybridization might occur at several points in the above sequence. Males might court only conspecific females. Females might enter only nests of conspecific males. Interspecific courting might occur but break off before spawning begins. Keenleyside (1967) tested the first of these possibilities and found that non-nesting and nesting males of both species distinguished conspecific from heterospecific females by directing more behaviour toward conspecifics although in both cases male longears gave a lower-level response, suggesting male conspecific choice of females may be an important barrier to hybridization. However, field observation of spawning longear colonies suggested that at the peak of spawning male longears often did not court the females with which they subsequently spawned; rather females initiated spawning by entering the nests without male inducement and began nest-circling with the resident male. Consequently it was felt that female choice of a spawning partner might be a significant factor affecting the incidence of hybridization in the study area. One method of testing this choice would be to conduct mate selection experiments.

Mate selection experiments of necessity must be performed by offering a ripe fish a choice of two ripe fish of the other sex and seeing with which of the two spawning occurs. Such an approach was taken by both Hagen (1967) and Semler (1971) conducting male and female mate selection experiments respectively in the threespine stickleback, *Gasterosteus aculeatus*.

Courtship preference tests were performed by Hagen (1967) and

McPhail (1969) by offering a ripe female threespine stickleback a choice of two kinds of courting male threespine stickleback and seeing which of the two the female responded to with the characteristic "head-up" display indicating receptiveness to spawning. This was not mate selection in the strictest sense as no mating occurred; nevertheless the assumption that the male to which the female responded would likely have been the male with which she spawned was reasonable.

In both the mate selection and courtship preference tests described above, both sexes were ripe and ready to spawn, as indicated by spawning actually occurring in the former case, and by behaviour indicating readiness to spawn occurring in the latter. To perform either of these types of experiments with any species of fish, several conditions must be met: 1) there must be an adequate supply of ripe fish of both sexes available, 2) fish must recover quickly from being handled, 3) a behavioural indicator of readiness to spawn must be given by the test fish if courtship preference tests are to be conducted. These conditions were extremely difficult to fulfill using sunfish.

Male sunfish from the study area can be induced to build nests throughout the year by subjecting them to an appropriate temperature and photoperiod (Smith 1970), but female sunfish have never been brought to sexual maturity in this laboratory despite repeated attempts to do so. Consequently availability of ripe female sunfish is limited to those caught in the field. Longears characteristically spawn synchronously every ten days to two weeks under optimum weather conditions. A continuous supply of female longears is not available however (though many would be available on spawning days), as ripe females transported to the lab quickly begin to resorb their eggs. For example, eggs stripped from females the day after their capture in the study area produced zero percent hatch although eggs stripped from similar females on the day of capture produced "normal" percent hatches (Table 2). Also, ripe females confined together in an 850 l cement tank became progressively slimmer until they reached pre-breeding abdominal size, indicating resorption of eggs. In short, wild female longears are exceedingly difficult to maintain in a state of spawning readiness. Ripe female pumpkinseeds were difficult to catch because: 1) they were somewhat scarcer than longear females, 2) large ripe females were found only in the backwaters which became more difficult to seine as the breeding season progressed and backwater vegetation increased, 3) small ripe female pumpkinseeds were often seined from the edges of spawning longear colonies but this was not a reliable source and was also subject to the periodic synchronous longear spawning limitations mentioned above. Hence a constant supply of ripe females of both species could not be obtained.

Neither males nor females of either species recover quickly from handling, particularly when introduced singly into a strange experimental environment. Often several hours elapse before the fish begins to swim about in the laboratory test enclosures. Male sunfish would recover in any experiment which permitted them to build nests; ripe female sunfish brought from the field and introduced into a strange environment might require so much recovery time that their eggs begin to be resorbed.

Neither males nor females of either sunfish species indicated readiness to spawn by any overt behaviour. While it is true that males of both species build nests, the presence of a nest is no indication that the male is ready to spawn. During the early stages of nest construction males of both species are highly aggressive and usually chase approaching females. Further, some sexually mature male longear sunfish do not even build nests, yet apparently spawn by intruding into the nests of other spawning males (Keenleyside 1972) so that, in those fish at least, a nest is not a necessary indicator of spawning readiness. Male sunfish court females with a characteristic behaviour called "courtship circle" but that behaviour is by no means an obligatory prerequisite for spawning to occur, at least in longear colonies (see below), so that while its performance by a nesting male would indicate male readiness to spawn, its non-performance would not preclude the possibility that the male was ready to spawn. Female sunfish indicate readiness to mate by entering the nests of males and spawning. Observations of spawning longear colonies, however, showed that many nests were entered by females who left again without spawning, so that even a female's entering a nest is no guarantee that spawning will occur. Consequently, it would be virtually impossible to perform courtship preference experiments as the necessary overt behaviour indicating choice of a potential spawning partner is not present in the behavioural repertoire of female sunfish, and not obligatory on the part of the males.

As a result of these difficulties in testing mate selection directly, sunfish in a variety of reproductive states were tested, with the rationale that if less-than-ripe fish indicated they could distinguish between two potential spawning partners, this ability would continue to be available when the fish were fully ripe, and could provide the basis for choice of one or the other available spawning partners. Furthermore, if the potential spawning partners were less than ripe but still provided the stimulus for a choice to be made, there is no reason to believe that the stimulus would not still be present when those potential spawning partners became ripe. In fact, the stimulus might well be enhanced by either the appearance or behaviour of the ripe stimulus fish. Male sunfish appear more brightly coloured when fully ripe and nesting, which may enhance their appearance. Liley (1966) used four species of female poeciliid fishes to test mate preference of males. Non-virgin females were used as "it was preferable to have females which were relatively unresponsive in their behaviour" (p. 104). Presumably using more responsive virgin females would have enhanced the behavioural stimulus to the males. Similarly, male sunfish reactions to conspecific and heterospecific females were determined using males which were both non-nesting (Keenleyside 1967, series A) and nesting (Keenleyside 1967, series B and C). Female abdominal swelling was comparable between species but female readiness to spawn was undeterminable. Female and male sunfish reactions to conspecifics and heterospecifics of the opposite sex were also performed in the non-breeding season (Steele and Keenleyside 1971). None of the fish tested was likely ripe as they had been held under natural photoperiod before testing. If non-ripe fish choose conspecifics there is no reason to expect that ripe fish would not make the same choice, as the alternative to making that choice (i.e. making no choice or choosing heterospecifics) would foster more interspecific mating than seems to occur in the study area. It is well known, however, that much behaviour depends on reproductive condition, so a non-choice by unripe fish would not preclude the possibility that those same fish when ripe might very well exercise a choice. With these constraints in mind it is possible to test sunfish of a variety of ripenesses with a choice of conspecific and heterospecific sunfish of the other sex, again of a variety of ripenesses, and to postulate that when both sexes of fish are ripe they would choose in a similar fashion. Such an approach while less than ideal is at least more direct than postulating ethological barriers to hybridization on the basis that: 1) hybridization is not frequent and 2) none of the other barriers accounted for the lack of hybridization (Nelson 1968).

To assess whether female sunfish could distinguish between different types of male sunfish, the females were offered a choice of two males in two sorts of choice apparatus. Male types included: longear and pumpkinseed males both nesting-sized (pumpkinseeds larger than longears) and equal-sized; longear and hybrid males both nesting-sized (hybrid larger than longear) and equal-sized; and different-sized pumpkinseed males. Longear males of all sizes used in these experiments have been seen nesting in the study area. Large pumpkinseed and hybrid males have been seen nesting, while those pumpkinseed and hybrid males of intermediate size may or may not nest. Small pumpkinseed and hybrid males have never been seen nesting. These observations on the relation between size and nesting have been made by several investigators from this laboratory over an eight-year period. Females were not presented with a choice between pumpkinseed and hybrid males as these males have never been observed nesting close to each other in the study area. The behaviour of males presented with a choice of the two species females was also recorded in the two sets of choice apparatus. If males and females demonstrated an ability to choose between stimulus fish in the laboratory, they could presumably utilize this ability to select among the different types of spawning partners available in the study area. If this ability consistently led to choice of a conspecific spawning partner, then ethological isolation would be an important barrier to hybridization.

Mayr's postmating mechanisms include: 1) gamete mortality, 2) zygote mortality, 3) hybrid inviability and 4) hybrid sterility. These mechanisms might also act to reduce the incidence of hybridization in spite of the fact that gametes are shed, because if interspecific gametes produce fewer or less successful offspring than intraspecific gametes, the parents contributing those interspecific gametes will ultimately be selected against. This should lead to the establishment or strengthening of premating isolating mechanisms (Dobzhansky 1970). Of the four postmating mechanisms only hybrid sterility was tested. As hybrid females have not been seen in the study area, only eggs from the two species were artificially crossed with milt from the three types of males in all combinations to ascertain if male hybrids produced as high a percent hatch of eggs as did the two species males. Production of a lower percent hatch of those eggs fertilized with hybrid milt would indicate the operation of hybrid sterility as a postmating isolating mechanism.

The presence of hybrids in the study area indicate that neither pre nor post-mating isolating mechanisms operate at maximum efficiency. If reproductive isolation is less than perfect between the two species, at what point in Mayr's hierarchy do the mechanisms break down? For instance, if conspecific mate selection can be inferred to be operating as an ethological barrier to hybridization, Keenleyside's (1967) results suggest that male conspecific choice exists and that male pumpkinseeds choose conspecifics more consistently and vigorously than do male longears. Answers to the following questions would help to better understand the barriers to sunfish hybridization and where they fail in the study area: 1) Does female conspecific choice exist? 2) Is female choice affected by the size of the presented male? (Size differences are striking between nesting males of the two species). 3) Where hybridization occurs, is it a result of a cross between a male longear and a female pumpkinseed or the reciprocal cross? 4) Are hybrids in the study area as fertile as the two species males? It was to answer the above that the present research was undertaken.

MATERIALS AND METHODS

1. Description of Fish

1.1 Longears

Adult male longears are small, deep-bodied sunfish with a brown dorsal surface which shades into yellow-orange ventrally. The lateral surfaces of the male longear are blue with orange speckling distributed so that the blue often appears as a series of vertical lines. The dorsal and anal fins contain a series of orange spots on the blue-grey inter-spine membrane. These spots often form horizontal orange lines on the fins. Male longears have a relatively large, black opercular flap set at an angle of 45° above the horizontal. These flaps have a narrow red posterior border, often with a thin white dorsal and ventral edge. Narrow disjointed wavy blue lines are found on the cheeks and opercula. In the breeding season the eye iris colour is bright red and the pelvic fins jet-black. Fork length measurement of fish used in this study ranged from 7.1 to 11.0 cm (Appendix I). For illustrations of male longears see Fig. 137, Trautman (1957) and Fig. 239, Hubbs and Lagler (1964).

Adult female longears are consistently smaller than males. In this study female fork lengths ranged from 5.9 to 10.3 cm (Appendix I). The body colouring is similar to that of the male, but the colours are duller. The ventral surface is pale yellow or white. The opercular flap is relatively much shorter than that of the male and although the red posterior spot is present, it is darker and less conspicuous than that of the male. Blue cheek-operculum lines are present, but are fewer in number, thicker and less conspicuous than those of the males. In the breeding season the eye colour is white or light pink and the pelvic fins are yellow rather than black.

1.2. Pumpkinseeds

Adult male pumpkinseeds are larger than male longears. In this study fork length ranged from 8.5 to 17.2 cm (Appendix I). The lateral surfaces of the male pumpkinseeds are grey-blue dorsally blending to blue laterally and yellow ventrally, the whole surface being covered with orange spots. The dorsal and anal fins have dark brown spots on a somewhat lighter brown interspine membrane with the result that these fins are much less conspicuous in pumpkinseed males than they are in longear males. The black opercular flaps are relatively smaller than those of male longears and a red spot is present on the posterior edge of the flap which extends horizontally from the operculum. The cheek-operculum pattern consists of fewer, thicker, more continuous blue lines than are present on male longears. Eye colour is brown and pelvic fins remain yellow throughout the year.

Adult female pumpkinseeds are very similar to the males in both size, ranging in fork length in this study from 8.5 to 16.4 cm (Appendix I), and colouration, although the colours of the female may be slightly duller than those of the male during the breeding season. Females have a larger urogenital aperture than males. For illustrations of pumpkinseeds see Fig. 139 and Plate IV, Fig. 1, Trautman (1957) and

Figs. 234-5, Hubbs and Lagler (1964).

1.3 Hybrids

Adult male hybrids are similar to pumpkinseeds in maximum size and body colouration. The dorsal and anal fins possess the orange-spotted blue inter-spine membrane characteristic of male longears. Hybrids have a black opercular flap that is relatively as long or longer than that of male longears although the angle of tilt is not as great. The posterior red patch of the opercular flap is larger and more conspicuous than that of the longear male, while the white dorsal and ventral edging of the flap may be present, absent or alternating with spots of red. The blue cheek-opercula lines are thick and continuous like those of the pumpkinseed although they seem brighter. Both eye colour and pelvic fin colour tend to be intermediate between those of the longear and pumpkinseed, ranging from red to brown and grey to yellow respectively. Females which could be clearly identified as hybrids have never been seen in the study area.

2. Collection, Transportation and Holding of Fish

The sunfish used in this study were collected in the study area by seining during the spring and summer months. They were transported to the laboratory in large polyethylene bags approximately 1/4 filled with water and 1/2 filled with a mixture of 92% O_2, 8% CO_2. Upon arrival the fish were either used immediately for stripping experiments or were separated by species and sex and held in five 850-litre cement holding tanks in a greenhouse before use in other experiments. Water temperature in the greenhouse tanks ranged from a summer maximum of 20C to a winter minimum of 15C. Photoperiod was natural (43°N lat.). New fish were caught each spring and summer. Those remaining in the greenhouse tanks from the previous season were fin-clipped (left pelvic) and released in the study area. Fish in the greenhouse tanks were fed largely a diet of Strike Fish Food #4 Crumbles (Country Best, Agway Inc., Syracuse, N.Y.), while the experimental fish were not fed except as noted below.

3. Observation of Longear Spawning

The longear breeding season began in late May and extended well into July in most years (personal observation). During this time, male longears periodically congregated to form colonies. These colonies usually started with a few males digging nests in the shallow water near the river's edge by vigorously sweeping the silt from the gravel substrate with powerful beats of their caudal fins. More males congregated until the final size of the colony may have ranged from less than a dozen to several hundred nesting longear males (Keenleyside 1972). These males were very aggressive in defense of their nest territories and chased out all intruders including females until such time as both the males and females were fully ripe, when females entered the colony to spawn. These females were sometimes courted by the nesting males in the colony. A courting male swam up toward the female from his hovering position over his nest, looped over her and quickly swam back to his nest to turn and face the female. This "courtship circling" was the most overt form of male longear courtship. If the female was receptive, she followed the male to the nest and the two fish swam in slow circles within the nest with the male usually on the outside. Periodically the female tilted at very nearly 90° to bring her urogenital aperture close to that of the male for a second or two, and shuddered while releasing eggs into the nest. Spawning finished when the female left the nest or was chased out by the resident male. The majority of spawning occurred in a colony over a two day period starting around mid-morning and peaking from mid to late afternoon.

At the height of spawning the paths of 92 females were traced as they entered a colony and both their behaviour and the behaviour of encountered males was noted until the females left the colony. These observations were made to test the hypothesis that male longears were not courting females to any great extent during peak spawning periods. Observations were made on five colonies in which spawning was frequent over a two day period. Recorded female behaviour consisted of the following acts:

Enter nest - The female swam into a male-occupied nest.

Circle - The female swam in circles within the nest with the male swimming beside her on the outside.

Spawn - The female periodically tilted on her side for a second or two, usually during circling, bringing her urogenital aperture close to that of the male and shuddered or vibrated while releasing eggs.

Each of the above female behaviours was subdivided into two groups: 1) male courtship circling preceded the female behaviour at least somewhere in the sequence: enter nest, circle, spawn; 2) no male courtship circling occurred at all. The relative numbers of female behaviours in these two groups therefore indicated the relative amount of male courtship occurring during the height of spawning.

Comparable observations of pumpkinseed spawning were not made. The lower numbers of nesting male pumpkinseeds combined with their nesting singly in the deeper weedier backwaters mitigated against the observation of pumpkinseed spawning.

4. Cement Tank Experiments

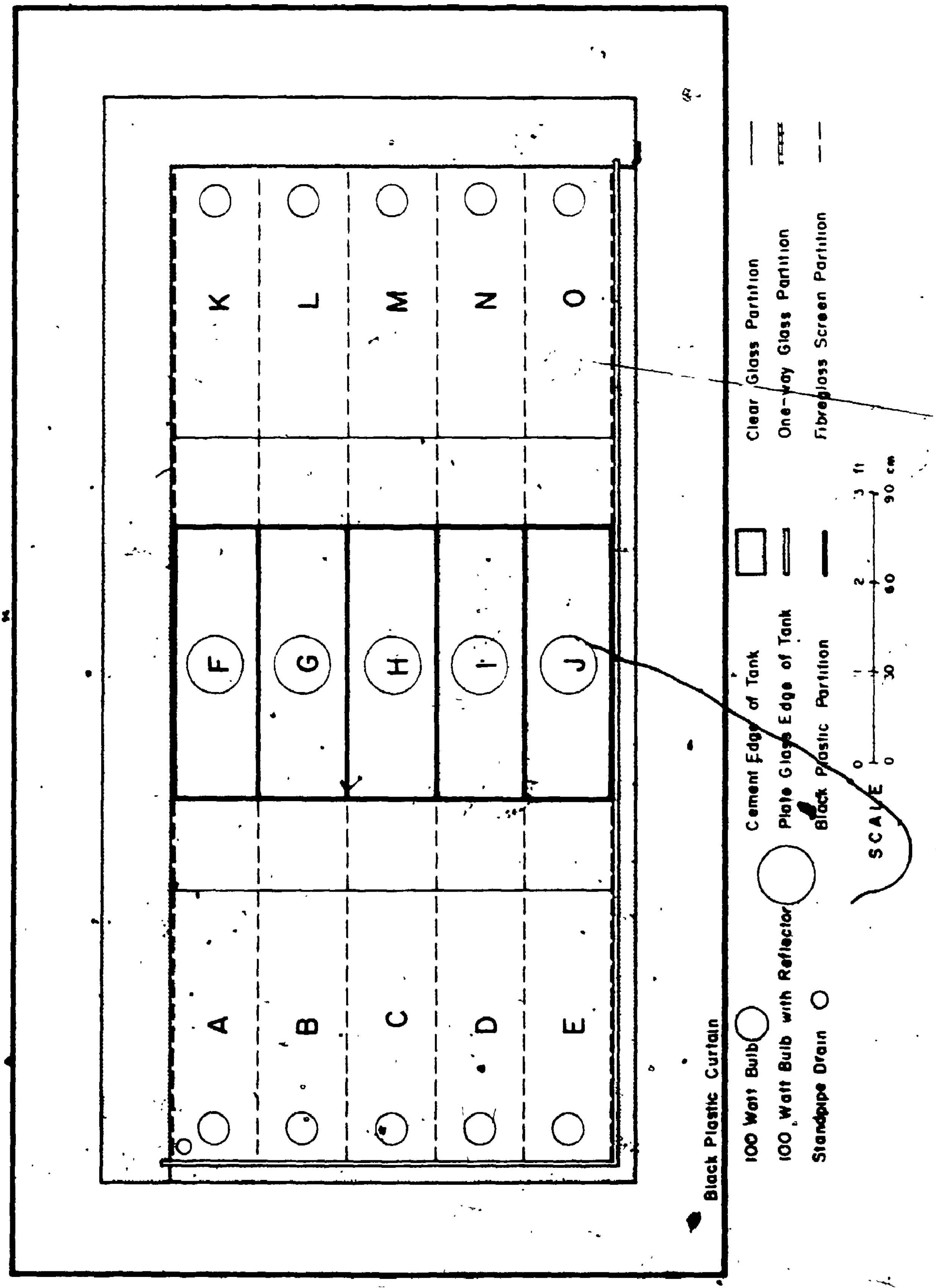
These experiments were conducted during the months of October and November. No attempt was made to match fish by size either within or between species, although the fish used did reflect the generalization that adult pumpkinseeds are larger than adult longears and longear males are larger than longear females (Trautman 1957).

4.1 Apparatus and Observations

A cement and plate glass tank (335 x 152 x 66 cm deep) was divided lengthwise into five equal compartments (Fig. 1). Each compartment was in turn divided into five sections by inserting a clear glass partition 91 cm from each end and a one-way glass partition 30 cm inward from the glass partitions towards the center of the compartment. The central sections (F-J, Fig. 1) were separated from each other by black plastic, while all other sections were separated by fine-mesh fiberglass screening. The tank bottom was covered by a layer of fine gravel to a depth of about 7 cm. Water dept[illegible]ove the gravel was about 24 cm.

Each end of each of the five compartments was illuminated by

Figure 1. Top view of cement tank showing subdivisions. Stimulus sections: A-E, K-O. Test sections: F-J.


K
L
M
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F
G
H
I
J
A
B
C
D
E
Black Plastic Curtain
100 Watt Bulb
100 Watt Bulb with Reflector
Standpipe Drain
Cement Edge of Tank
Plate Glass Edge of Tank
Black Plastic Partition
Clear Glass Partition
One-way Glass Partition
Fibreglass Screen Partition
SCALE
0
1
2
3 ft
0
30
60
90 cm

a 100-W bulb. The central sections were each illuminated by a 100-W bulb surrounded with an aluminum foil reflector. All bulbs were suspended 30 cm above the water surface. The central bulbs were connected to a powerstat variable autotransformer (type 3PN116B, Superior Electric Co., Bristol, Conn.). All lights were connected to an interval timer which provided a 16 L: 8D cycle beginning at 0700 hrs to approximate the local June photoperiod when spawning of both species normally occurs.

The entire tank was surrounded by a black plastic curtain about 250 cm high and 30 cm from the edge of the tank. Tap water slowly trickled into each compartment at one end, and water level was maintained by a stand-pipe drain at the other end of the tank. Water temperature was 21.5 ± 2°C at the inflow end and 24 ± 3°C at the outflow end of the experimental tank for 90% of the experimental time. Two inflow adjustments to cool the water in the tank when it rose above 27°C caused brief declines of 7 and 8°.

Observations of test fish position and behaviour were made through a narrow slit in the black plastic curtain while watching reflections of the fish in a mirror suspended over the test sections. These observations were made under two lighting conditions: control (C) with the central lights on, allowing each test fish to see only its own mirror image in the one-way glass partition at each end of the test section, and experimental (E) with the central lights off, allowing the test fish to see through the one-way glass partitions into the stimulus sections at each end of the test sections. The lights over the stimulus sections remained on during both C and E conditions. Under the C condition, stimulus fish could see into the central section, while under the

E condition, stimulus fish could see their mirror images in the one-way glass partition.

Under lighting condition C, a test fish was observed for a metronome-timed 2-s interval during which its position and behaviour were recorded (see below). Then the test fish in the next compartment was observed for a 2-s interval and its position and behaviour recorded. This continued until all five test fish had been observed, at which time the first test fish was again observed for a 2-s interval. This procedure was repeated until a total of 200 observations had been recorded for each of the five test fish. The intensity of the lights over the test sections was then reduced to zero during a 30-s period (producing lighting condition E) and the entire observation and recording procedure was repeated until a further 200 observations per test fish had been recorded. The five test fish were then discarded; no fish was used as a test fish more than once during the experiments. Fish behaviour and position were recorded on five 9-unit laboratory counters (Clay-Adams Inc., New York, N.Y.). All data were collected between 11 a.m. and 12:30 p.m.

Position and behaviour of test fish were recorded as one of the following categories:

1. Pointing conspecifically (PC) was recorded if the test fish was within one fish length of, and oriented toward, the one-way glass partition beyond which were the three conspecifics of the opposite sex (the conspecific partition).

2. Pointing heterospecifically (PH) was recorded if the test fish behaved as in 1, but oriented towards the heterospecific partition.

3. Nosing conspecifically (NC) was recorded if the test fish touched the

conspecific partition with its snout, and was either stationary or swimming slowly along the partition.

4. Nosing heterospecifically (NH) was recorded if the test fish behaved as in 3 but contacted the heterospecific partition.

5. Fluttering conspecifically (FC) was recorded if the test fish in addition to touching the conspecific partition with its snout, also engaged in rapid horizontal beats of its tail, as if trying to swim through the partition.

6. Fluttering heterospecifically (FH) was recorded if the test fish behaved as in 5 against the heterospecific partition.

7. Neutral (N) was recorded if the test fish was not recorded in one of the above six categories. Such fish usually hovered close to the bottom gravel in the central part of the test section.

8. Crossover (XO) was recorded each time the test fish swam from one end of the test section to the other.

The XO data were collected at the same time as the data for the first seven behaviour acts; however, only the latter were included in the 200 observations per test fish recorded under each of lighting conditions C and E.

4.2 Testing Procedure

Reactions of males and females of both species to conspecifics and to heterospecifics of the opposite sex were recorded in two experiments. In both, a single fish to be tested was introduced into the central section of each compartment (test sections F-J, Fig. 1), given a day to settle down, and observed the following day. Conspecific or heterospecific fish of the opposite sex were introduced into the end sections (stimulus sections A-E and K-O, Fig. 1) in groups of three. Longear females do not normally encounter singly nesting male longears, and it was felt that the addition of more than three fish might unduly crowd the stimulus sections. These groups remained in the stimulus sections for the duration of each experiment. In each longitudinal compartment, then, there was a single fish under observation in the test section, three conspecific fish of the opposite sex in one stimulus section, and three heterospecific fish of the opposite sex in the other stimulus section.

4.3 Experiments

4.3.1 Females as Test Fish

Stimulus fish were alternated with each other by introducing three male pumpkinseeds into each of stimulus sections A, C, E, L, and N, and three male longears into each of stimulus sections B, D, K, M, and O (Fig. 1). All 10 groups of males remained in these sections for the duration of the experiment, and were fed dried fish food each afternoon.

For the first day of observations, single female pumpkinseeds had been placed in test sections F, H, and J, and female longears in test sections G and I (Fig. 1). For the second day the allocation of test sections was reversed; longear females being placed in sections F, H, and J, and pumpkinseed females in sections G and I. Naive female test fish of the two species were alternated in this manner until 20 females of each species had been tested which required eight observation days. Test fish were not fed while in the experimental tank. The apparatus was then dismantled, cleaned, set up again as before, and the water aged for one week before male tests began.

4.3.2 Males as Test Fish

The testing procedure for male sunfish responses to conspecific and heterospecific females was similar to that described for females but with the positions of the sexes reversed.

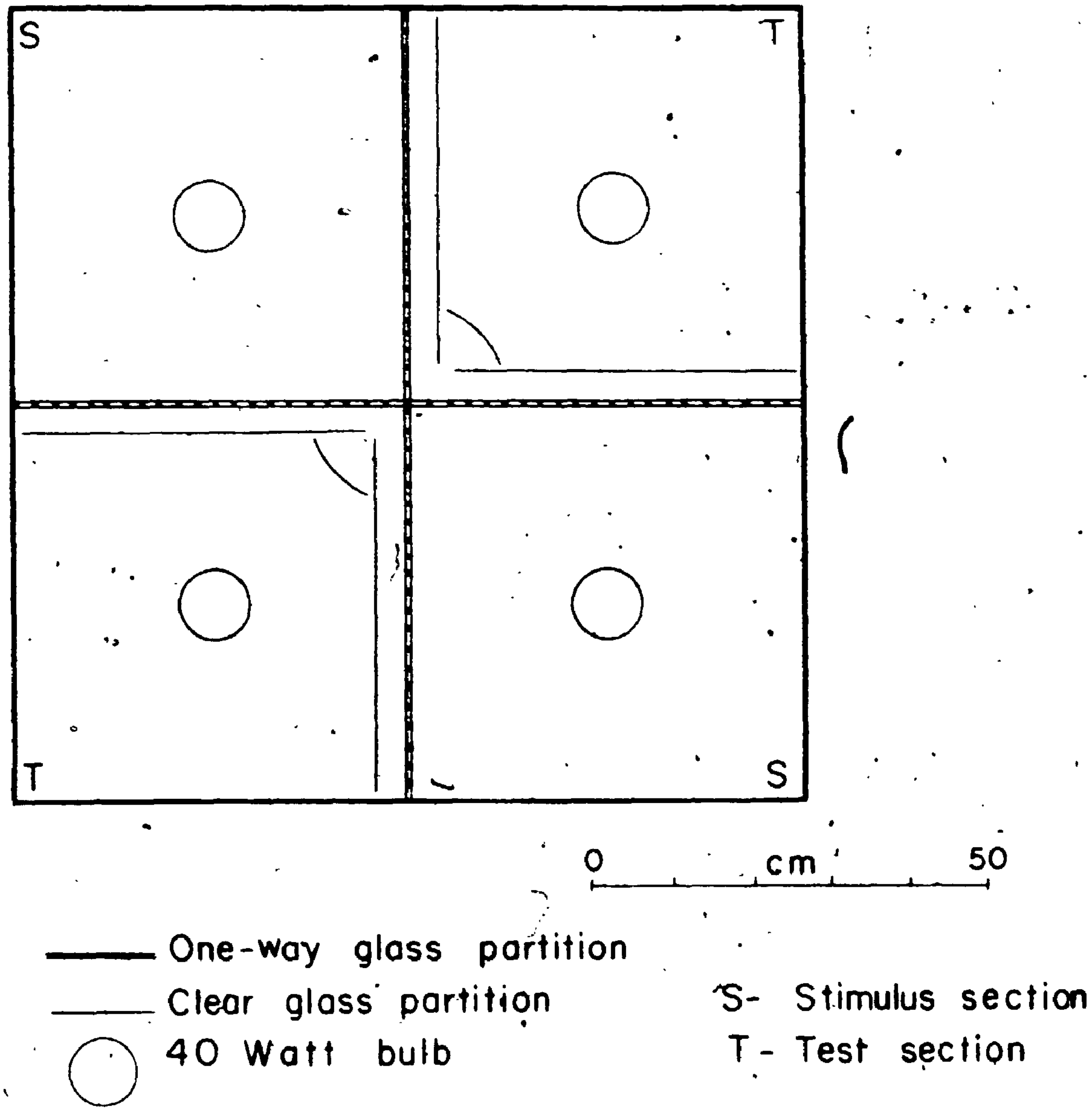
5. Activity Box Experiments

The sunfish used in these experiments were collected in the study area during the spring and summer and held in the greenhouse tanks before use. Fish were chosen to represent the adult sizes of the species used although in some of these experiments fish were matched for size. The apparatus was again designed to allow the fish under investigation (the test fish) to exercise a choice between two dissimilar fish of the opposite sex (the stimulus fish).

5.1 Apparatus and Observations

Five grey-painted plywood boxes (90 x 90 x 45 cm deep) were lined with clear polyethylene sheeting and partially filled with gravel and tap water to a depth of 2 cm and 20 cm respectively. One-way glass partitions divided each box into quadrants. Diagonally opposite quadrants were designated as either "test" or "stimulus" quadrants and contained either test or stimulus fish respectively (Fig. 2). Clear glass partitions

Figure 2. Top view of activity box apparatus. (Connection of clear glass partition to event recorder not shown.)


S
T
T
S
0 cm 50
One-way glass partition
Clear glass partition
40 Watt bulb
S- Stimulus section
T - Test section

were suspended in the test quadrants parallel to and about 1 cm from the oneway glass partitions. These clear glass partitions swung freely when contacted. Attached to each clear partition was a copper wire which passed through a hole in a copper strip. Both the wire and strip were wired to an Esterline Angus 20 channel event recorder so that when the clear glass partition was moved by the test fish contacting it, a connection occurred between wire and strip causing the recording pen to fire. Thus the Esterline Angus recorder indicated test fish activity relative to the clear glass partitions.

Suspended centrally 10 cm above the water level of each quadrant was a 40 watt light bulb. Two lighting conditions were used; control (C) with the lights on over the test fish and off over the stimulus fish, allowing the test fish to see only its own mirror image in the one-way glass partitions, and experimental (E) with the lights on over the stimulus fish and off over the test fish, allowing the test fish to see through the one-way glass partition into the stimulus quadrants.

Test fish activity was monitored over a 24 hr. control period followed by a 24 hr. experimental period. The control period started at 1400 hrs and consisted of 8 hrs. of C light, 8 hrs. of total darkness and 8 hrs. of C light. The lighting was then switched to the E phase and the experimental period commenced. It consisted of 8 hrs. of E light, 8 hrs. of total darkness and 8 hrs. of E light, following which the experiment was terminated. After each experiment, fork lengths of all fish used were recorded (Appendix I). In addition, nesting of male fish was recorded each time a male swept a depression in the gravel of sufficient depth to expose the clear plastic lining the bottom of the activity box. The gravel bottom of each quadrant was leveled between experiments if necessary. Fish tested in these experiments were used once, fin-clipped and released in the study area. Naive test fish were used in every experiment and were introduced into the boxes the day before the control period began to gain familiarity with the apparatus. During this "settling down" period the light regime was identical to that of the control period.

The event recorder was set to run at a speed of 3 inches per hr. for the 48 hour period of the experiment. Each inch of chart was subdivided into 10 units so that in one hour 30 chart units passed the recording pens. An "activity unit" was defined as one or more pen firings in a chart unit. Consequently a maximum of 30 activity units could be recorded per fish per hour. An indication of test fish activity relative to the clear glass partitions was obtained by totalling the number of activity units recorded for each of the 48 hours of the experiment.

Some fish activity records were discarded for one of the following reasons:

a) Very little activity was recorded (less than five activity units in the entire control or experimental period). This may have occurred either because the test fish was not active or because the glass partition-copper wire-copper strip arrangement was not sensitive enough to record the fish's activity. This occurred most frequently when small fish (female longears and small female pumpkinseeds) were being tested. Occasionally it also occurred while larger fish were being tested and may have resulted from the test fish shifting the gravel so that it prevented the clear glass partition from moving freely. Subsequent contacting of the clear partition by the test fish no longer caused the recorder pen to fire. b) Occasionally the copper wire-copper strip arrangement became supersensitive and fired either spontaneously or in response to slight rippling of the water surface caused by the aristone present in each quadrant in the corner furthest from the glass partitions. Records of this situation were recognized by there being as much or more activity recorded during the 8 hr. period of darkness as there was during the light periods preceding or following the dark periods. The same type of chart record also occurred if a test fish squeezed between the clear glass and one-way glass partitions, when slight fin movements were enough to move the clear partition and to fire the recording pen. This situation was discouraged by having the clear partitions close to both the sides of the box and the gravel; nevertheless test fish were occasionally seen behind the partitions. c) A recording pen ran out of ink. d) A light bulb burnt out during the experiment. e) A fish died during the experiment. The most common reason for discarding activity records was a) above.

Retained records were analysed by calculating the differences in hourly activity units directed toward each stimulus fish by each test fish and subjecting those differences to a t-test. This test took the form:

$$t = \frac{\bar{d}_{ij}}{\sqrt{\left(\frac{\dfrac{\sum\limits_{i=1}^{8} \sum\limits_{j=1}^{n} d_{ij} - \dfrac{\sum\limits_{j}^{n} \left(\sum\limits_{i}^{8} d_{ij} \right)^2}{8}}{7n}}{8n} \right)}}$$

where $\bar{d}_{ij}$ = average difference in response for the ith hour, jth fish

n = number of fish tested.

Use of this test is subject to certain limitations described in the Results (Section 3.2.1).

5.2 Testing Procedures

Fish tested in the activity box experiments were subjected to one of two methods of testing:

Method 1 used both naive stimulus fish and naive test fish for each experiment. In addition test fish of two species were usually tested in each activity box at the same time. The positions of both stimulus and test fish were systematically alternated between experiments. Neither test nor stimulus fish were fed while in the boxes.

Method 2 used naive test fish for each experiment, but the same stimulus fish during a number of experiments, although they were alternated between stimulus quadrants between experiments. In addition only test fish of one species were tested per experiment. While test fish were again not fed during the experiments, the stimulus fish which were sometimes in the boxes for as much as a month, were fed a diet of live mosquito larvae at least thrice-weekly. Unless otherwise specified all experiments used method 2 and were conducted in the summer months.

5.3 Female Longears as Test Fish

5.3.1 Responses to Different-sized Longear and Pumpkinseed Males

Ten, ten and nine female longears were tested with male longears and pumpkinseeds as stimulus fish using method 1, 2 and 1 in summer, summer and winter respectively. See Appendix I-1, 2 and 3 for fork lengths of fish used in these experiments.

5.3.2 Responses to Equal-sized Longear and Pumpkinseed Males

Sixteen female longears were tested with male longears and pumpkinseeds matched for length so that the difference in stimulus males never exceeded 0.4 cm (Appendix I-4).

5.3.3 Responses to Different-sized Longear and Hybrid Males

Twelve female longears were presented with a choice between male longears and larger male hybrids (Appendix I-5).

5.3.4 Responses to Equal-sized Longear and Hybrid Males

Fifteen female longears were offered a choice between male longears and hybrids matched for length so that the difference in stimulus males never exceeded 0.4 cm (Appendix I-6).

5.3.5 Responses to Different-sized Pumpkinseed Males

Twelve female longears were tested with pairs of large and small pumpkinseed males (Appendix I-7). Both sizes of males had similar body colouring and markings.

5.4 Female Pumpkinseeds as Test Fish

5.4.1 Responses to Different-sized Longear and Pumpkinseed Males

Twelve, fourteen and seven female pumpkinseeds were tested with male longears and pumpkinseeds as stimulus fish using methods 1, 2 and 1 in summer, summer and winter respectively. See Appendix I-8, 9 and 10 for lengths of fish used in these experiments.

5.4.2 Responses to Different-sized Pumpkinseed Males

For pumpkinseeds, the categories small, medium and large were arbitrarily defined as: small -<11.0 cm; medium - 11.0 to 13.0 cm; large ->13.0 cm.

Ten small, eighteen medium and twelve large female pumpkinseeds were offered a choice of large and small male pumpkinseeds. See Appendix I-11, 12 and 13 for lengths of fish used in these experiments.

5.4.3 Responses to Different-sized Longear and Hybrid Males

Sixteen female pumpkinseeds were tested with longear and large hybrid males as stimulus fish (Appendix I-14.)

5.5 Male Longears as Test Fish

Twenty male longears were tested with females of the two species in the summer using method 1. (Appendix I-15).

5.6 Male Pumpkinseeds as Test Fish

Twenty male pumpkinseeds were tested with females of the two species in the summer using method 1. (Appendix I-16).

6. Stripping Experiments

6.1 Egg Ripeness and Order of Extrusion

To determine if differences in the quality of egg ripeness varied with the order in which eggs were extruded from the female, eggs from six of each species of female were stripped into 6 to 12 round plexiglass containers (described in 6.3) and immediately fertilized with conspecific milt by either fertilizing several dishes with several males (female pumpkinseeds Series A) or by fertilizing all eggs with the same male (female pumpkinseeds Series B, female longears).

Females used for these experiments were seined from spawning longear colonies. The criterion for using these females was that the eggs flowed freely with slight abdominal pressure. Females were chosen which seemed to be fully ripe at the time of capture although the last four female longears' abdomens were not as enlarged as those of the first two as they had probably been spawning before being captured.

6.2 Egg Size

Diameters of 20 eggs from each of five pumpkinseed females and eight longear females were measured within an hour of fertilization using an ocular micrometer to confirm the visual impression that longear eggs were larger than pumpkinseed eggs. Both species of eggs were approximately spherical and only one measurement of diameter was usually taken. When an egg appeared distorted, the average of its maximum and minimum diameter was recorded.

6.3 Crossing Experiments

To assess the possible success of all the crosses which might occur in the study area, eggs from longear and pumpkinseed females were fertilized with milt from longear, pumpkinseed and hybrid males in the laboratory. Fish used in these experiments were seined from the study area, transported to the laboratory and stripped immediately. Eggs were fertilized in round plexiglass containers (7.5 cm in diameter) with fine nylon mesh bottoms resting in finger-bowls containing 100 ml of water. Five minutes after fertilization these containers were transferred to an aerated aquarium (62 x 32 x 31 cm deep). Each container had a styrofoam collar so the containers floated in the aquarium with 2 cm of water covering the eggs. Both the water in which the eggs were fertilized and the aquarium water in which they were subsequently placed was river water from the study area. Water temperature in the aquarium was 21 ± 3°C throughout the experiments which approximates spawning temperatures in the study area. Dead eggs were counted and removed at least daily. After hatching (2-3 days after fertilization), fry were counted and discarded.

Eggs and milt were stripped into the plexiglass containers using one of two methods:

Method A

Eggs from one species of female were stripped into three containers and eggs from the other species female were stripped into three additional containers. Then each of the three males (longear, pumpkinseed and hybrid) had at least 2 drops of milt squeezed into one pair of containers each of which contained one of the species eggs. The order of stripping therefore was: one species female followed by the second species female followed in turn by each of the 3 types of males. No more than 2 minutes elapsed between stripping of the first eggs and fertilization of the last eggs stripped.

Care was taken to rinse the hands well between male strippings so sperm of one male did not contaminate the eggs fertilized by another male. The order in which females were stripped was varied so that sometimes the female pumpkinseed was first stripped, sometimes the female longear was first stripped. In addition the order of fertilization by the males was varied so that each type of male was stripped first, second or third an approximately equal number of times.

After 10 such replicates, Method A was altered slightly to eliminate the possibility that the unequal times between stripping of eggs and fertilization might affect the hatching success of the eggs in the six containers.

Method B

Again the eggs of both species of female were each stripped into three plexiglass containers. However, the eggs which were stripped were immediately fertilized by the three types of males. The order of stripping for Method B was therefore: one species female fertilized immediately by the three types of males followed by the other species female again fertilized immediately by the same three males. As in Method A, the hands were well rinsed between fertilizations, and the order of male fertilization was varied. Method B was replicated 15 times, 10 in the same summer as the Method A replicates, five in the following summer.

RESULTS

1. Observations of Longear Spawning

Female longears at the height of spawning in a longear colony frequently entered the colony, entered male nests, circled, spawned and left the colony with little or no preliminary courtship circling on the part of the males. The thicker arrows on the left-hand side of Figures 3 and 4 clearly indicate that most of the observed females performed the indicated behaviour acts in the absence of male courtship circling. All 43 of those females which entered the colony and eventually left without spawning, initially entered nests without courtship (Fig. 3). Further, a total of 98 nests (43 + 55) was entered without male courtship, while only 4 nests were entered with male courtship (Fig. 3). In addition, of the 49 females which entered a colony and eventually spawned (Fig. 4), 42 initially entered nests without male courtship, only 7 with male courtship. Further, a total of 93 nests (42 + 51) was entered without courtship, only 12 (7 + 5) with courtship (Fig. 4).

Once circling in the nest by both male and female takes place, there is a good chance that spawning will occur, regardless of whether or not there has been previous courtship circling by the male. Of the total of 102 nests entered by females who eventually left the colony without spawning, only 14 (11 + 3, i.e. 10.8%) were circled in (Fig. 3), while of the total of 105 nests entered by females who eventually spawned, 68 (50 + 6 + 12; i.e. 64.8%) were circled in (Fig. 4). Figure 4 further illustrates that of 50 non-courted females who circled in the nest, 35 (70.0%) spawned, and that of 18 courted females who circled in the nest,

Figure 3. Frequency of behaviour acts of female longears observed entering and leaving five longear colonies without spawning. Left-hand acts occurred in nests of non-courting males, right-hand acts in nests of courting males. Thickness of arrows is proportional to frequency of acts.

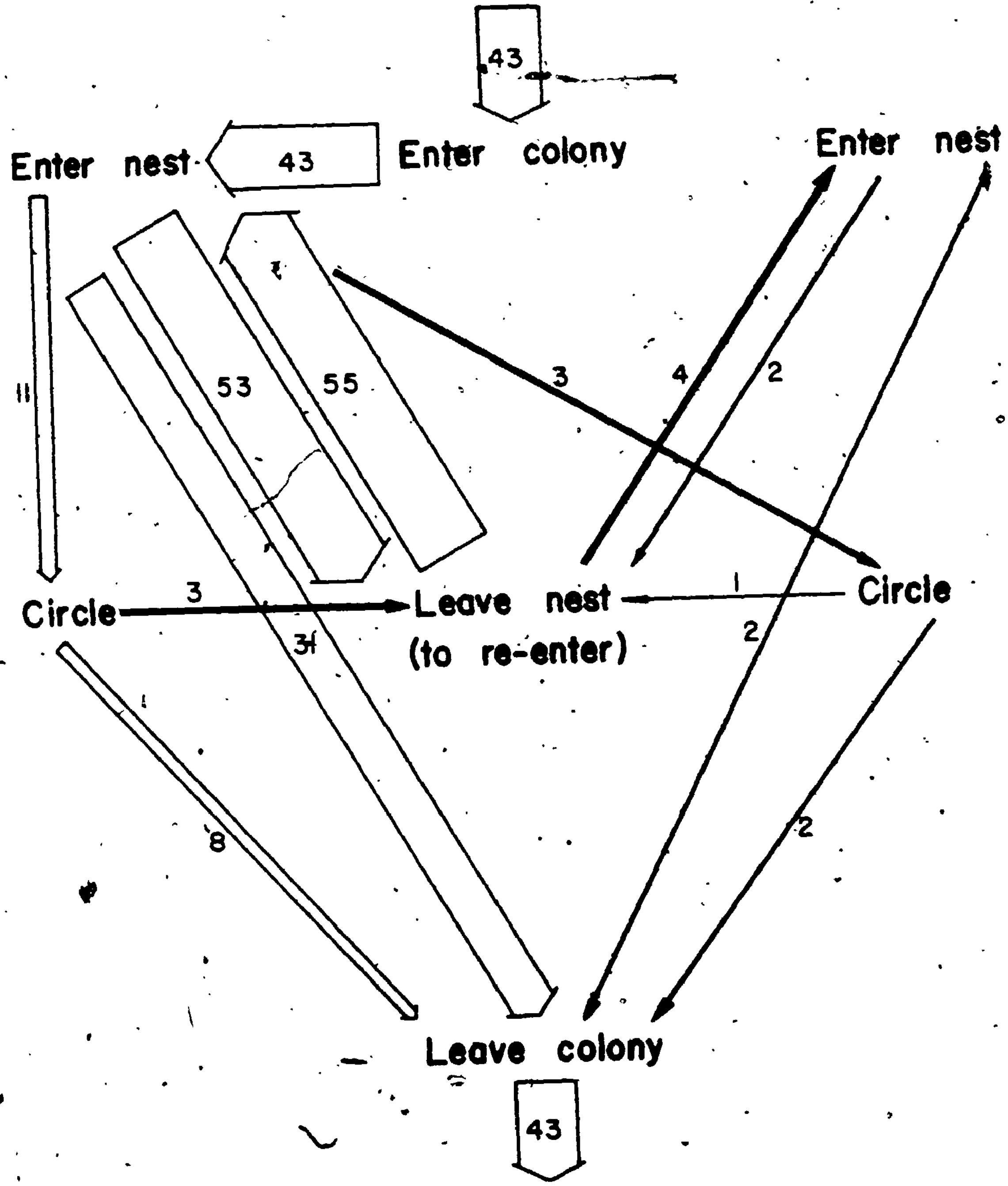
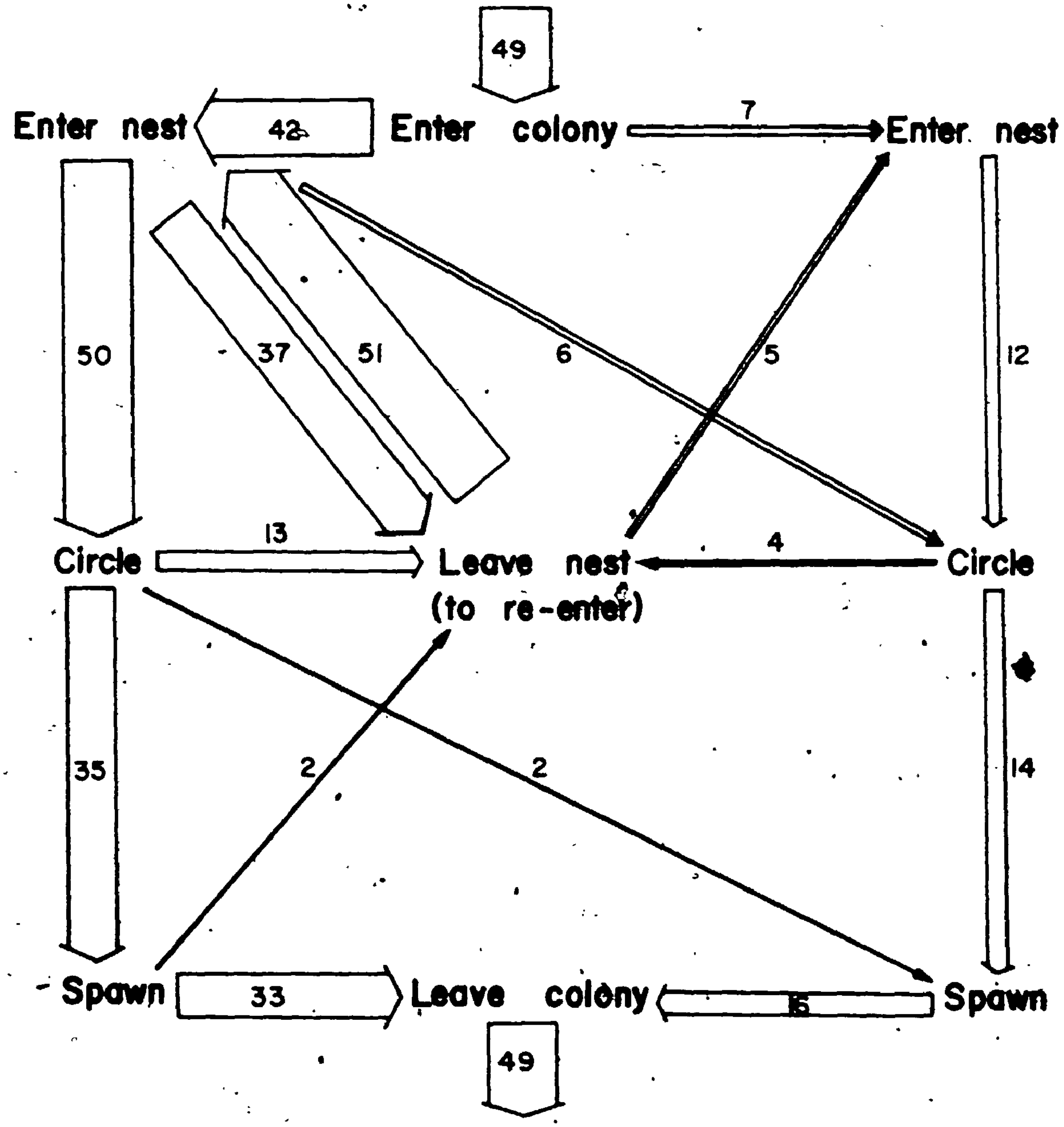

43
Enter nest
43
Enter colony
Enter nest
11
53
55
3
4
2
Circle
3
Leave nest
(to re-enter)
1
Circle
31
2
8
2
Leave colony
43

Figure 4. Frequency of behaviour acts of female longears observed entering five longear colonies and spawning before leaving. Remainder of legend as for Figure 3.


49
Enter nest
42
Enter colony
7
Enter nest
50
37
51
6
5
12
Circle
13
Leave nest
(to re-enter)
4
Circle
35
2
2
14
Spawn
33
Leave colony
15
Spawn
49

about the same proportion spawned (14 of 18 or 77.8%).

The function of male courtship circling may be to induce females to circle in the nest and hence improve the chance of spawning occurring. Of the 7 females who entered the colony and were initially courted, 7 (100%) spawned (Fig. 4) while of the total of 85 females who entered the colony and were not initially courted, only 42 (49.4%) spawned (Figs. 3 and 4).

2. Cement Tank Experiments

Table 1 presents the sums of behaviour acts for males and females of each species under lighting conditions C and E. These values were greater in the E phase than in the C phase of the experiments for every category except N, indicating an increase in test fish activity under the E lighting conditions. In every case PH > NH > FH, and in every case but one (male pumpkinseeds, E phase) PC > NC > FC, indicating that these fish pointed more than they nosed, and nosed more than they fluttered under both C and E conditions.

A comparison of comparable behaviour acts (FC and FH, NC and NH, PC and PH) in the C phase, using an analysis of variance[1] (Appendix V-1) revealed a significant difference only between PC and PH for female pumpkinseeds ($P < 0.025$). This difference indicated that female pumpkinseeds discriminated between the two ends of the test section by pointing significantly more towards the conspecific end, in spite of the fact that under the C conditions they could not see the stimulus males behind the one-way glass partition.

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[1] Populations, acts, and experimental conditions were treated as fixed effects, and fish as random effect for the analysis of comparable behavior acts.

Table 1. Totals of behaviour acts in cement tank experiments. FC, NC, PC, - flutter, nose, point conspecifically; N, neutral, PH, NH, FH,-point, nose, flutter heterospecifically; XO, crossover; C, control; E, experimental.

FEMALE

PUMPKINSEEDS (n=20)

	FC	NC	PC	N	PH	NH	FH	XO
C	3	32	828	2640	464	31	2	76
E	105	156	999	1533	849	202	156	254

LONGEARS (n=20)

	FC	NC	PC	N	PH	NH	FH	XO
C	8	27	381	3050	500	27	7	46
E	85	389	1248	1588	612	61	17	184

MALE

PUMPKINSEEDS (n=20)

	FC	NC	PC	N	PH	NH	FH	XO
C	15	32	271	3167	454	49	12	105
E	153	106	797	1731	951	157	105	248

LONGEARS (n=20)

	FC	NC	PC	N	PH	NH	FH	XO
C	5	18	574	2840	539	15	9	178
E	39	146	872	1772	958	141	72	285

A comparison of comparable behaviour acts in the E phase of the experiments revealed significant differences between PC and Ph, and between NC and NH in female longears ($P < 0.001$). This indicated that of the four kinds of fish tested only female longears clearly distinguished between the two ends of the test sections in the E phase by orienting toward and contacting the conspecific one-way glass partition more than they did the heterospecific partition. Although FC was not significantly larger than FH for female longears in the E phase the trend to conspecific choice persisted. Certainly when all the conspecific acts (FC, NC, and PC) were compared with all the heterospecific acts (PH, NH, and FH) under both conditions C and E, only female longears performed significantly more conspecific than heterospecific acts ($P < 0.001$).

Figure 5 was derived from the data of Table 1 and illustrates the percentage increase in behaviour acts between the C and E phases of the two experiments. Males scored higher XO values than females in the C phase (Table 1), but the percentage increase in XO from phase C to phase E was higher for females than for males (Fig. 5). A comparison of XO values using an analysis of variance[2] (Appendix V-2) revealed no significant differences between females of the two species, or between males of the two species, or between females and males, when differences between the E and C conditions were considered. When mean XO values were

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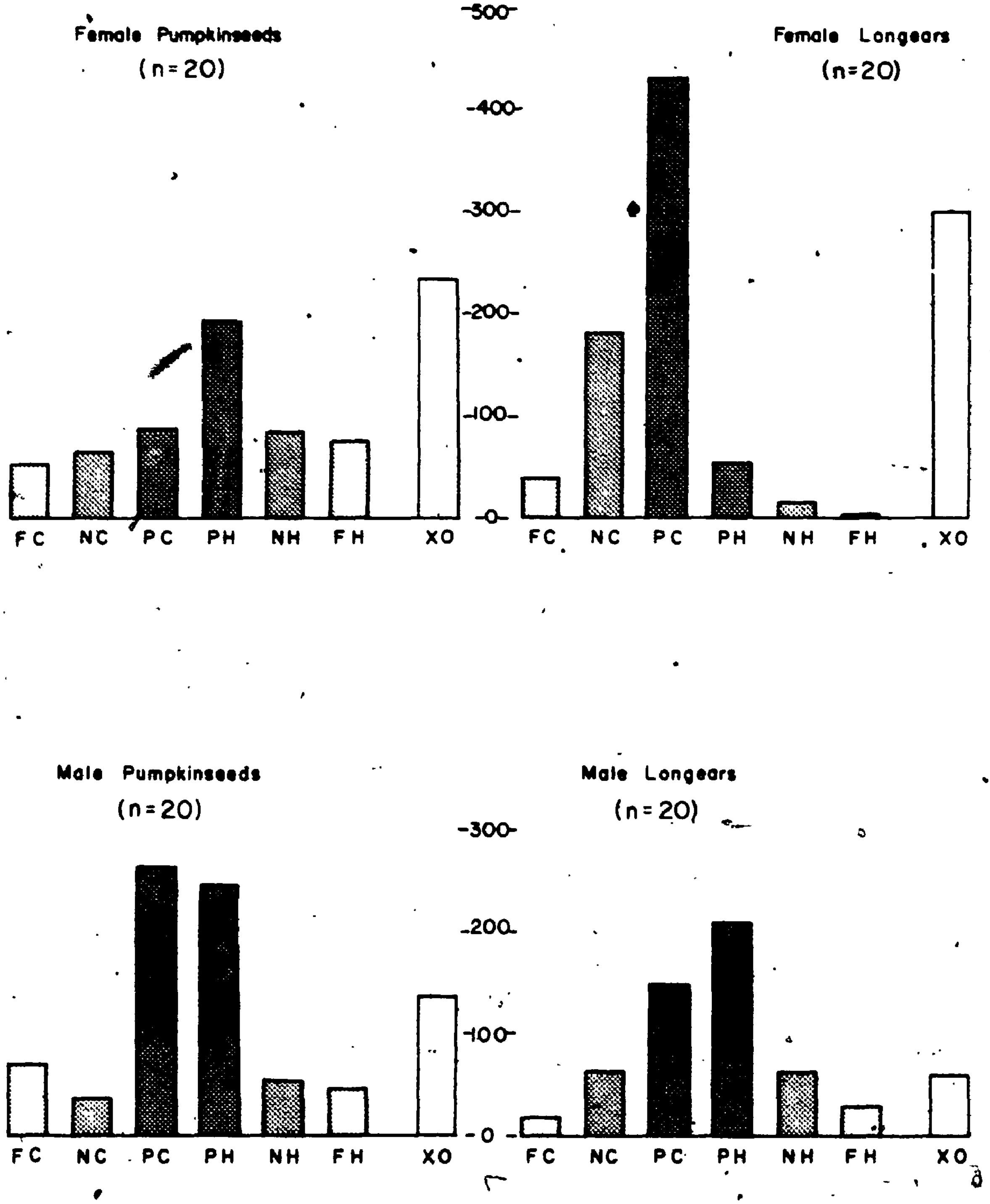
[2] Populations and experimental conditions were treated as fixed effects, and fish as random effects for analysis of the XO data which was transformed to the square root of the observed value plus 0.5.

Figure 5. Percentage increases (vertical scale) in behaviour acts between control and experimental conditions. Rest of legend as Table 1.


Female Pumpkinseeds
(n=20)
Female Longears
(n=20)
-500-
-400-
-300-
-200-
-100-
-0-
FC
NC
PC
PH
NH
FH
XO
FC
NC
PC
PH
NH
FH
XO
Male Pumpkinseeds
(n=20)
Male Longears
(n=20)
-300-
-200-
-100-
-0-
FC
NC
PC
PH
NH
FH
XO
FC
NC
PC
PH
NH
FH
XO

considered, no significant differences were found between females of the two species, or between males of the two species, but the mean XO value for females was significantly lower ($P < 0.025$) than it was for males. This difference resulted from females having lower XO values than males during the C phase, and from male longears having higher XO values than female longears in the E phase (Table 1).

Figure 5 also shows that female longears exhibited both the largest percentage increase in a single behaviour act (PC), and the greatest differences between comparable behaviour acts (PC and PH, NC and NH), when they were able to perceive fish of the opposite sex under lighting condition E.

3. Activity Box Experiments

3.1 General Activity Levels

Test fish activity declined sharply when the lights went out and increased sharply when the lights came on in most of the experiments conducted. T-tests comparing means of total activity directed toward each stimulus fish in 8 hr blocks revealed that activity in each of the lights-on periods was significantly greater than in the lights-off period for both C and E phases, with the following exceptions: C-ON 2 vs C-OFF [1](Figs. 7, 11, 20) and E-ON 2 vs E-OFF (Figs. 7, 12). In short, test fish activity was greater when lights were on than when they were off in both the C and E phases of most experiments.

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[1] C-ON 1 - first lights-on period, control;
C-OFF - lights-off period, control;
C-ON 2 - second lights-on period, control;
E-ON 1 - first lights-on period, experimental;
E-OFF - lights-off period, experimental;
E-ON 2 - second lights-on period, experimental.

3.2 Female Longears as Test Fish

3.2.1 Responses to Different-sized Longear and Pumpkinseed Males

Figures 6 and 7 illustrate the responses of 10 female longears tested in the breeding season using Method 1 (Fig. 6) and Method 2 (Fig. 7).

An examination of figure 6 shows that the t-test described in the Methods (Section 5.1) revealed significant differences in each of the six 8 hr periods. This test, which is designed to give more weight to strongly responding fish than to weakly responding fish, makes several simplifying assumptions: (a) normality of error variance; (b) homogeneity of variance between fish, and (c) independence of hourly differences.

The t-test used has the virtue of removing the variation in response among the test fish from the total variation and considering the remainder as variation due to differences in response to the 2 stimulus fish. As a result of the removal of among-fish variation, the t-test identifies significant differences when the average difference in response of all the test fish to the two stimulus fish is significantly different from zero in the direction indicated. For example, the following table illustrates the direction chosen by the test fish in each of the six 8 hr periods of figure 6 as indicated by their difference score totals:

	C - ON 1	C - OFF	C - ON 2	E - ON 1	E - OFF	E - ON 2
ML	161 (2)	358 (6)	181 (4)	558 (6)	137 (6)	297 (6)
MP	425 (8)	119 (4)	519 (6)	233 (4)	34 (4)	135 (4)

Note: - Bracketed numbers indicate the number of female longears whose total response differences in activity units suggest a preference for the male shown. ML, male longear; ML male pumpkinseed.

The graphic presentation (Fig. 6) and the t-test indicate that the "average" female longear chose male pumpkinseeds in C-ON 1 and C-ON 2 and chose male longears in the other periods. The t-test, however, does not show that a significant number of test fish chose the same male stimulus fish (6 of 10 test fish choosing one type of stimulus fish could not be interpreted as significant). Figures 6-23 should be viewed with this constraint and the above assumptions in mind.

Both figures 6 and 7 show a switch from a choice of male pumpkinseed in C-ON 2 to male longears in E-ON 1. Figure 6 also shows a lights-on choice for male pumpkinseeds throughout the C phase and for male longears throughout the E phase. As method 1 and 2 produced similar results, the data of figures 6 and 7 were pooled to produce a composite picture of the response of longear females to longear and pumpkinseed males during the breeding season (Fig. 8). These results suggest that male pumpkinseeds may have emitted a non-visual stimulus to which the female longears responded during the C phase but that when the females could see both the males in the E phase, the females responded more to conspecific males than to heterospecific males.

Figure 9 illustrates the response of 9 female longears tested in the non-breeding season using Method 1. Again these females consistently directed more activity toward male pumpkinseeds in C-ON 1 and C-OFF, then directed more activity toward male longears in E-ON 1 and E-ON 2. The general similarity of these results to those shown in figure 8 suggests that female longears responded to conspecific and heterospecific males in a similar manner in both the breeding and non-breeding season. Further, the switch in direction of activity from male pumpkinseeds during the C phase to male longears during the E phase (Figs. 8 and 9) is similar to the results [illegible]tained in the cement tank expe[illegible]ts (Table 1).

Figure 6. Mean response of 10 longear females to different-sized longear and pumpkinseed males. Summer, Method 1. For this and figures 7-23, lights-off phase indicated by dark bars at hours 8-16 and 32-40; lights-on phase at all other times.

Asterisks below the x - axis indicate significance levels for the data of each 8 hr block treated with a t-test for paired differences.

*, $0.05 > P > 0.01$; **, $0.01 > P > 0.001$; ***, $P < 0.001$

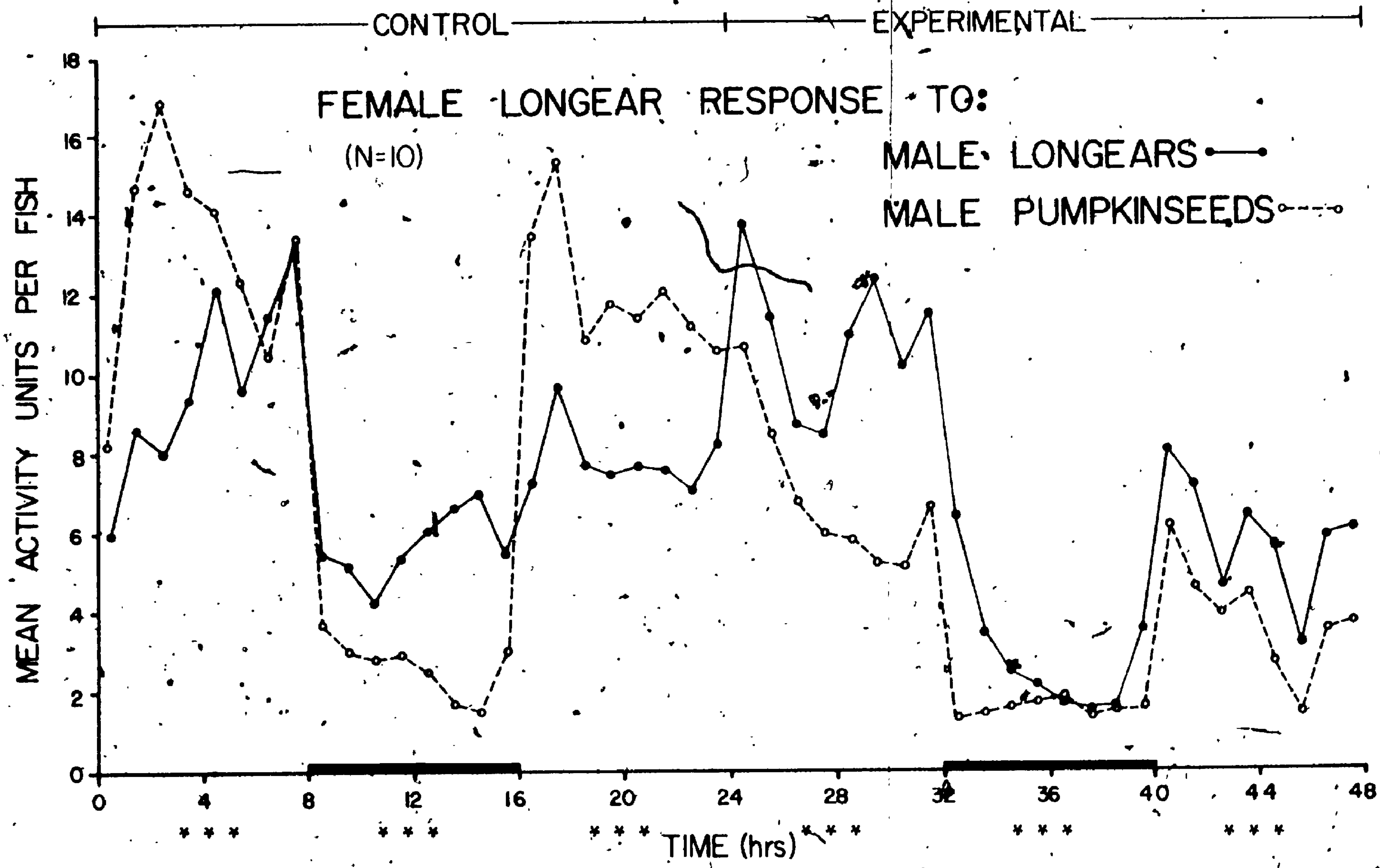

CONTROL
EXPERIMENTAL
FEMALE LONGEAR RESPONSE TO:
(N=10)
MALE LONGEARS
MALE PUMPKINSEEDS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
16
18
0
4
8
12
16
20
24
28
32
36
40
44
48
* * *
* * *
* * *
* * *
* * *
* * *
TIME (hrs)

Figure 7. Mean responses of 10 longear females to different-sized longear and pumpkinseed males. Summer, Method 2.

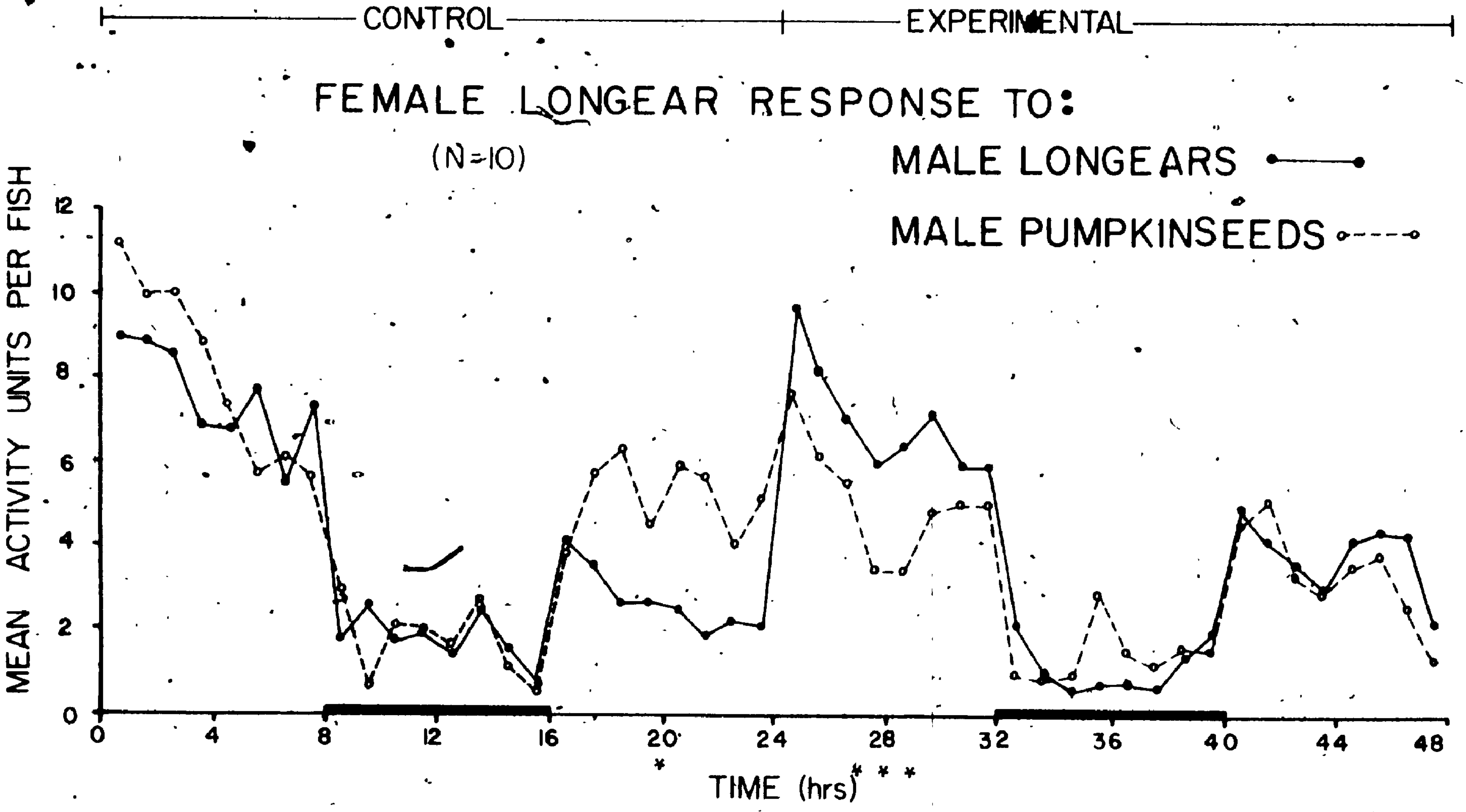

CONTROL
EXPERIMENTAL
FEMALE LONGEAR RESPONSE TO:
(N=10)
MALE LONGEARS
MALE PUMPKINSEEDS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
0
4
8
12
16
20
24
28
32
36
40
44
48
*
* * *
TIME (hrs)

Figure 8. Mean response of 20 longear females to different-sized longear and pumpkinseed males. Summer, Methods 1 and 2 combined.

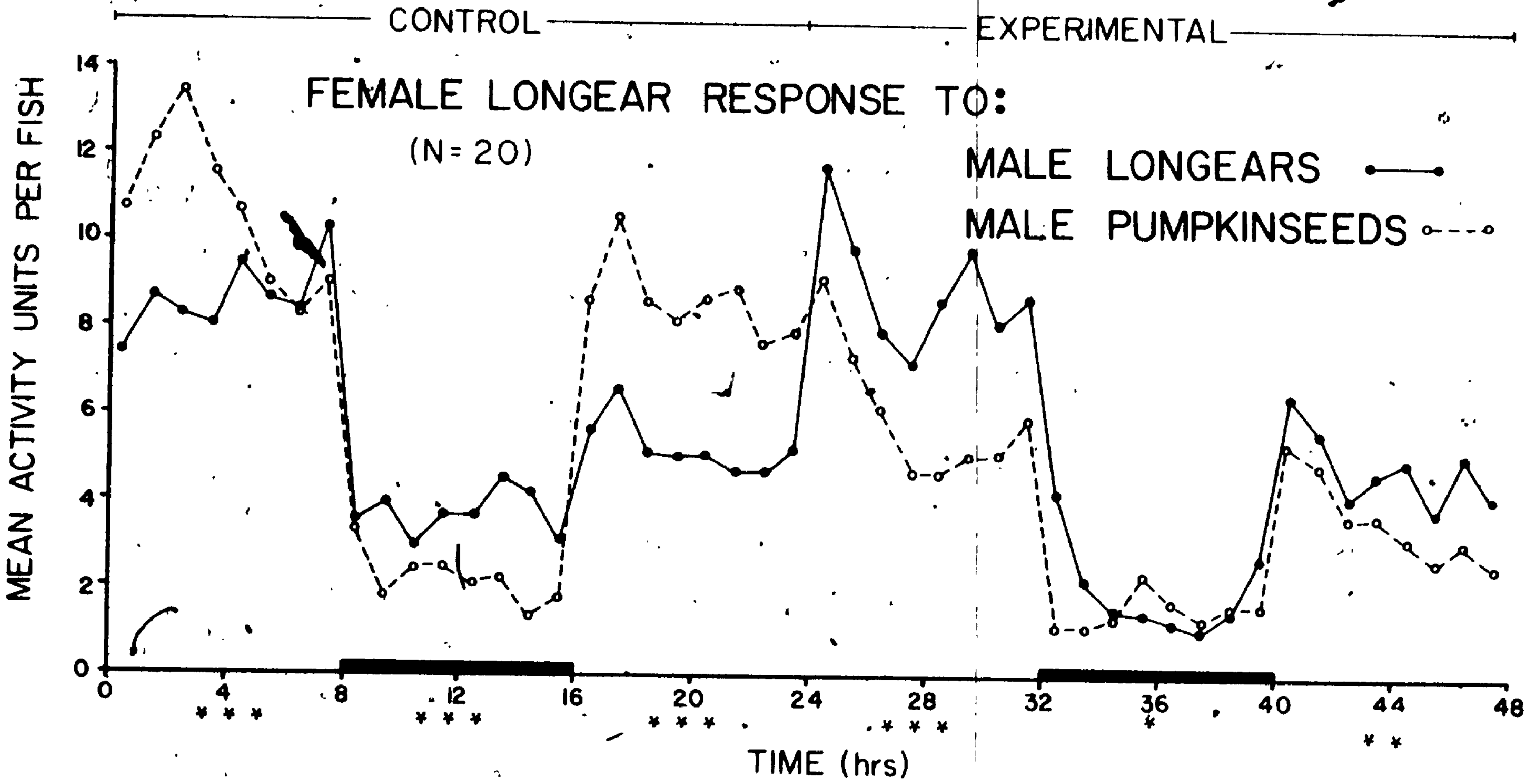
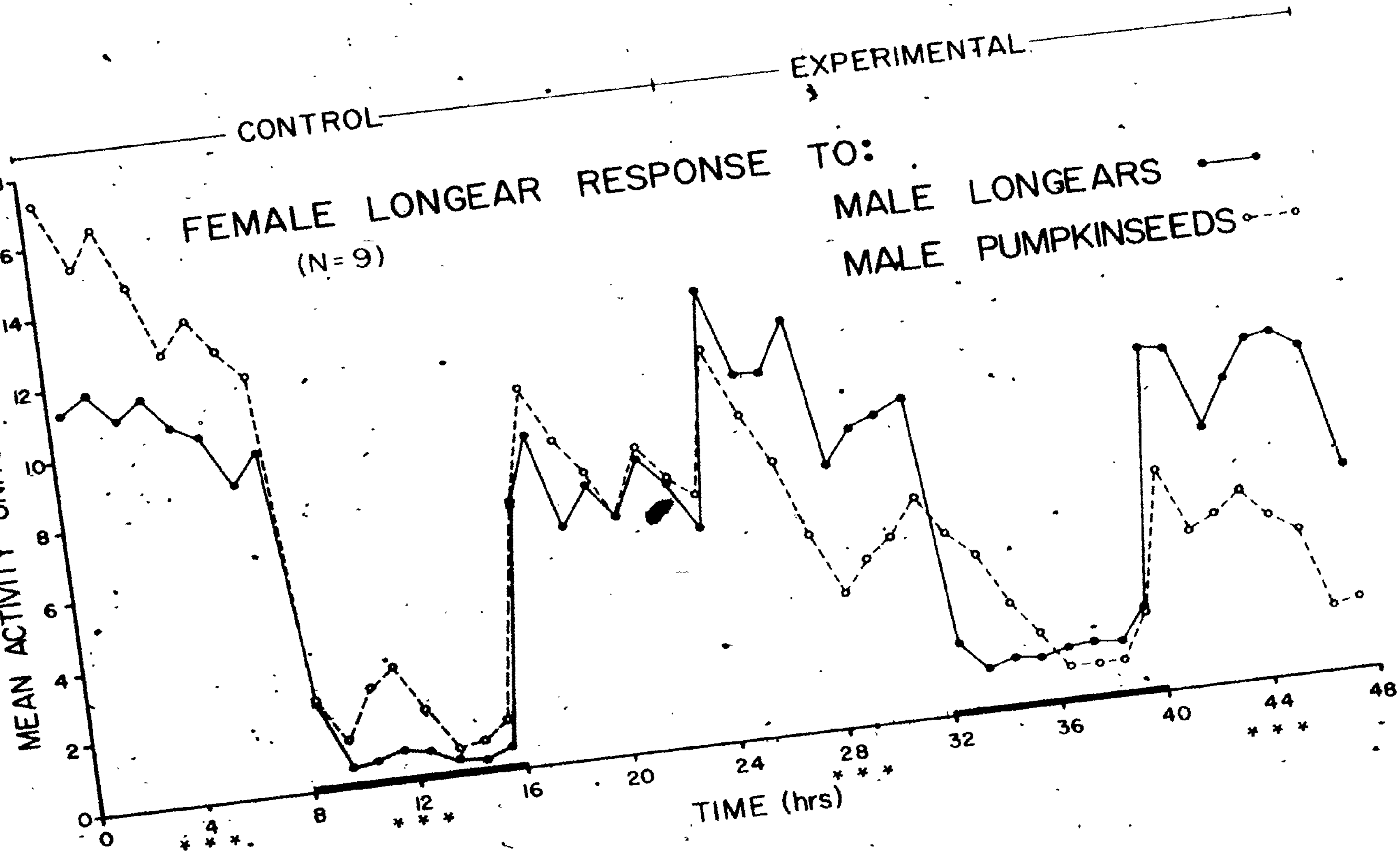

CONTROL
EXPERIMENTAL
FEMALE LONGEAR RESPONSE TO:
(N= 20)
MALE LONGEARS
MALE PUMPKINSEEDS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
0
4
8
12
16
20
24
28
32
36
40
44
48
* * *
* * *
* * *
* * *
*
* *
TIME (hrs)

Figure 9. Mean response of nine longear females to different-sized longear and pumpkinseed males. Winter, Method 1.


CONTROL
EXPERIMENTAL
FEMALE LONGEAR RESPONSE TO:
(N=9)
MALE LONGEARS ●—●
MALE PUMPKINSEEDS ○- - -○
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
16
18
TIME (hrs)
0
4
8
12
16
20
24
28
32
36
40
44
48
* * *
* * *
* * *
* * *

3.2.2. Responses to Equal-sized Longear and Pumpkinseed Males

When 16 female longears were tested with longear and pumpkinseed males of similar lengths they again showed a non-visual preference for male pumpkinseeds in the C phase (although not significant in C-ON 1), then maintained this preference throughout the E phase (Fig. 10). These results suggest that female longears given a choice between males of similar size may not visually choose between them as the E phase choice may be a continuation of the non-visual C phase choice. Further, they suggest that the criterion for visual choice of conspecific males by longear females (Figs. 8 and 9) may have been based on size.

3.2.3 Responses to Different-sized Longear and Hybrid Males

When 12 female longears were tested with longear and hybrid males as stimulus fish, neither male was chosen in the C phase, but more activity was directed toward conspecifics throughout the E phase (Fig. 11). Longear males were shorter than hybrid males (Appendix I-5) again suggesting that the visual choice of conspecifics might have been based on their shorter length, as some of the other criteria which might have been used for distinguishing previously between male longears and male pumpkinseeds (size of operculum, colour of dorsal and anal fins) were either more pronounced or equally as pronounced on the hybrid males as on the longear males.

3.2.4 Responses to Equal-sized Longear and Hybrid Males

When 15 female longears were tested with longear and hybrid males of similar lengths (Appendix I-6) hybrids were chosen in C-OFF, C-ON 2, E-ON 1 and E-ON 2 (Fig. 12). Again the E phase choice may be a continuation of the C phase choice suggesting a reduction of choice as length differences

Figure 10. Mean response of 16 longear females to equal-sized longear and pumpkinseed males.

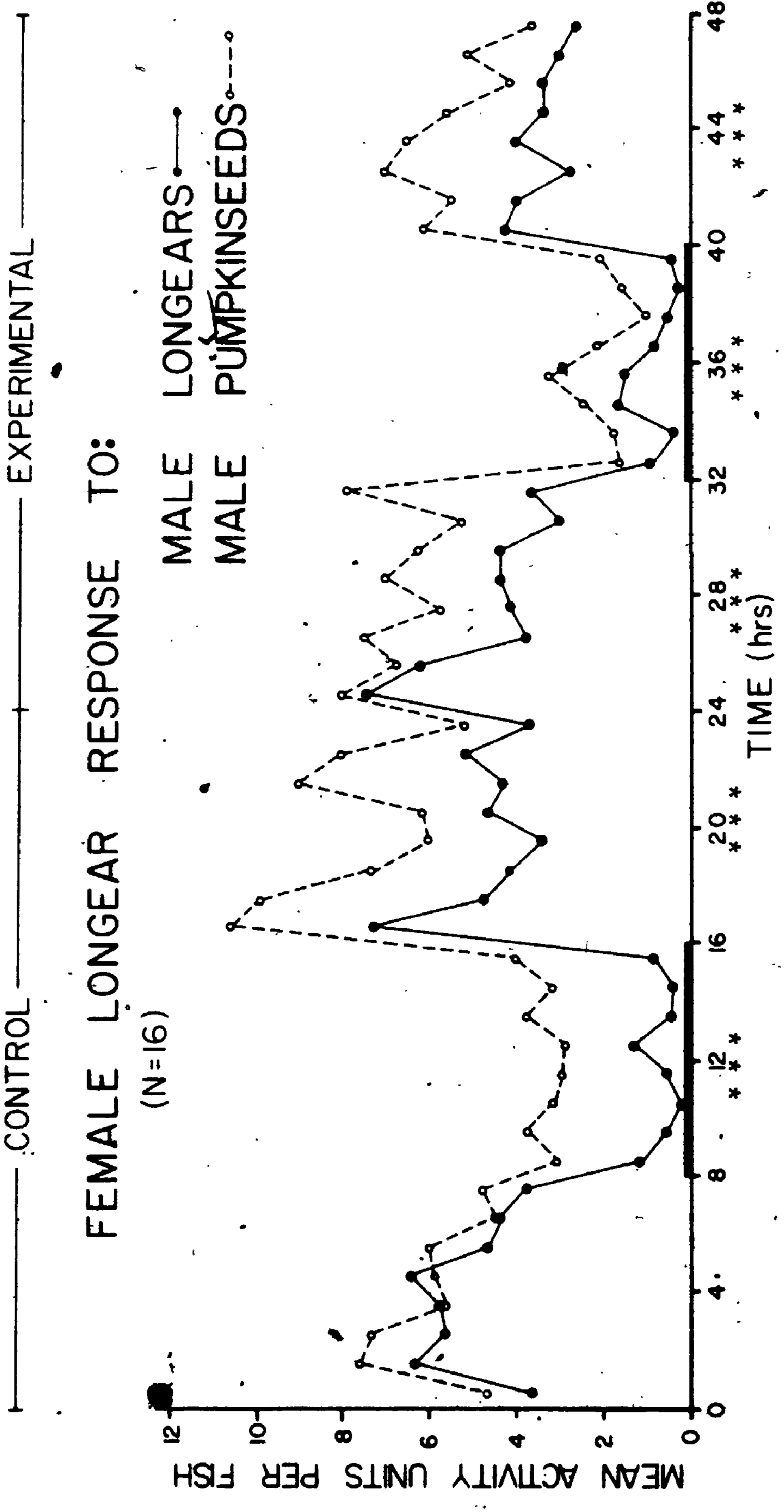

CONTROL
EXPERIMENTAL
FEMALE LONGEAR RESPONSE TO:
(N=16)
MALE LONGEARS
MALE PUMPKINSEEDS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
TIME (hrs)
0
4
8
12
16
20
24
28
32
36
40
44
48
***
***
***
***
***

Figure 11. Mean response of 12 longear females to different-sized longear and hybrid males.

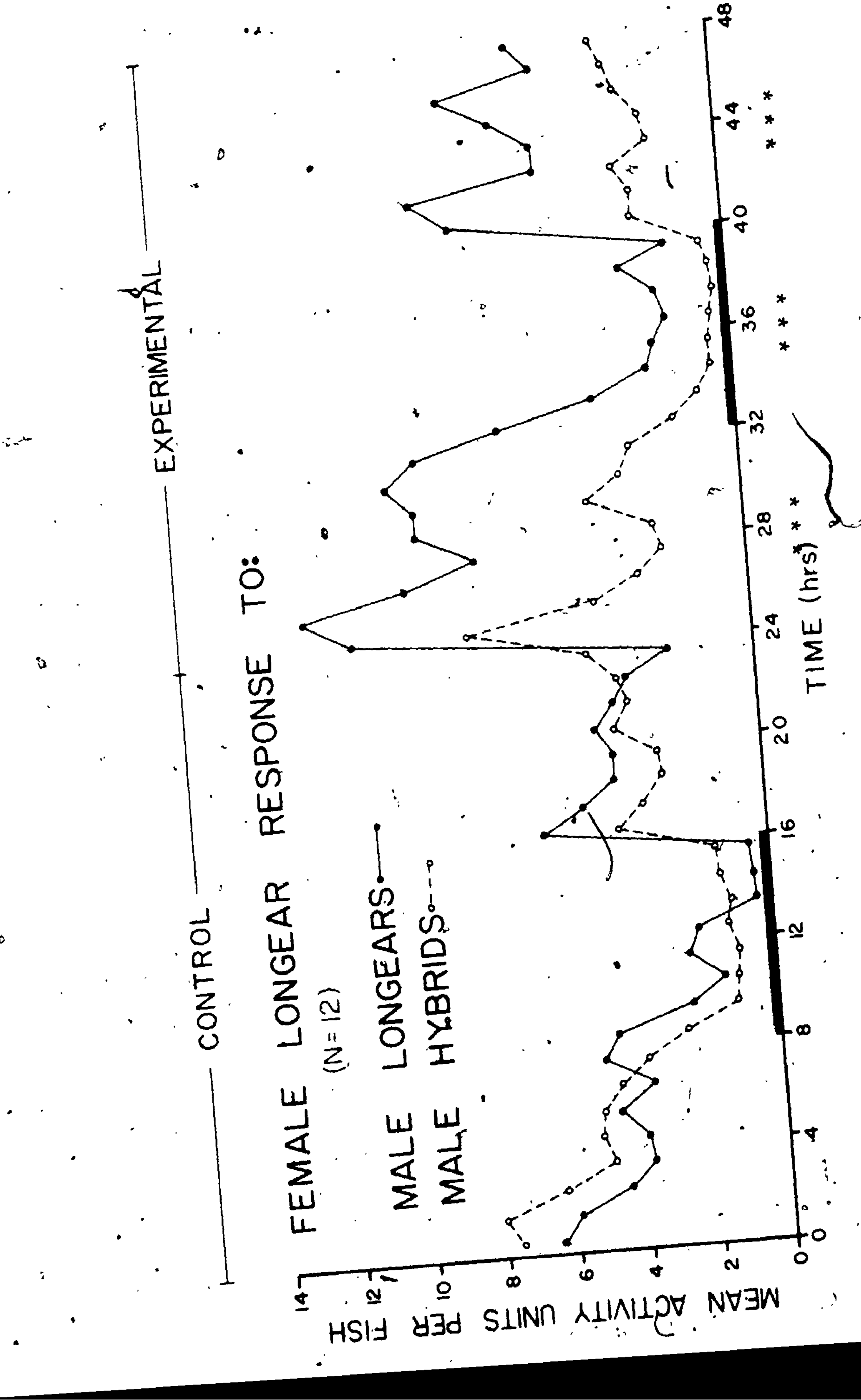
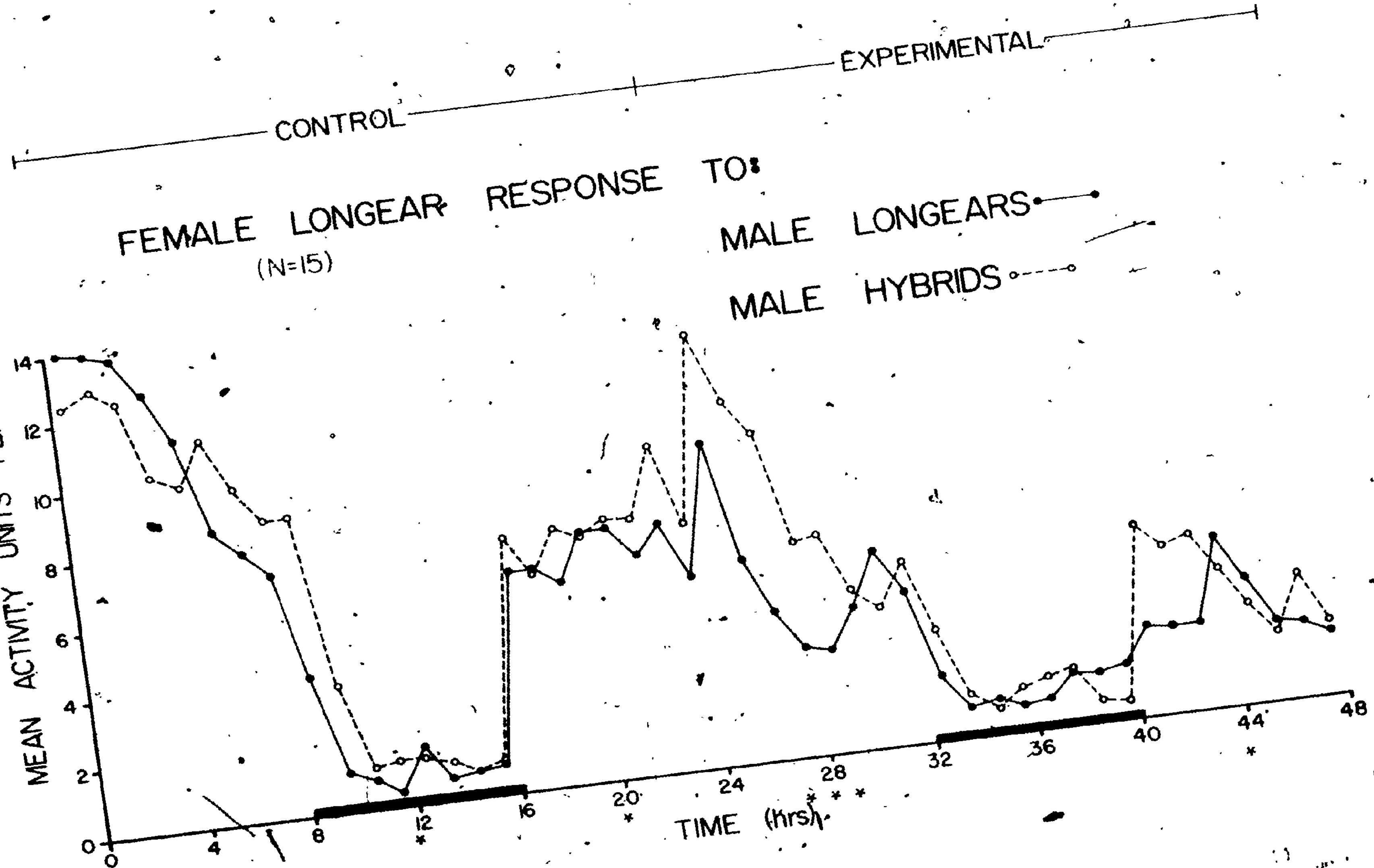
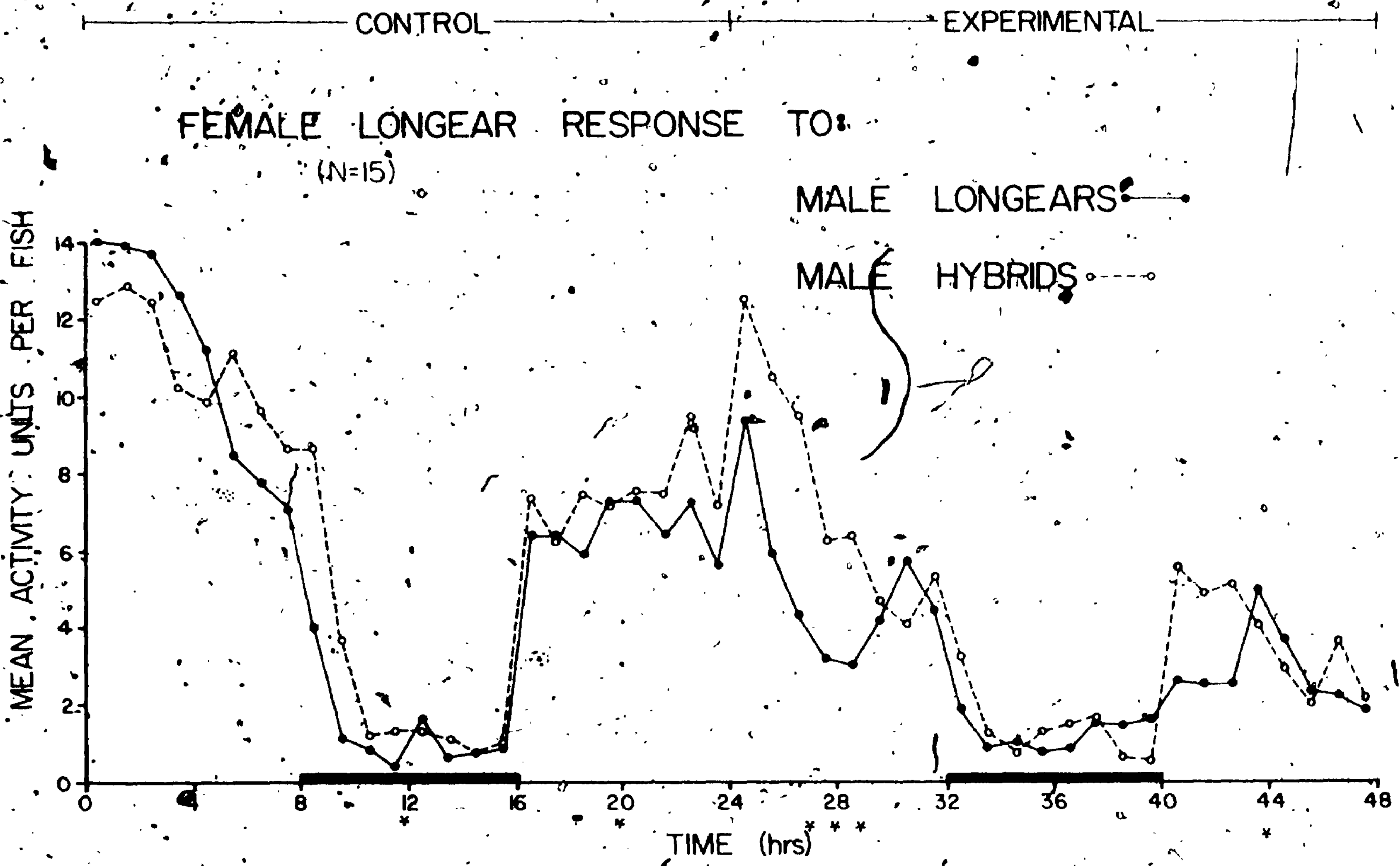

CONTROL
EXPERIMENTAL
FEMALE LONGEAR RESPONSE TO:
(N=12)
MALE LONGEARS
MALE HYBRIDS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
TIME (hrs)
0
4
8
12
16
20
24
28
32
36
40
44
48
* * *
* * *
* * *

Figure 12. Mean response of 15 longear females to equal-sized longear and hybrid males.


CONTROL
EXPERIMENTAL
FEMALE LONGEAR RESPONSE TO:
(N=15)
MALE LONGEARS
MALE HYBRIDS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
TIME (hrs)
0
4
8
12
16
20
24
28
32
36
40
44
48
*
*
* * *
*


CONTROL
EXPERIMENTAL
FEMALE LONGEAR RESPONSE TO:
(N=15)
MALE LONGEARS
MALE HYBRIDS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
0
4
8
12
16
20
24
28
32
36
40
44
48
TIME (hrs)
*
*
*
* * *
*

between the males decreased (Figs. 11 and 12).

3.2.5 Responses to Different-sized Pumpkinseed Males

When 12 female longears were tested with small and large male pumpkinseeds (Appendix I-7), the females directed more activity toward large males in C-ON 1 and C-ON 2 but showed no preference for either size of male in the E phase until E-ON 2, when small males were chosen (Fig. 13). The non-persistence of choice for large males in the E phase coupled with the preference for small males exhibited in the E phase again suggests that female longears, given a choice of different-sized males, tended to visually choose males whose size resembled that of conspecific males.

3.3 Female Pumpkinseeds as Test Fish

3.3.1 Responses to Different-sized Longear and Pumpkinseed Males

Female pumpkinseeds tested with longear and pumpkinseed males in the breeding season showed similar responses whether Method 1 (Fig. 14) or Method 2 (Fig. 15) was used. Consequently the data were pooled to provide a composite picture of those responses (Fig. 16). In each case female pumpkinseeds either did not choose or showed a preference for conspecific males during the C phase. The conspecific choice persisted and became more pronounced during the E phase although an hour was required at the beginning of both E-ON 1 and E-ON 2 before it developed, suggesting that visual choice of conspecific males may be part of the behavioural repertoire of female pumpkinseeds.

The above results are similar to those of the cement tank exper-

Figure 13. Mean response of 12 longear females to different-sized pumpkinseed males.

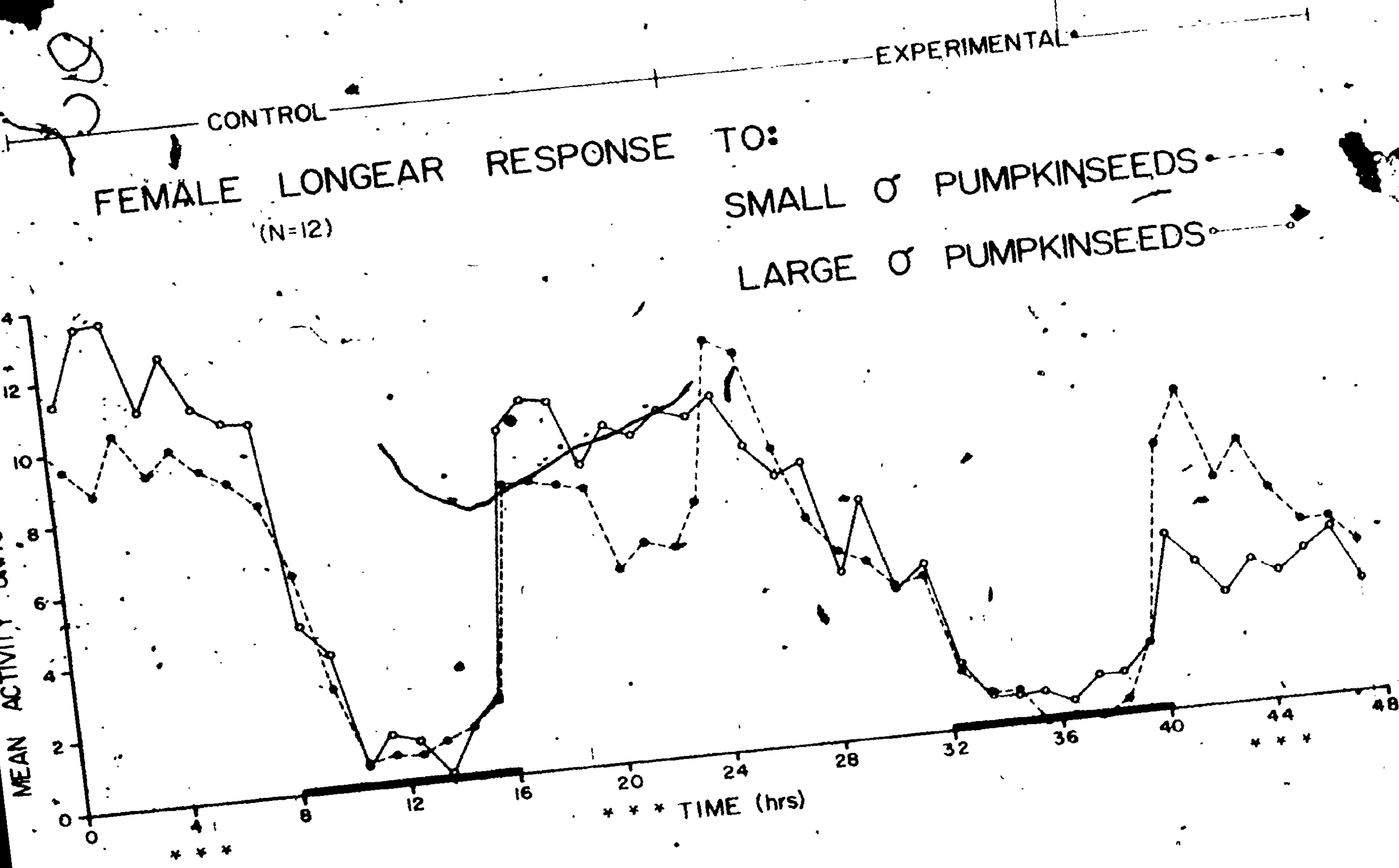

CONTROL
EXPERIMENTAL
FEMALE LONGEAR RESPONSE TO:
(N=12)
SMALL ♂ PUMPKINSEEDS
LARGE ♂ PUMPKINSEEDS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
0
4
8
12
16
20
24
28
32
36
40
44
48
* * * TIME (hrs)
* * *
* * *

Figure 14. Mean response of 12 pumpkinseed females to different-sized longear and pumpkinseed males. Summer, Method 1.

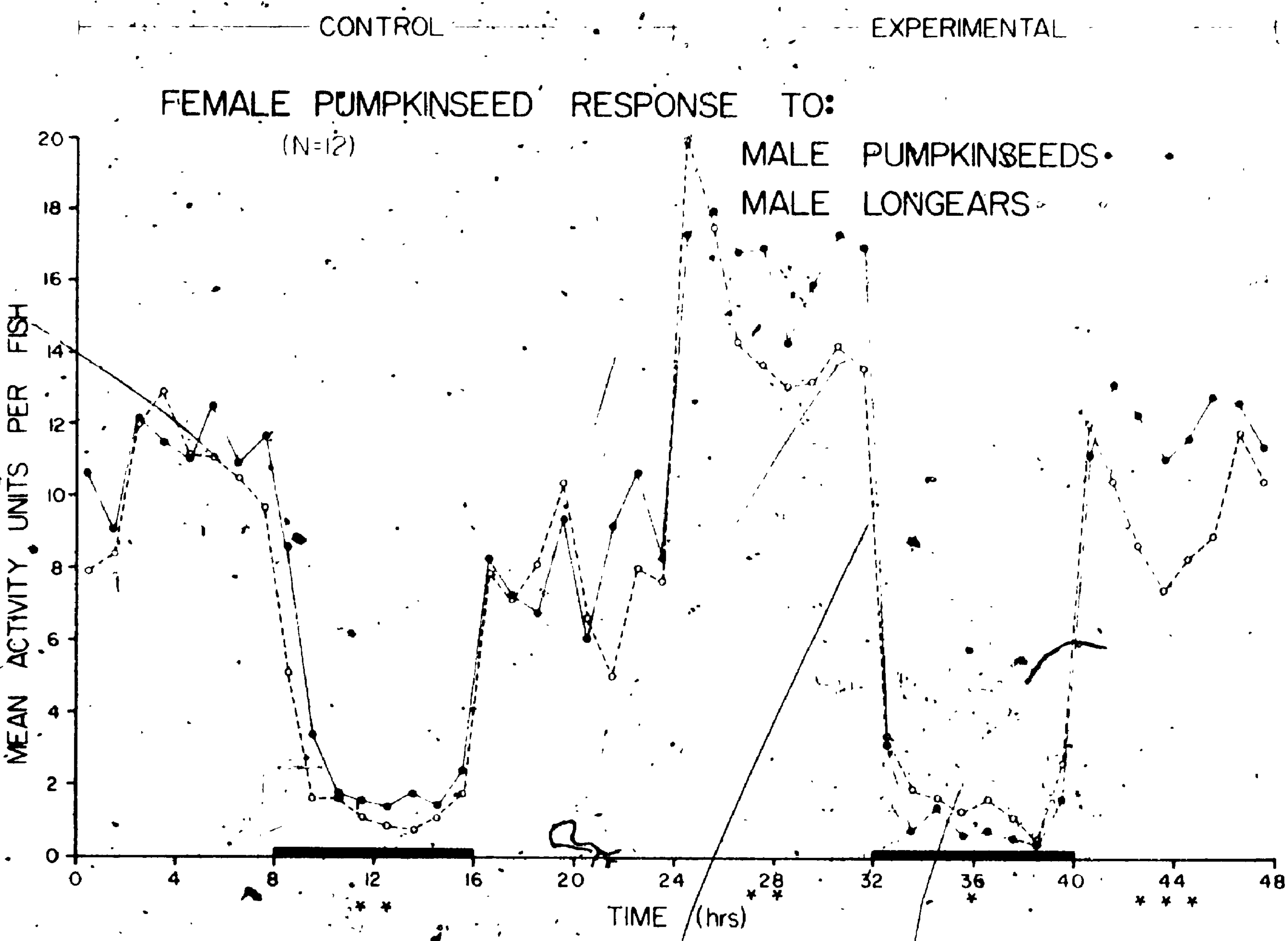

CONTROL
EXPERIMENTAL
FEMALE PUMPKINSEED RESPONSE TO:
(N=12)
MALE PUMPKINSEEDS •
MALE LONGEARS ○
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
16
18
20
TIME (hrs)
0
4
8
12
16
20
24
28
32
36
40
44
48
* *
* *
*
* * *

Figure 15. Mean response of 14 pumpkinseed females to different-sized longear and pumpkinseed males. Summer, Method 2.

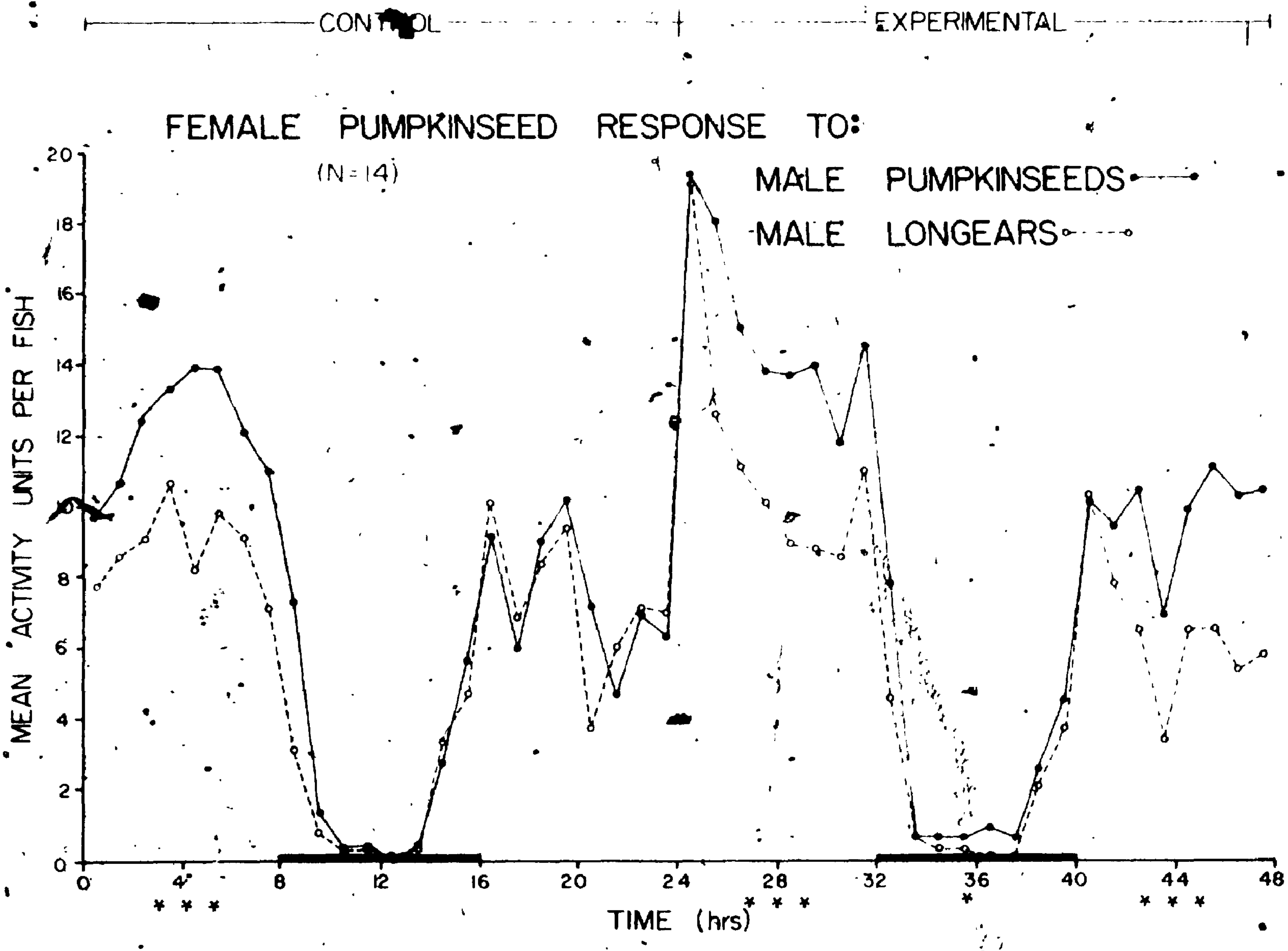
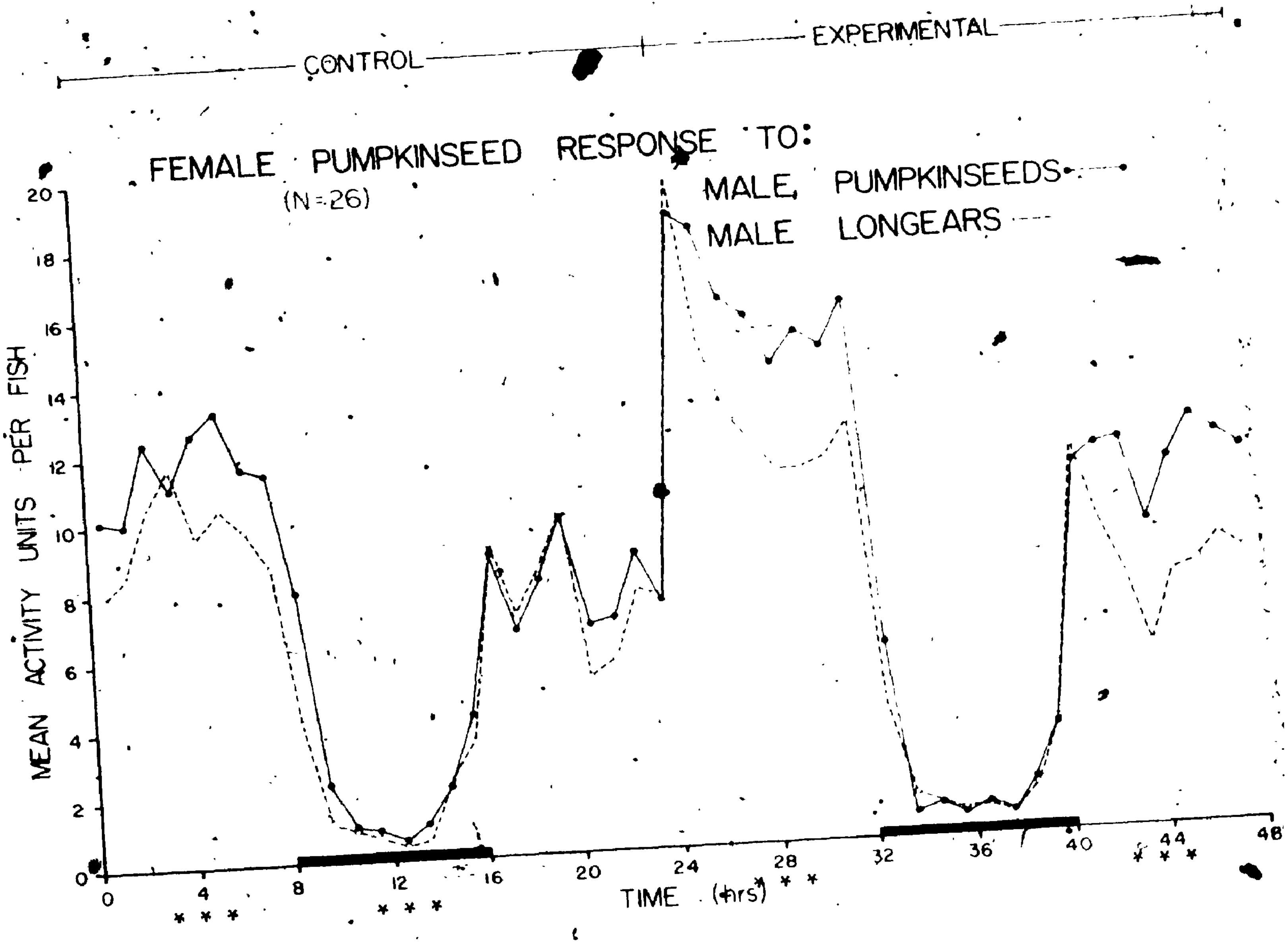

CONTROL
EXPERIMENTAL
FEMALE PUMPKINSEED RESPONSE TO:
(N=14)
MALE PUMPKINSEEDS
MALE LONGEARS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
16
18
20
0
4
8
12
16
20
24
28
32
36
40
44
48
* * *
* * *
*
* * *
TIME (hrs)

Figure 16. Mean response of 26 pumpkinseed females to different-sized longear and pumpkinseed males. Summer, Methods 1 and 2 combined.


CONTROL
EXPERIMENTAL
FEMALE PUMPKINSEED RESPONSE TO:
(N=26)
MALE PUMPKINSEEDS
MALE LONGEARS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
16
18
20
TIME (hrs)
0
4
8
12
16
20
24
28
32
36
40
44
48
* * *
* * *
* * *
* * *

iments (Table 1) in that there is an indication on non-visual choice of conspecific males in the C phase of both types of experiment. The conspecific choice in the E phase of these activity box results is not apparent in the cement tank results. However, females were only observed for slightly more than 1/2 hr in the latter case and the conspecific choice may not have had a chance to develop.

When seven female pumpkinseeds were tested in the non-breeding season using Method 1 (Fig. 17), they exhibited no choice in C-ON 1, C-ON 2 or E-ON 2 and a heterospecific choice in C-OFF, E-ON 1 and E-OFF. These results somewhat contradicted both the previous activity box results (Fig. 16) and the cement tank results (Table 1) where either conspecific or no choice occurred, suggesting that the females did not exhibit the same choice in the non-breeding season as they did in the breeding season, and that female pumpkinseeds did not respond in the same manner to the same types of stimulus fish in the two different types of apparatus.

3.2 Responses to Different-sized Pumpkinseed Males

When female pumpkinseeds were tested with large and small male pumpkinseeds very little consistent or persistent non-visual choice was exhibited in the C phase, but both small and medium females chose small males while large females chose large males in the E phase.

Ten small female pumpkinseeds were tested with large and small male pumpkinseeds (Appendix I-11, Fig. 18). These females showed a preference for large males in C-ON 1 and a preference for small males in C-OFF and C-ON 2 indicating an inconsistent pattern of choosing in the C phase as a whole. The preference for small males persisted and increased in the E phase suggesting that small female pumpkinseeds were visually

Figure 17. Mean response of seven pumpkinseed females to different-sized longear and pumpkinseed males. Winter, Method 1.

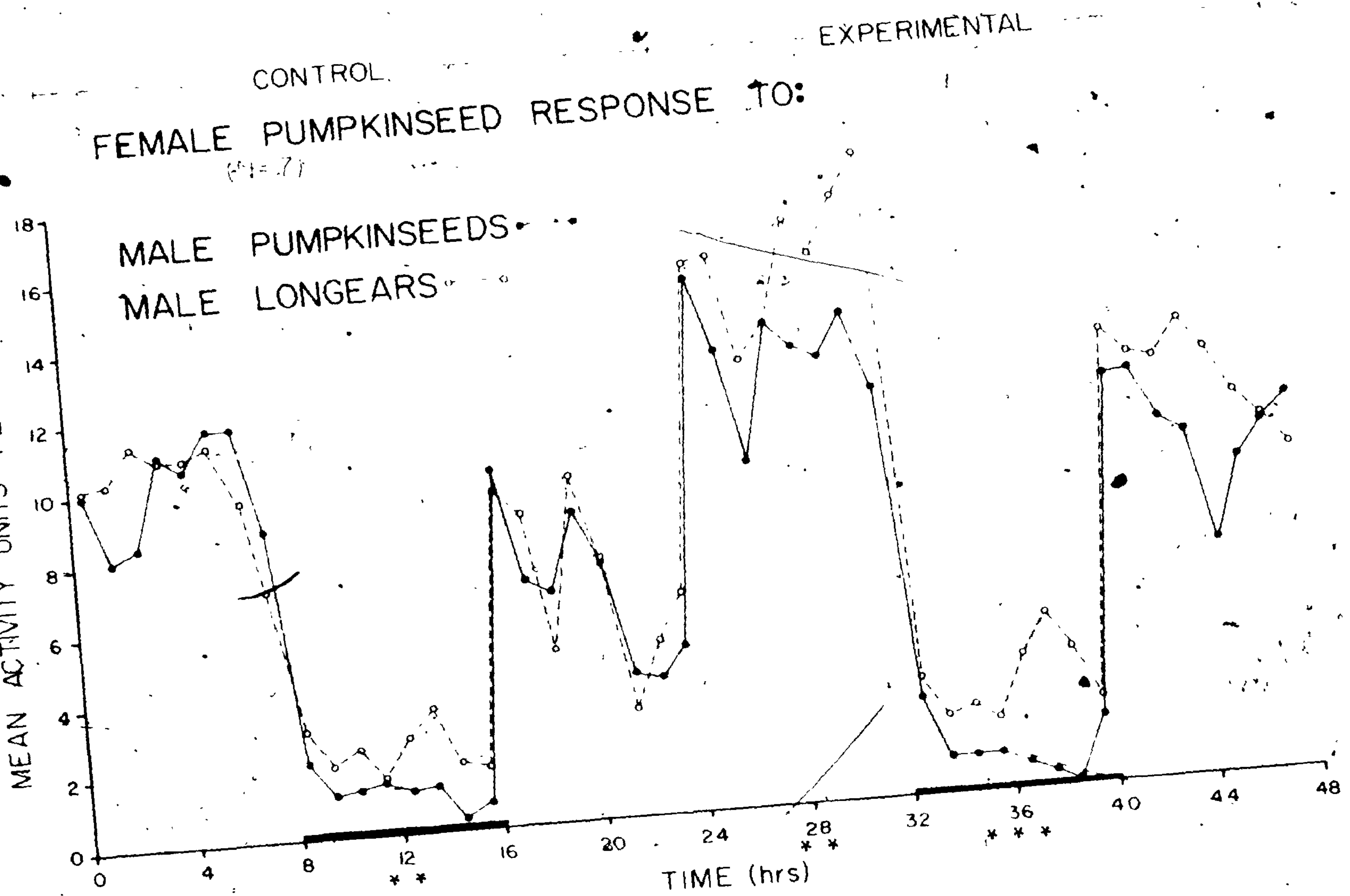
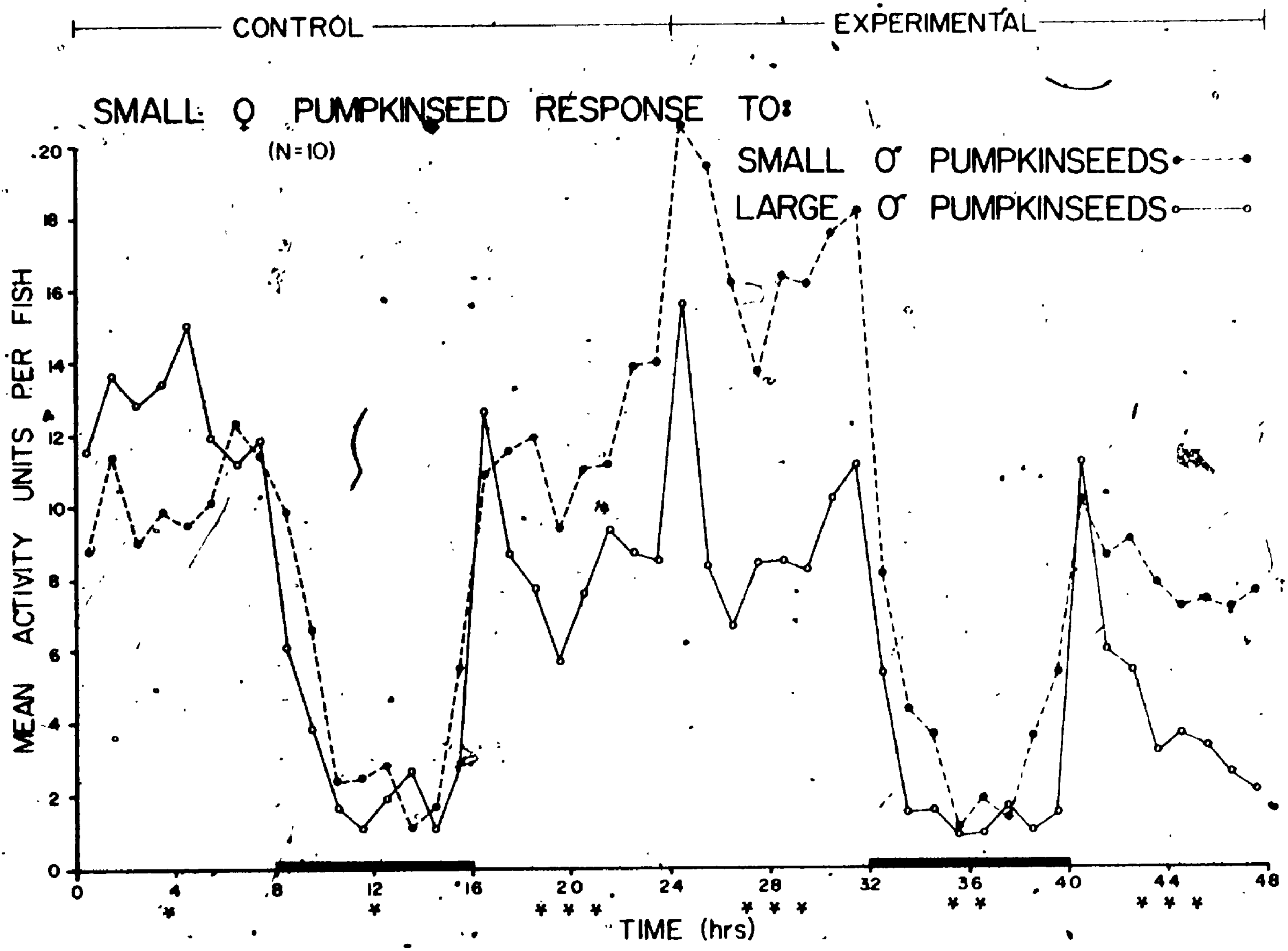
CONTROL
EXPERIMENTAL
FEMALE PUMPKINSEED RESPONSE TO:
(N=7)
MALE PUMPKINSEEDS
MALE LONGEARS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
16
18
0
4
8
12
16
20
24
28
32
36
40
44
48
TIME (hrs)
* *
* *
* * *

Figure 18. Mean response of 10 small pumpkinseed females to small and large pumpkinseed males.

2 OF/DE 2


CONTROL
EXPERIMENTAL
SMALL ♀ PUMPKINSEED RESPONSE TO:
(N=10)
SMALL ♂ PUMPKINSEEDS
LARGE ♂ PUMPKINSEEDS
MEAN ACTIVITY UNITS PER FISH
.20
18
16
14
12
10
8
6
4
2
0
0
4
8
12
16
20
24
28
32
36
40
44
48
*
*
* * *
* * *
* *
* * *
TIME (hrs)

choosing small male pumpkinseeds (Fig. 18).

When eighteen medium-sized female pumpkinseeds were tested with large and small male pumpkinseeds, a choice of small males was evident in C-ON 1 which persisted in E-ON 1 and increased in E-ON 2 (Fig. 19). These results indicate that again female pumpkinseeds visually chose males whose size most closely resembled their own, as test fish length was closer to the small than to the large stimulus fish length in every case (Appendix I-12).

When twelve large female pumpkinseeds were tested with large and small male pumpkinseeds, no choice was exhibited until E-ON 2 when large males were chosen (Fig. 20) indicating that again female pumpkinseeds which chose visually, chose males closer in size to their own length.

3.3.3 Responses to Different-sized Longear and Hybrid Males

Sixteen female pumpkinseeds tested with longear and hybrid males directed more activity toward hybrid males in C-ON 2 but consistently directed more activity toward longear males in both E-ON 1 and E-ON 2 (Fig. 21). These results indicate that the test female pumpkinseeds, all of which were small or medium in size, when confronted with a choice of males, both rather dissimilar from their own species males, visually chose males closer to their own length. Again these results suggest that in female pumpkinseed choice of males, male size is probably an important factor.

3.4 Male Longears as Test Fish

Twenty male longears tested with longear and pumpkinseed females as stimulus fish showed a heterospecific choice in both lights-off periods,

Figure 19. Mean response of 18 medium pumpkinseed females to small and large pumpkinseed males.

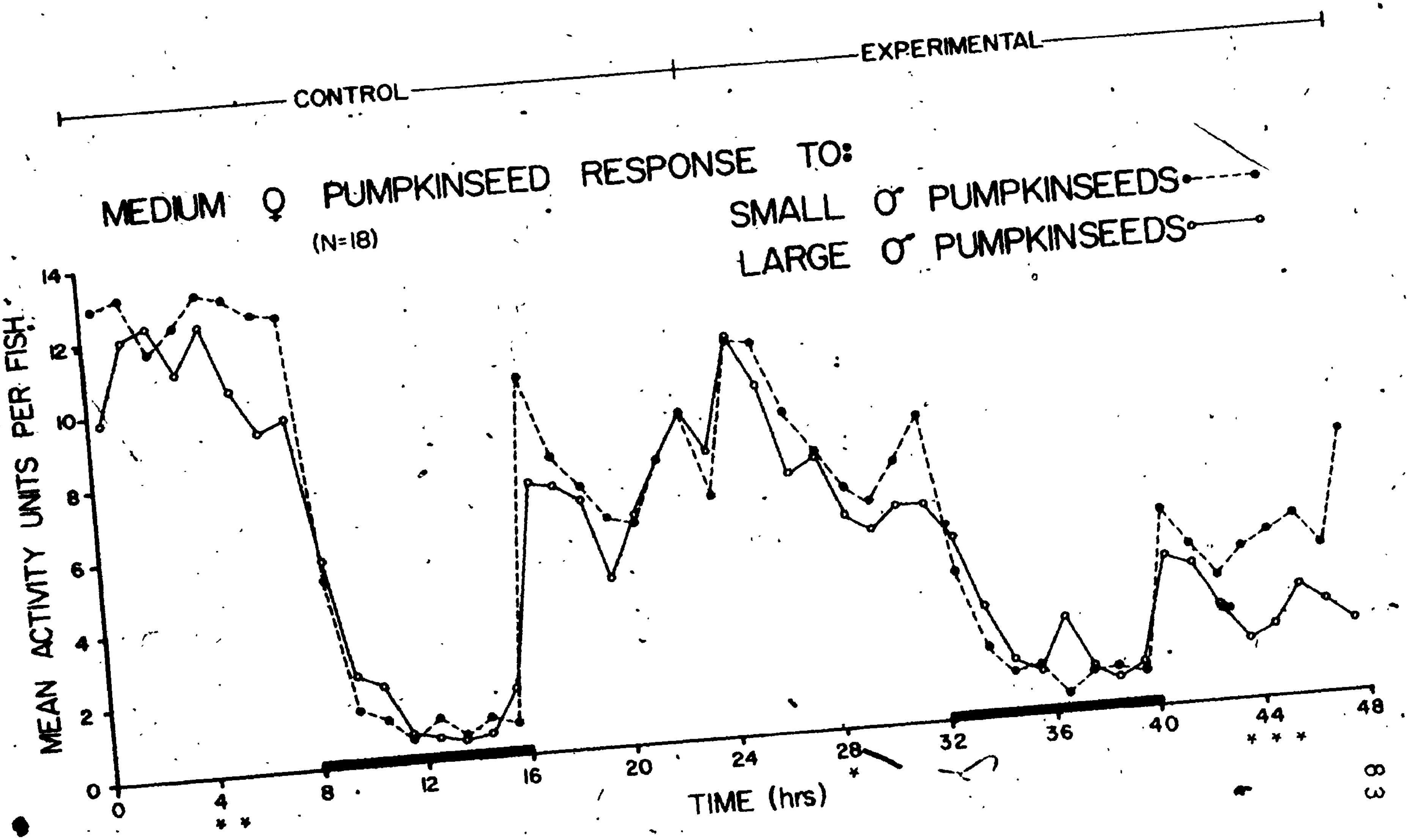

CONTROL
EXPERIMENTAL
MEDIUM ♀ PUMPKINSEED RESPONSE TO:
(N=18)
SMALL ♂ PUMPKINSEEDS
LARGE ♂ PUMPKINSEEDS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
TIME (hrs)
0
4
8
12
16
20
24
28
32
36
40
44
48
* *
*
* * *

Figure 20. Mean response of 12 large pumpkinseed females to small and large pumpkinseed males.

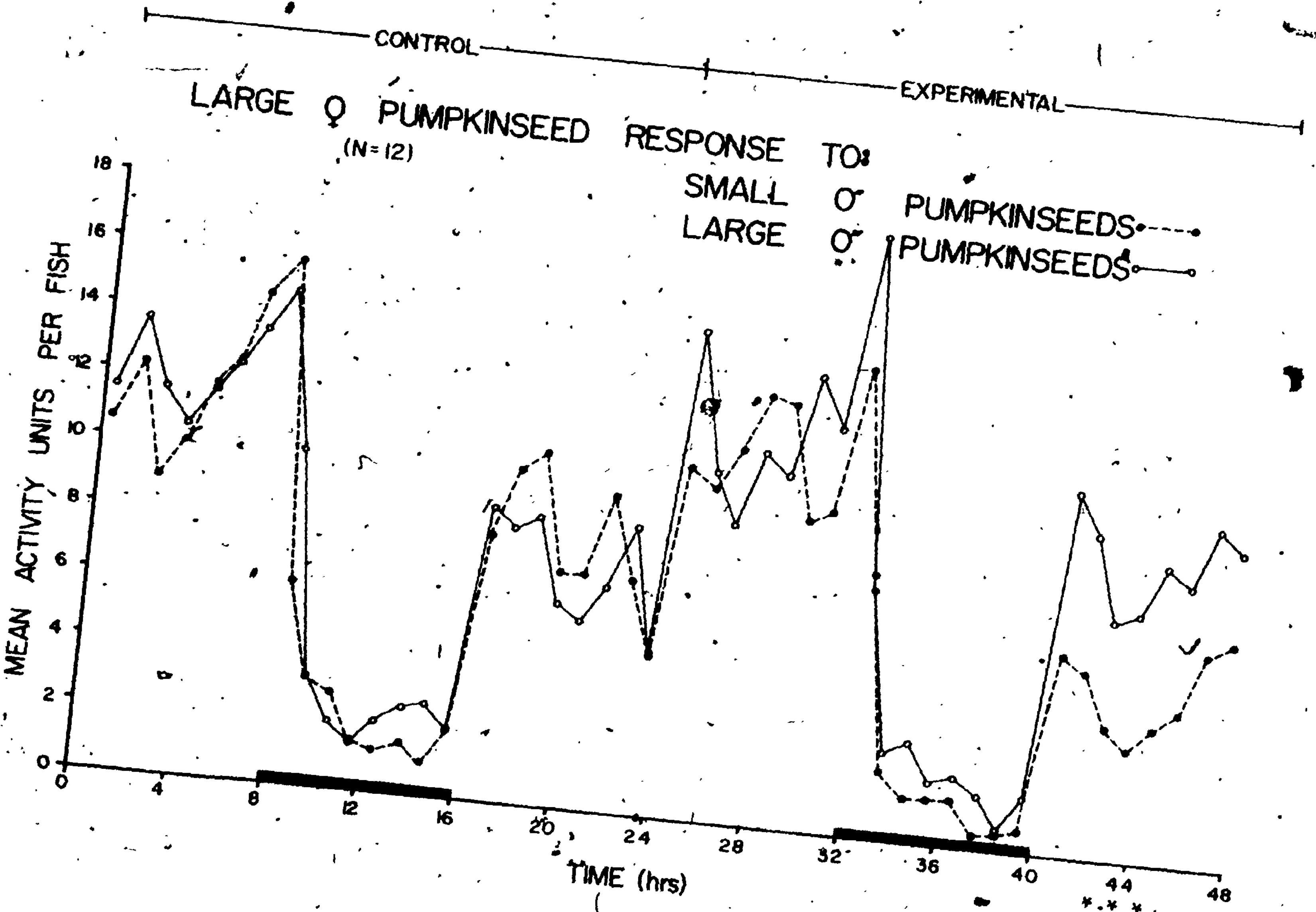
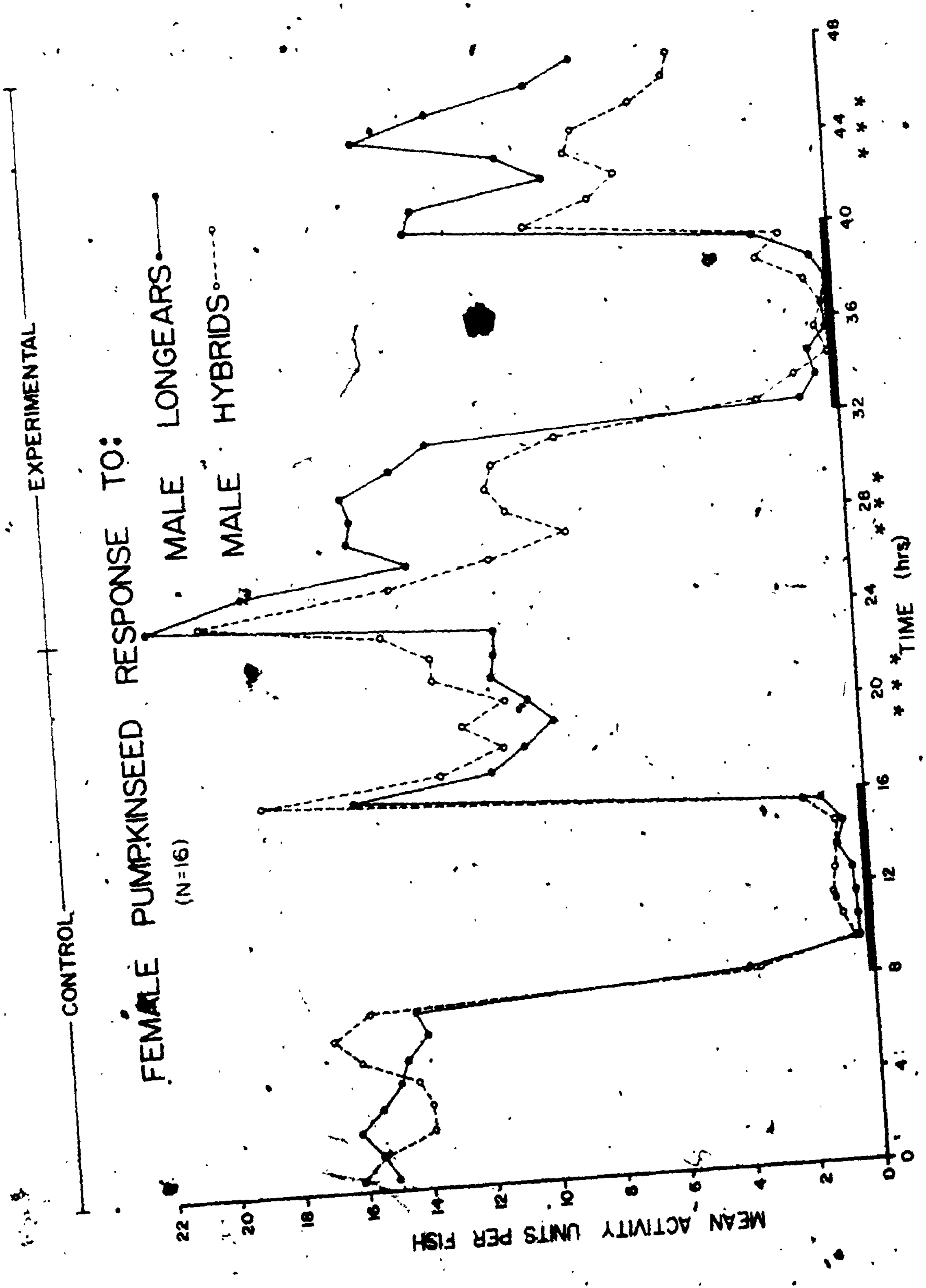

CONTROL
EXPERIMENTAL
LARGE ♀ PUMPKINSEED RESPONSE TO:
(N=12)
SMALL ♂ PUMPKINSEEDS
LARGE ♂ PUMPKINSEEDS
MEAN ACTIVITY UNITS PER FISH
18
16
14
12
10
8
6
4
2
0
0
4
8
12
16
20
24
28
32
36
40
44
48
TIME (hrs)

Figure 21. Mean response of 16 pumpkinseed females to different-sized male longears and male hybrids.


FEMALE PUMPKINSEED RESPONSE TO:
(N=16)
MALE LONGEARS
MALE HYBRIDS
CONTROL
EXPERIMENTAL
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
16
18
20
22
TIME (hrs)
0
4
8
12
16
20
24
28
32
36
40
44
48
* * *
* * *
* * *

of the experiment and a conspecific choice in E-ON 1 (Fig. 22).

3.2 Male Pumpkinseeds as Test Fish

Twenty male pumpkinseeds tested with longear and pumpkinseed females as stimulus fish showed a choice of female pumpkinseeds in both lights-on periods of the C phase which persisted throughout the E phase (Fig. 23). The results of both male longear and pumpkinseed experiments are inconsistent with those of the cement tank experiments where no choice was made under either lighting condition C or E (Table 1) perhaps indicating that neither male chose between conspecific and heterospecific females in the non-breeding season (when the cement tank experiments were performed), but did choose in the breeding season (when the above activity box experiments were performed), or that male choice differed with the apparatus used.

4. Stripping Experiments

4.1 Egg Ripeness and Order of Extrusion

When a varying number of eggs was stripped from females into a series of plexiglass containers and fertilized with conspecific milt neither female pumpkinseeds (Fig. 24) nor female longears (Fig. 25) showed consistent between-fish variation in ripeness of eggs as indicated by percentage hatched (Appendix II-1, 2). Hence no "order effect" could be ascribed to unequal ripeness of eggs as they emerged from the females.

4.2 Egg Size

Mean egg diameter of 160 fertilized eggs from eight female

Figure 22. Mean response of 20 longear males to longear and pumpkinseed females.

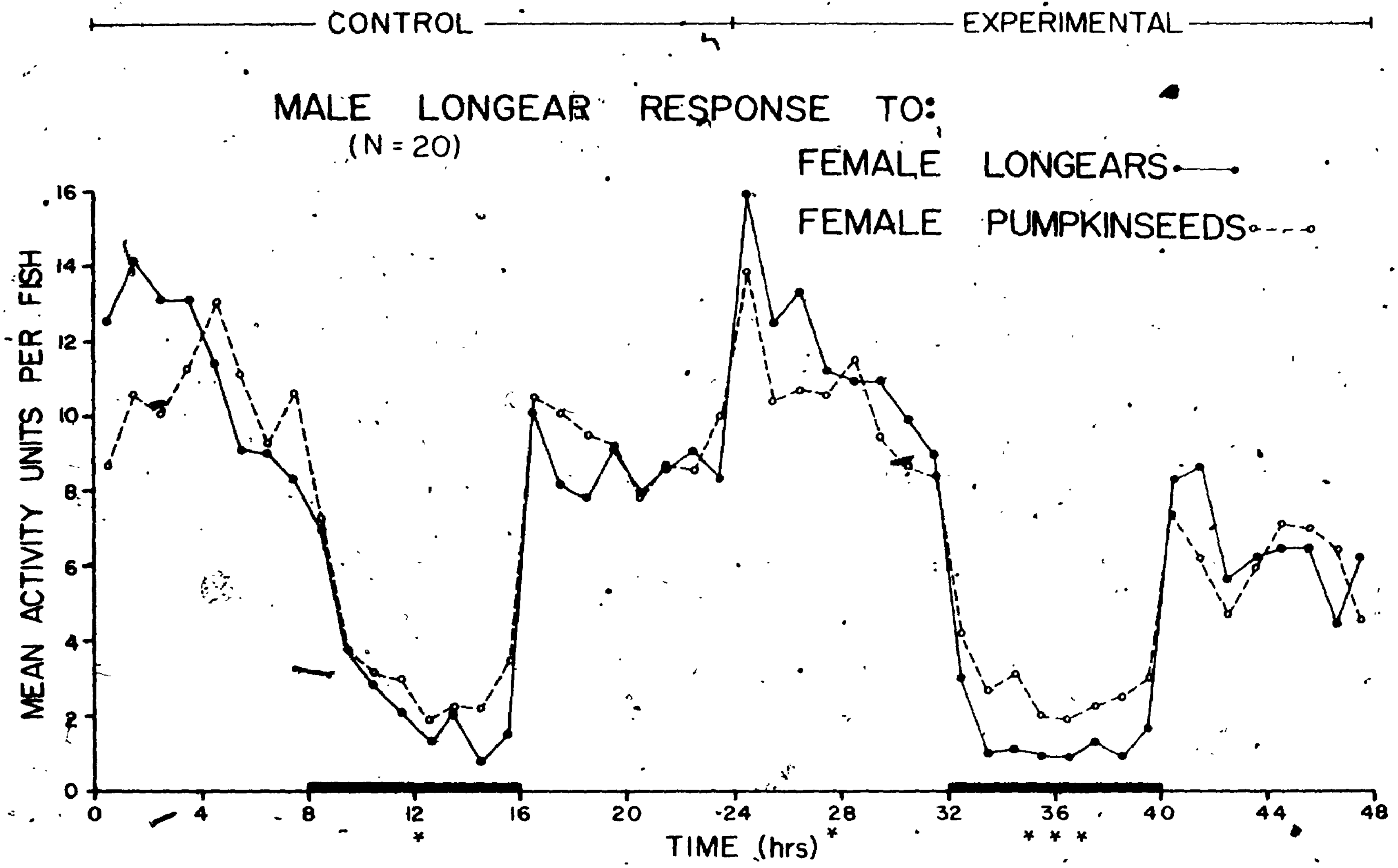

CONTROL
EXPERIMENTAL
MALE LONGEAR RESPONSE TO:
(N = 20)
FEMALE LONGEARS
FEMALE PUMPKINSEEDS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
16
TIME (hrs)
0
4
8
12
16
20
-24
28
32
36
40
44
48
*
*
* * *

Figure 23. Mean response of 20 pumpkinseed males to longear and pumpkinseed females.

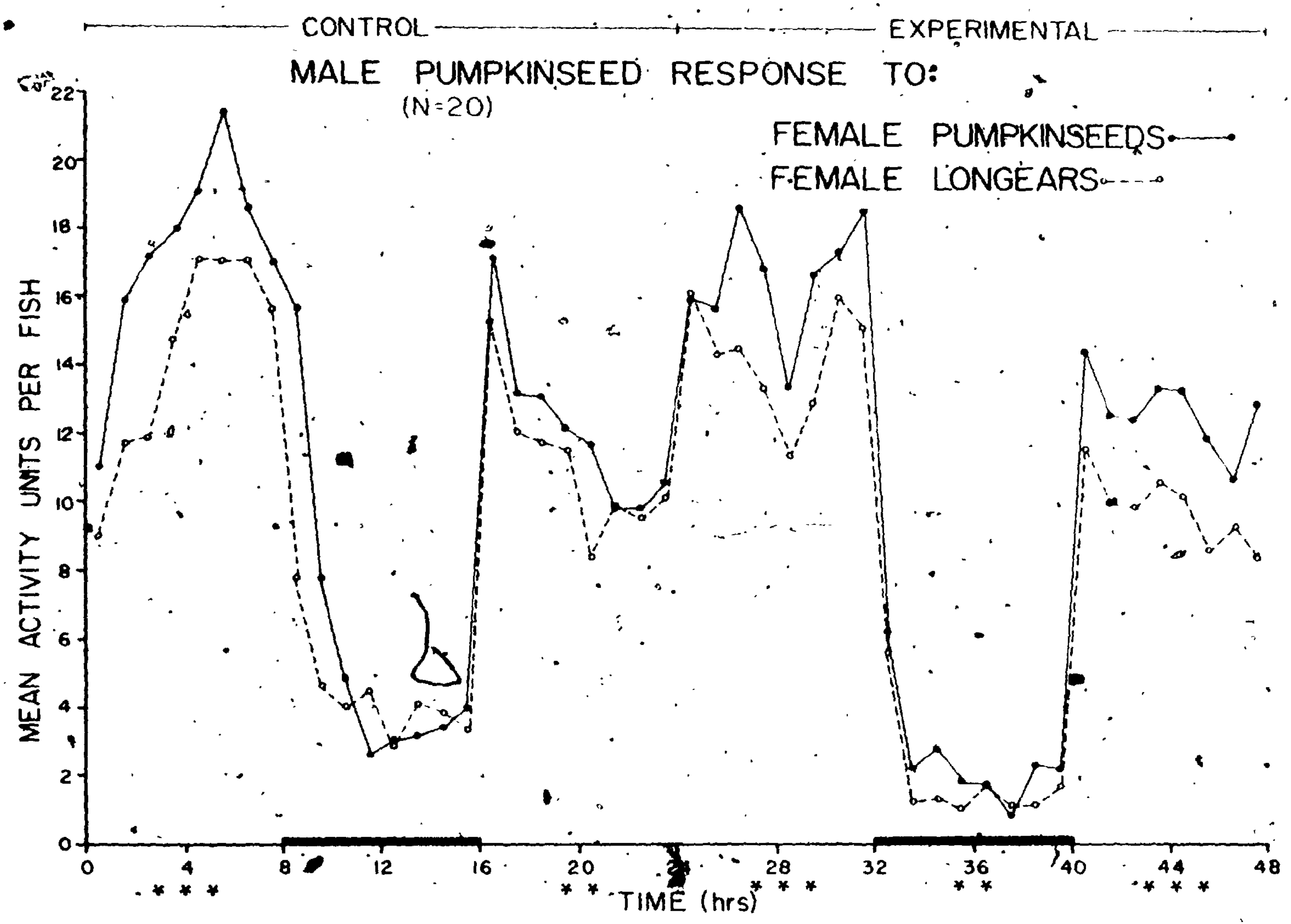

CONTROL
EXPERIMENTAL
MALE PUMPKINSEED RESPONSE TO:
(N=20)
FEMALE PUMPKINSEEDS
FEMALE LONGEARS
MEAN ACTIVITY UNITS PER FISH
0
2
4
6
8
10
12
14
16
18
20
22
0
4
8
12
16
20
24
28
32
36
40
44
48
TIME (hrs)
* * *
* *
* * *
* *
* * *

Figure 24. Percent hatch of eggs from six pumpkinseed females. Eggs from each of three females were fertilized with sperm from several males (Series A); eggs from each of three other females were fertilized with sperm from one male each (Series B). Eggs from each female were stripped successively into 10 to 12 plexiglass containers; arrows indicate the egg containers fertilized by each male.

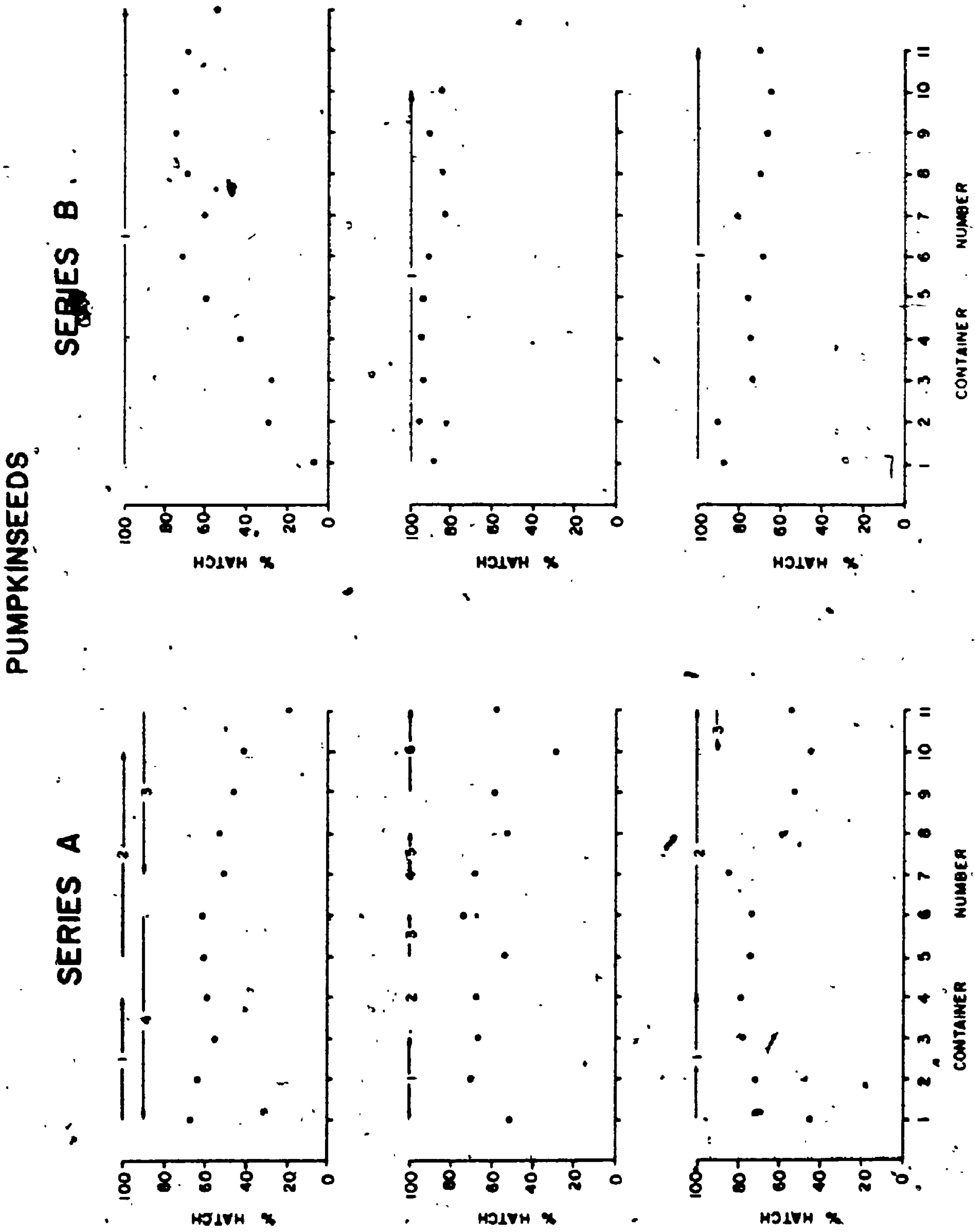
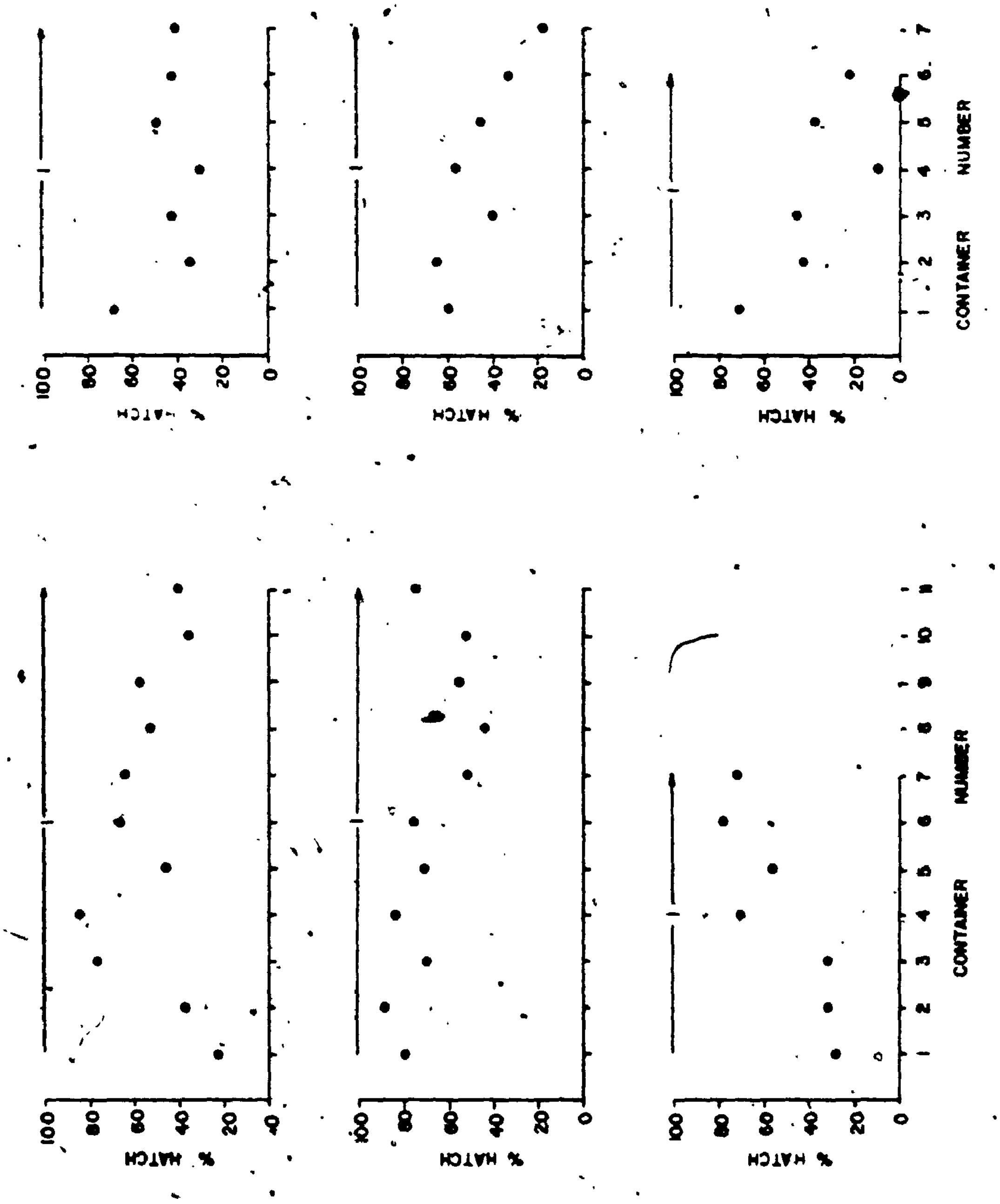

PUMPKINSEEDS
SERIES A
SERIES B
% HATCH
100
80
60
40
20
0
CONTAINER NUMBER
1
2
3
4
5
6
7
8
9
10
11

Figure 25. Percent hatch of eggs from six longear females. Eggs from each female were stripped successively into 6 to 11 plexiglass containers and fertilized with sperm from a different longear male as indicated by the arrows.

LONGEARS



longears was 1.35 ± 0.08 cm (Appendix III-1) while the mean egg diameter of 100 fertilized eggs from five female pumpkinseeds was 1.16 ± 0.05 cm (Appendix III-2).

4.3 Crossing Experiments

No differences existed between years when the 10 replicates using stripping Method B in one summer were compared with the 5 replicates using Method B the following summer[1]. (Appendix V-3). However, when the 10 replicates using stripping Method A were compared with the combined 15 replicates using Method B by means of the same two-way analysis of variance[2] (Appendix V-4) highly significant differences ($P < 0.01$) were found between the two methods. In both cases highly significant differences ($P < 0.01$) occurred among the crosses. As the percent hatched were much higher (Table 2) and the standard deviations lower for Method B, only the 15 replicates of Method B were further analysed. A one-way analysis of variance of these 15 replicates again showed highly significant differences ($P < 0.01$) among the six crosses (Appendix V-5).

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[1] A two-way analysis of variance with unequal but proportional subclass numbers (Sokal and Rohlf 1969, p. 334) was performed on the percentage hatch data (Appendix IV) treated with an arcsine transformation. Crosses were treated as a fixed effect, years as a random effect for the analysis.

[2] Both crosses and methods were treated as fixed effects for the analysis.

Table 2. Total eggs stripped and their percent hatch from 10 (Method A) and 15 (Method B) pumpkinseed and longear females fertilized by pumpkinseed, longear and hybrid males.

Method A

N=10

		♂P	♂L	♂H
Total eggs	♀P	2900	3015	3144
% Hatch		50.6	57.5	53.6
Total eggs	♀L	2121	2093	2178
% Hatch		17.6	29.1	19.3

Method B

N=15

		♂P	♂L	♂H
Total eggs	♀P	4875	5047	5184
% Hatch		86.6	86.6	65.2
Total eggs	♀L	3785	3760	3625
% Hatch		64.4	76.9	47.5

When differences were analysed between the crosses, the following results were obtained:

(P x P) vs (P x L)	ns
(P x P) vs (P x H)	*
(P x L) vs (P x H)	*
(L x P) vs (L x L)	**
(L x P) vs (L x H)	ns
(L x L) vs (L x H)	**
(P x P) + (P x L) + (P x H) vs (L x P) + (L x L) + (L x H)	**
(P x P) vs (L x P)	*
(P x L) vs (L x L)	ns
(P x H) vs (L x H)	**

Note: ns, not significant; *, $P < 0.05$; **, $P < 0.01$; first bracketed letter represents the female member of the cross; P, pumpkinseed; L, longear; H, hybrid.

These results indicate that there was no difference between longear and pumpkinseed males in their ability to fertilize pumpkinseed eggs and that hybrid males produced a significantly lower percentage hatch than either of the other two males when the hybrids were used to fertilize eggs from the same female pumpkinseeds. When female longear eggs were being fertilized by the same males however, there was a highly significant difference in percentage hatch between pumpkinseed and longear males on the one hand and longear and hybrid males on the other hand; the longear males producing the higher percentage hatch in both cases. The pumpkinseed and hybrid males were not significantly different in the percentage hatch produced when they were used to fertilize longear eggs. Although the percentage hatch of pumpkinseed eggs was very significantly higher than the percentage hatch of longear eggs when the data from all the males were pooled, the difference between the two species' eggs within male pumpkinseeds was significant, within male longears was not significant, and only between male hybrids was the difference in percentage hatch highly significant.

DISCUSSION

1. Observation of Spawning

Courtship is often a necessary preliminary to spawning and may serve to reduce hybridization between species (Tinbergen 1953, Bastock 1967). This courtship might be expected to be male-initiated if male choice of a conspecific female were an important ethological isolating mechanism. Observations in the study area, however, suggested that the frequency of male courtship decreased in longear colonies as the incidence of spawning increased. Certainly, at the height of spawning, male courtship occurred only infrequently in five observed longear colonies (Figs. 3 and 4).

Other factors might also mitigate against the prevention of hybridization by male longears choosing conspecific spawning partners. Female recognition of conspecific males is often more important than the reverse when females produce fewer gametes than males, and when males tend to be promiscuous (Liley 1966, Bastock 1967). This must be particularly true in colonially nesting species where the preoccupation of territorial males with nest-defending, male-male aggressive encounters, probably reduces the time available for male discrimination. Furthermore, if the pre-copulatory courtship period is short, conspecific recognition must occur rapidly if hybridization is to be avoided (Sibley 1957).

Male longears are promiscuous, nest colonially, are aggressive in defense of their nesting territories (Keenleyside 1971, 1972), and their courtship is short (personal observations) or non-existent (Figs. 3 and 4). As a result of the colonial nesting habit of longears, every male in the colony has neighbouring males with which to interact. As spawning increases in a colony, males lacking spawning partners spend more and more time intruding into the nests of spawning neighbours, while spawning males spend more and more time chasing intruders out of their nests (Keenleyside 1971, 1972). Both behaviours increase the incidence of male-male interactions thereby leaving less time for male-female courtship. Hunter (1963) suggested that males of green sunfish (L. cyanellus) which, like longears, nest colonially and are sexually dimorphic, may recognize females by their nest-circling behaviour rather than morphologically. Male longears may also rely on female behaviour to identify a potential spawning partner, and consequently spawn with any female that behaves appropriately (entering and remaining in the nest even though threatened), regardless of her species. Under these circumstances, male courtship might be curtailed or even deleted from the males' behavioural repertoire at the height of spawning.

Females, on the other hand, swim along the edges of longear colonies and do not appear to participate in female-female interactions. They therefore appear to have more time than males to choose a spawning partner. Consequently, if behaviour in the form of choice of a conspecific spawning partner operates as a reproductive isolating mechanism in longear colonies, female choice is probably more important than male choice.

Pumpkinseed spawning was rarely seen and then only after male and female nest-circling and female tilting had begun. Hence female approach to the male and male courtship, if any, were not seen. However, as the average nearest-nest distance of 81 pumpkinseed nests was 1 m

(Keenleyside unpublished) compared to a nearest-nest distance of 20 cm in a sample of 345 longear nests (Keenleyside 1972), pumpkinseed nests were further apart than longear nests in the study area. Furthermore, any casual observer would recognize that longears nest colonially and pumpkinseeds singly in the study area. Consequently male pumpkinseeds were freer from frequent aggressive interactions with nesting neighbours than longear males and hence should have had more time to observe and react to approaching females. Single male pumpkinseeds in aquarium tests clearly discriminated between the two species females ~~by~~ directing more aggressive and courtship behaviour at conspecifics (Keenleyside 1967). Those aquarium experiments more closely resembled the field situation of pumpkinseeds than of longears with respect to inter-nest distances so it is likely that male pumpkinseeds in the field could also discriminate between approaching females. If this ability were used to choose conspecific spawning partners then male pumpkinseed mate selection would be an important ethological isolating mechanism.

Male pumpkinseeds have, however, been induced to nest-circle with a wide variety of appropriately manipulated objects ranging from a dead, formalinized male to equal-size leaves (Noble 1934). This suggests male pumpkinseeds are receptive to the set of visual and perhaps tactile cues provided by an object circling in the nest and that those cues are sufficient to initiate spawning. Male pumpkinseeds, like longears, defend their nest against any approaching fish regardless of sex, but if a female persists and manages to enter the nest and keep moving in a circular path, she might be spawned with, no matter what her species. Which female spawns in a pumpkinseed nest may thus be largely a function of which female approaches and persists in entering that nest. Again, in pumpkinseeds as in longears, female choice of a spawning partner might be more important than male choice in determining whether or not hybridization is prevented.

2. Behaviour Experiments

2.1 General Activity

Both longears and pumpkinseeds are more active during the day than at night (Miller 1963, Boyer 1969, Kapoor 1971, Emery 1973). This was also shown in the activity box experiments by the relatively high levels of activity recorded during the lights-on periods. Increased daylight activity is characteristic of diurnal animals and such animals are usually profoundly influenced by visual stimuli. If recognition and choice of a conspecific spawning partner is an important isolating mechanism, that recognition might well be based on visual cues.

Considering XO data of the cement tank experiments, male test fish of both species performed more crossovers than female test fish during the C phase of the experiments (Table 1). This may have been caused by the stronger response of males to their mirror images in the end walls of the test section. Male sunfish are generally aggressive towards conspecifics, both in aquaria and in the field (Keenleyside 1967, 1971), and during the C phase male test fish occasionally moved across the center of the test section as though responding to their mirror image. That this response was not more pronounced may have been ~~a~~ result of their having habituated to their mirror images under lighting condition C for a day before testing.

Stimulus fish in the cement tank experiments do not appear to respond to either the sight of the test fish in the C phase or their mirror images in the one-way glass in the E phase. This lack of response was probably a result of three factors: (1) stimulus fish had a longer time to habituate to the experimental conditions, (2) the clear glass partition prevented the stimulus fish from approaching the one-way glass partition closer than 30 cm (Fig. 1), (3) stimulus fish, because they were introduced in groups of three, could interact among themselves.

During the breeding season free-living adult males of both species confine their movements to a relatively small area surrounding their nests. Females swim up and down the river and backwaters visiting longear colonies or pumpkinseed nests, and hence cover greater distances. This may explain why females in this study showed a relatively greater increase in XO behaviour than males when exposed to the E condition.

2.2 Specific Experiments

Test fish in these experiments often responded positively to one or the other types of stimulus fish under both C and E lighting conditions. Possible cues which might direct such responses include visual, auditory, chemical, tactile and electrical stimuli. There is no evidence that sunfish possess an electric organ, and tactile communication is prevented in these experiments (Figs. 1 and 2). The one-way glass and the lighting arrangement were designed to permit test fish to visually perceive stimulus fish only during the E phase of the experiments. No attempt was made, however, to isolate the test fish from chemical or auditory cues. Hence these sunfish might use visual, chemical or auditory cues (or any combination of these) to communicate both in the present experiments, and, with the addition of tactile cues, in the field.

Chemical cues in these experiments could only reach test fish by diffusing around the one-way glass partitions (Figs. 1 and 2), a rather indirect source. In the field, olfaction may be used by longear sunfish in homing (Gunning 1959) and in nest colony formation (Gerald 1970), but its role in pumpkinseed reproduction is unknown. In the study area pumpkinseeds nest in quiet weedy backwaters where water flow in the breeding season is usually negligible, whereas longears nest in the river's current. Since chemical cues provide poor localization except in moving water (Kleerekoper 1969), such cues would be more useful to female longears than to female pumpkinseeds in locating a conspecific nest.

Auditory cues provide good localization (Tavolga 1971). Sound discrimination was demonstrated in males of two species of cyprinid fish by Delco (1960) who suggested it operated as an important sexual isolating mechanism. Gerald (1971) demonstrated grunt-like sound production by courting males in six out of seven species of sunfish tested. The central longear (L. m. megalotis) was one of the six species which produced sounds; pumpkinseeds were not tested. He suggested the function of male courtship sounds was the attraction of conspecific females. Sound production in the present experiments might result from the fish contacting the one-way glass, from rapid swimming movements, from gravel disturbance by nest-digging males or from male courtship sounds. The latter two are the most likely sources of male-produced sounds in the field.

2.2.1 Female Longears as Test Fish

Female longears in the C phase responded non-visually to male pumpkinseeds by directing more activity toward them than toward male longears in both the cement tank (Table 1) and activity box experiments (Figs. 6-10). (This differential activity is referred to as a "choice" throughout the following discussion.) Method 1 females chose male pumpkinseeds in both C-ON 1 and C-ON 2 but chose longears in C-OFF (Fig. 6), while method 2 females did not choose until C-ON 2, when male pumpkinseeds were chosen (Fig. 7). Choice in the C phase was based on non-visual stimuli. C phase lighting permitted the male stimulus fish of both species to see both test females through the one-way glass. Method 1 male pumpkinseeds saw both species of females as test fish (see Materials and Methods section 5.2). If the male pumpkinseed responded to the sight of a conspecific female test fish by producing some or all of the sounds mentioned above, and there is evidence for visual choice of conspecific females by male pumpkinseeds (Keenleyside 1967), those sounds might have evoked a choice from the female longears. If the non-visual stimulus was emitted only when the lights were on, the females might have responded during the lights-off period to some non-visual stimulus emitted by the male longears (perhaps a chemical cue).

Method 2 male pumpkinseeds saw only female longear test fish in the C phase (Materials and Methods section 5.2) and may not have been as active and hence as stimulating to the female longears. Evidence for this is shown in figure 7 where female longear preference for male pumpkinseeds is not shown until C-ON 2.

Female longears in the non-breeding season may have responded to male pumpkinseeds in the C phase for the same reasons female longears responded to them in the breeding season (Figs. 9 and 6), although the response in the winter experiments declined to no choice in C-ON 2 (Fig. 9).

Female longears offered a choice of equal-sized males of both species chose male pumpkinseeds in the C phase (Fig. 10). This choice was similar to the response shown to different-sized males (Fig. 7), perhaps for the same reasons.

The role of non-visual communication among spawning sunfish in the study area is unknown, but it is possible that auditory cues are more important to pumpkinseeds than to longears. In the study area male pumpkinseeds might enhance their chances of attracting a potential spawning partner by emitting a non-visual stimulus as their nests tend to be surrounded by dense aquatic vegetation perhaps rendering visual cues unsatisfactory. Male longears nest in colonies along the sparsely vegetated edge of the river. These colonies of brightly coloured, extremely active fish could provide a powerful visual stimulus to attract ripe females. Under such breeding conditions, auditory stimuli may be of relatively little importance in longear reproduction.

When presented with an E phase visual choice of different-sized males of the two species, female longears chose male longears in both cement tank (Table 1) and activity box experiments (Figs. 6-9). In every case there was a switch from a C phase choice of male pumpkinseeds to an E phase choice of male longears, strongly suggesting the female longear test fish were responding to the sight of the two species of stimulus fish by visually discriminating between them and choosing male longears. For this to happen, the effect of the visual stimulus of the male longears on the female longears must have been sufficiently strong to outweigh the effect of any possible non-visual stimulus emitted by the male pumpkinseeds.

On the other hand, when the stimulus males were matched for size (Appendix I-4) conspecific males were not chosen by female longears (Fig. 10). In fact, the E phase choice was for male pumpkinseeds. As the C phase choice was also for male pumpkinseeds it is impossible to determine the basis for the E phase choice in this experiment as it may have resulted either from a choice based primarily on visual cues, or from a continued response to non-visual cues in the preceding C phase (Fig. 10). The former explanation is unlikely, however, as the results shown in figures 6-9 clearly demonstrate that female longears visually distinguish between males of the two species by choosing male longears. Hence female longears do not appear to distinguish visually between males of the two species when they are the same size.

Female longears offered a choice of different-sized longear and hybrid males, did not chose either in the C phase, but chose male longears in the E phase (Fig. 11). This choice was made in spite of the fact that a male hybrid, because of his larger size, larger opercula, and brighter colours, might be regarded as a "supernormal stimulus" (Tinbergen 1951). When longear and hybrid males were matched for size (Appendix I-6), the C phase choice of male hybrids, which may have resulted from their nest digging sounds, persisted in the E phase (Fig. 12). This persistence of choice suggests that female longears did not visually distinguish between equal-sized male longears and hybrids for the same reasons they did not distinguish between equal-sized male longears and pumpkinseeds (above).

Presented with large and small male pumpkinseeds, female longears chose large males in the C phase but chose small males in E-ON 2 (Fig. 13). This visual choice was for the smaller of the two males; that is, the male closer in size to the female's own size, and to the size of a male longear.

Summarizing results of all experiments in which female longears were the test fish; female longears visually chose the smaller stimulus male when presented with a choice of unequal-sized males, and tended not to choose visually between equal-sized males, regardless of which of the three types of males were used as stimulus fish.

In the study area, female longears choosing the smaller of two nesting males would almost certainly spawn with a conspecific partner. Male longears nest in the river and backwaters. If other males nest nearby, these will be either hybrids or pumpkinseeds, both of which are larger than nesting longear males. Hence the choice of small males by female longears demonstrated in these activity box experiments would tend to minimize hybridization or backcrossing if the same choice were made in the field.

2.2.2. Female Pumpkinseeds as Test Fish

Female pumpkinseeds offered a choice of males of the two species tended to either choose conspecific males or not choose in the C phase of both cement tank (Table 1) and activity box experiments (Figs. 14-17). Where a non-visual conspecific choice was made, it may well have been for the same reasons suggested above for the female longears' non-visual choice of male pumpkinseeds.

A visual choice for male pumpkinseeds in the E phase was made in the breeding season (Figs, 14-17) but in the non-breeding season, heterospecifics were chosen in E-ON 1 and E-OFF of the activity box experiments (Fig. 17), and no choice was exercised in the cement tank experiments (Table 1). This lack of visual choice of conspecifics in the winter may have occurred either because female pumpkinseeds were not motivated to choose the same way as in the summer, or, in the case of the cement tank results, because there was insufficient time for a choice to develop.

When small, medium and large female pumpkinseeds were offered a choice of small and large male pumpkinseeds, small females chose inconsistently in the C phase (Fig. 18); medium females chose small males in C-ON 1 (Fig.19); large females did not choose either male in the C phase. These results are difficult to explain individually, but collectively suggest that none of the three sizes of females showed a strong or persistent non-visual choice for either size of male. The same females in the E phase, however, consistently chose males closest in size to their own; small and medium females chose small males, large females chose large males (Figs. 18-20).

In the study area sexually mature male pumpkinseeds probably have to reach a certain threshold size before they begin to construct nests. Indirect evidence for this is the apparent excess of suitable spawning habitat where small ripe males could nest, and the fact that small male pumpkinseeds rarely built nests in the activity boxes, while large males often did (see, for example, Appendix I-12, 13). Direct evidence is the absence of small male pumpkinseeds on nests, though such males were seined from the backwaters. Hence small and medium female pumpkinseeds, which in these experiments chose small male pumpkinseeds, are in all probability unable to spawn with such males in the field. These females, if they spawn at all, would have to spawn with large male pumpkinseeds in the backwaters, or spawn with another species of sunfish more similar in size (i.e. male longears) in the river. Large female pumpkinseeds chose large male pumpkinseeds in the activity boxes and hence were likely to spawn with the large males if they made the same choice in the field. The larger of two fish usually attains dominance over the smaller fish in a competitive situation (Braddock 1945, Magnuson 1962, Miller 1963, Erickson 1967, Huck and Gunning 1967, Hadley 1969). Hence if a competition for large male spawning partners did develop, the large females would likely win, leaving no other choice for the small and medium-sized female pumpkinseeds but to seek heterospecific spawning partners. That they do so is supported by the fact that small and medium-sized ripe female pumpkinseeds have frequently been seined from spawning longear colonies and occasionally observed spawning in longear nests in the river where male pumpkinseeds never nest, while large ripe female pumpkinseeds have never been seined from these colonies.

When small and medium female pumpkinseeds were presented with a choice of nesting-sized male longears and hybrids, they chose male hybrids in C-ON 2, possibly in response to the nest-digging sounds of the hybrids (Appendix I-14), but switched over to choose male longears in the E phase (Fig. 21). The male longears were the smaller of the two stimulus fish and closest in length to the female pumpkinseeds (Appendix I-14).

Small and medium-sized female pumpkinseeds in the river seeking spawning partners encounter both colonially nesting male longears and male hybrids nesting either singly or with the longear males. If the same choice was exercised in the field as in the above experiment, female pumpkinseeds in the river would likely attempt to spawn with male longears rather than with male hybrids. The same spawning is also favoured by the considerable excess of longear nests over hybrid nests.

Summarizing results of all experiments using female pumpkinseeds as test fish shows that when female pumpkinseeds were offered a choice of conspecific males of different sizes (but presumably with other morphological features similar), the females visually chose males closest to their own size (Figs. 18-20). When offered a choice of different-sized males both of which possessed morphological characters rather different from those of conspecific males (i.e. male longears and hybrids), female pumpkinseeds again chose males closest to their own size (Fig. 21). When offered conspecific and heterospecific males, however, female pumpkinseeds chose conspecifics both visually and non-visually (Figs. 14-16) although on the basis of size alone (and assuming a choice of male closest in size to their own) they might have been expected to choose each species equally often (Appendix I-8, 9). This suggests that there may be factors in addition to size mediating female pumpkinseed responses to males.

2.2.3. Male Longears as Test Fish

Male longears offered a choice of female longears and pumpkinseeds did not choose either female in the cement tank experiments (Table 1) but chose heterospecific females in both lights-off periods and conspecific females in E-ON 1 of the activity box experiments (Fig. 22). While the latter weak conspecific visual choice agrees with the conspecific choice demonstrated by Keenleyside (1967), the cement tank results do not. Conditions in the cement tank experiments may have prevented males from approaching females closely enough to choose between them (Fig. 1). Alternately, male choice of females may be related to the state of male sexual maturity. Nest-digging in sunfish, one index of sexual maturity, is dependent on gonadal development which in turn is dependent on temperature and photoperiod (Smith 1969, 1970). Temperature differences between experiments were probably small. The cement tank experiments, however, were conducted in the late fall 3 to 4 months after the breeding season and fish had been held under natural photoperiod before use. None of the males tested dug nests in the apparatus and were not likely sexually mature. The experiments in the breeding season (Keenleyside 1967 and Fig. 22 of the present study) demonstrate a weak choice of conspecific females by male longears. While again nest digging was not always exhibited (Keenleyside 1967 series A and Appendix I-15 of the present study), males had been held under natural photoperiod before testing and male gonads may have been sufficiently ripe to promote the observed conspecific choice.

2.2.4. Male Pumpkinseeds as Test Fish

When offered a choice of longear and pumpkinseed females, male pumpkinseeds made no choice in either the C or E phase of the cement tank experiments (Table 1), but chose conspecific females in both the

C and E phase of the activity box experiments (Fig. 23). The activity box results are in conformity with Keenleyside's (1967) results while the cement tank results are not. The same explanation for the non-conformity of cement tank results may be offered for male pumpkinseeds as was offered for male longears. The fact that several of the activity box males tested did nest (Appendix I-16) may indicate that gonad development of the pumpkinseeds was at a more advanced stage than that of the longear males during testing, which might promote a stronger response to conspecific females than to heterospecific females if such a response again depended on sexual maturity.

In short, female longears chose conspecific males when offered a choice between longear and other males (pumpkinseeds or hybrids) in the size range of males nesting in the study area. Female pumpkinseeds usually chose conspecifics when offered a choice between pumpkinseed and longear males, but chose males closest in size to their own size when offered either a choice of large and small male pumpkinseeds or of male longears and hybrids. Male pumpkinseeds chose conspecific females when offered a choice of pumpkinseed and longear females in the breeding season while male longears weakly chose conspecifics when offered the same choice.

All of the fish tested in these experiments (female longears and pumpkinseeds, male longears and pumpkinseeds) demonstrated some ability to discriminate between conspecific and heterospecific fish of the other sex and to visually choose conspecifics. Furthermore the choice seemed influenced in the case of females by the size of the presented males. This ability is undoubtedly also present in sexually mature fish seeking spawning partners in the field. If this ability is used by such fish to choose conspecific spawning partners, then visual mate selection is an important barrier to hybridization between these two species of sunfish.

The presence of hybrids in the study area however indicates crossing does occur between the two species. The clear choice of conspecifics by male pumpkinseeds and female longears under these experimental conditions strongly suggests that hybridization is unlikely to occur between them if the choice in the above experiments persists in the field. Large female pumpkinseeds chose large male pumpkinseeds and in the study area would likely compete successfully with small and medium-sized pumpkinseed females for large male pumpkinseed spawning partners. This would necessitate small and medium-sized pumpkinseed females looking elsewhere for spawning partners and they might well enter male longear nests. Presumably they would be able to spawn successfully as male longears tended not, in these experiments, to exercise a strong conspecific choice of females. Hybridization therefore, most likely results from crosses between small and medium-sized pumpkinseed females and longear males. That such crosses actually occur in the study area was inferred by catching small and medium female pumpkinseeds when seining through longear colonies and confirmed by seeing such spawnings in longear nests.

3. Stripping Experiments

There appeared to be no consistent pattern in percent hatch of eggs manually stripped from either longear or pumpkinseed females when they were fertilized with conspecific milt (Figs. 24, 25). This lack of consistency likely resulted from the experimental method used to strip the eggs, as spawning females probably extrude only eggs which are fully ripe and ready to be fertilized. While experimental females were seined from spawning colonies and were used only if eggs flowed freely with slight abdominal pressure, as it has been suggested that otherwise eggs are generally not ripe (Smitherman and Hester 1962), the possibility existed that some or all of the eggs were either under- or over-ripe. If female sunfish spawn over extended periods of time, all the eggs contained in the ovary at any one time may not be in the same state of ripeness. Consequently the manual stripping of all of a female's eggs in less than two minutes would tend to produce a less than maximum percentage hatch in some of the containers into which the eggs were stripped. Ripeness differences between captured females may therefore have accounted for some of the differences between females in percent hatch of eggs. Further, manual stripping cannot insure that the eggs are necessarily squeezed out in the order in which they naturally would have been laid. Although the first eggs stripped were likely those that would have been first laid, some of the middle containers may have contained eggs which would not ordinarily have been extruded until the end of a female's spawning. This may have accounted for some of the fluctuations within females in percentage hatch. In the face of these inconsistencies between and within females in percent hatch of artificially stripped eggs, no "order effect" could be ascribed to ripeness of eggs from the females used in these stripping experiments; i.e percent hatch of eggs did not appear to vary consistently with the order in which those eggs were stripped from the females.

Female hybrids were not observed in the study area, which was not surprising in view of the many reports of male predominance among hybrid Lepomis sunfish ranging from 80-100% F_1 males (Hubbs and Hubbs 1933, Bailey and Lagler 1938, Ricker 1948, Hubbs 1955, Lagler and Steinmetz 1957, Childers and Bennett 1961, Childers 1967, Birdsong and Yerger 1967, Etnier 1968). In the apparent absence of female hybrids, only the five identifiable types of sunfish present in the study were artificially crossed to determine if post-mating isolating mechanisms were operating against hybridization.

Childers (1967) found the average functional life spans of gametes of four species of sunfish were approximately 1 hour for ova and 1 minute for sperm. As the time until the last eggs laid were exposed to milt was no more than 2 minutes using stripping Method A (Materials and Methods section 6.3), all ova should have been receptive to fertilization. The milt was stripped directly on the eggs, so it was assumed that essentially no time elapsed between milt stripping and fertilization. In spite of this, however, the percentage hatch of fertilized eggs tended to be rather low when stripping Method A was used (Table 2). Further, 15 of 20 of the first and last fertilized containers had the highest and lowest percent hatches respectively (Appendix IV-1) and if replicate 2 was eliminated because the male pumpkinseed was not very fertile (producing only 1 hatched fry from 468 eggs), then 15 of 18 of the first and last fertilized containers had the highest and lowest percent hatches respectively. This suggested that the more time which had elapsed between stripping of eggs and application of milt, the lower the percent hatch of the eggs. Certainly when the time delay between stripping of eggs and stripping of sperm was eliminated by using Method B, percent hatch increased (Table 2), confirming that for maximum hatching success, eggs should be fertilized as soon after they are extruded as possible. When spawning occurs in the study area, fertilization must be virtually instantaneous as the resident male shudders and neighbouring males intrude, both behaviours presumably signalling sperm release (Keenleyside 1972), when the spawning female tilts releasing eggs. Hence Method B was superior to Method A not only because it produced a greater percent hatch but also because it more closely simulated the timing of natural fertilization.

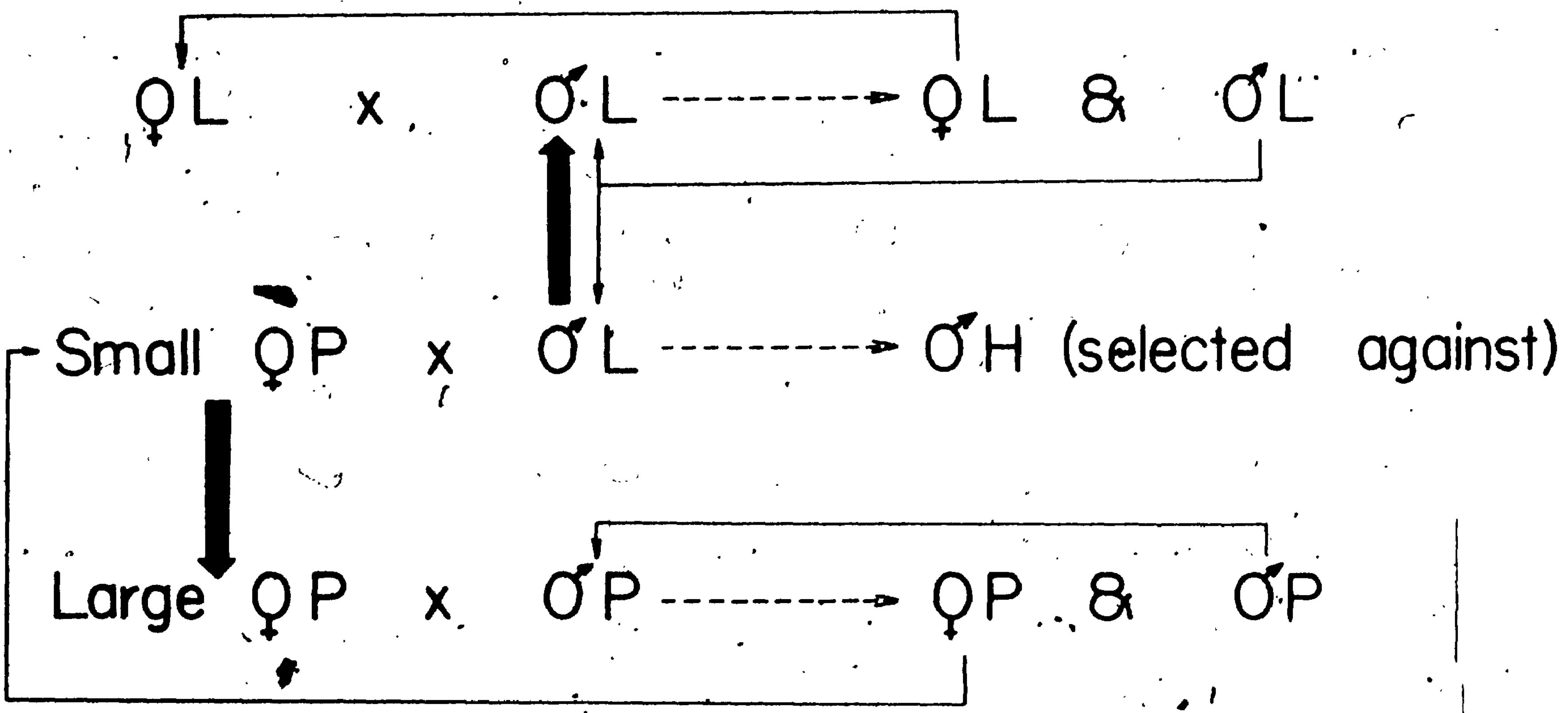
When pumpkinseed eggs were fertilized with milt of the three types of males using Method B, the percent hatch of the hybrid cross was significantly lower than the other two crosses (Table 2). When longear eggs were fertilized with milt of the same males, the longear male cross was significantly higher than the other two crosses. Pumpkinseed females would therefore maximize their chances of leaving offspring by spawning with either pumpkinseed or longear males while longear females would maximize their chances of leaving offspring by spawning with longear males. Thus maximization of offspring is achieved if females spawning in the study area choose as spawning partners the same males they chose in the cement tank and activity box experiments.

Hybrid backcrosses in these experiments produced significantly lower percent hatches than either of the conspecific crosses (Table 2). Hubbs and Hubbs (1933) suggested F_1 sunfish hybrids were sterile and Hubbs later reiterated that belief (1955). While others have also reported sterile male *Lepomis* hybrids (Birdsong 1963, Birdsong and Yerger 1967, West 1970) and suggested abnormal gonad development and/or abnormal spermatogenesis as the cause of this sterility, others have found F_1 Lepomis hybrids to be fully fertile with a sex ratio often approaching 1:1 (Lagler and Steinmetz 1957, Childers and Bennett 1961, Smitherman and Hester 1962, Childers 1967, West 1970). The hybrid males in this study had well developed gonads and cursory microscopic examination of the sperm revealed no striking abnormalities in size and motility. Microscopic examination of the eggs shortly after fertilization revealed that all had been fertilized, so death of the developing embryos occurred sometime between fertilization and hatching. This death may have occurred during gastrulation, a critical stage in embryo development when "nuclear genetic factors (the genes), dormant throughout cleavage, begin to manifest themselves" (Balinsky 1970: 242).

Pumpkinseed eggs had a consistently higher percent hatch than longear eggs, regardless of the type of male or method used to fertilize the eggs (Table 2). As the experimental conditions under which the eggs were stripped, fertilized and hatched were the same for both species' eggs, the difference in their hatchability must be due either to an inherent difference in the eggs themselves or to there being a greater difference between the natural and experimental conditions for longear eggs than for pumpkinseed eggs. Considering the former, the only readily observed morphological difference in the eggs of the two species was that pumpkinseed eggs were consistently smaller than longear eggs. As success of crossing was not affected by egg size when four different genera of centrarchids were crossed (Merriner 1971) nor did survival rate seem to be correlated with egg size within a species (Lindsey and Ali 1971), it is unlikely that size differences between the species' eggs accounted for the differences in percent hatch. Consequently the difference in percent hatch of the two species eggs is more likely attributable to greater differences between natural and experimental conditions for longear eggs than for pumpkinseed eggs.

In short, postmating isolating mechanisms appear to be operating between these two species of sunfish. Reduced hybrid fertility in backcrosses, acting through zygote mortality, should act to reduce the incidence of hybridization, as those parental genotypes which originally contributed to the formation of the hybrid should ultimately be selected against. This selection should spare genotypes of fish which chose conspecific spawning partners and encourage the development of premating isolating mechanisms as suggested by Dobzhansky (1970). As the majority of the interbreeding between the species most likely occurs between small female pumpkinseeds and male longears, it should be those parental genotypes which would be selected against. The small female pumpkinseeds, however, may return to breed in subsequent years, and as they become larger from year to year will likely breed with male pumpkinseeds. This cross would of course produce pumpkinseed offspring in which case the female parent's genotype would not be selected against. The female pumpkinseed offspring resulting from this cross might well choose a male longear spawning partner the first year or two that she becomes ripe and ready to spawn as her mother did before her (Fig. 26). Furthermore, both male and female longears are polygamous; nesting males spawn with more than one female and vice versa during peak spawning periods. Considering that female longears probably choose male longear spawning partners, and that

Figure 26. Model of hybrid perpetuation. Genotypes of hybrid-producing parents are not rapidly selected against because they both may mate with conspecifics either previously ([illegible] longears) or subsequently (small female pumpkinseeds, male longears) thereby preserving their genotypes. Thick arrows, previous or subsequent spawnings; thin arrows, possible crosses of F_1 offspring upon reaching sexual maturity; dashed arrows, F_1 produced by the parental generation.


♀L x ♂L → ♀L & ♂L
Small ♀P x ♂L → ♂H (selected against)
Large ♀P x ♂P → ♀P & ♂P

there are about three times as many female longears as female pumpkinseeds in the study area (Keenleyside unpublished), the male longear parent of the hybrid almost certainly spawns with conspecific females either before or after the heterospecific spawning. In either case, conspecific spawning occurs and the male genotype is perpetuated (Fig. 26). Under these circumstances, hybridization may continue for a long time in the study area even though the hybrids are at a reproductive disadvantage relative to the two parental species.

Sibley (1957) postulated that when populations come secondarily into contact, either intrinsic isolating mechanisms have been established, in which case no hybridization occurs, or isolating mechanisms have not been established, in which case hybridization does occur. If the latter, either the hybrids are not selected against, in which case introgression and subsequent selection may cause the two populations to converge to form a single population or hybrids are selected against, in which case incipient isolating mechanisms between the two species are reinforced "until gene exchange between them is virtually or entirely stopped" (Sibley 1957, p. 170).

The sunfish model of hybridization proposed in Figure 26 does not fit either of the hybridization models proposed by Sibley (1957). Rather it seems to be intermediate in that interbreeding will tend to continue although selection is acting against the hybrids. This situation is likely to continue until small female pumpkinseeds spawn with male pumpkinseeds, or until male longears begin actively discriminating against female pumpkinseeds as spawning partners, or until other premating barriers to hybridization become more effective.

SUMMARY

1. Female and male sunfish, both longears and pumpkinseeds were offered a choice of male and female sunfish in two kinds of choice apparatus. Results of one choice apparatus, in conjunction with the results of the other choice apparatus which employed simplifying assumptions in data analysis, lead to the following tentative conclusions regarding approach to a member of the opposite sex:
    a) Female longears offered a choice of nesting-sized longear males and either nesting-sized pumpkinseed or hybrid males (both larger than the longear males) chose consepcifics. Size apparently influenced this choice as no choice was exercised when female longears were presented with male longears and equal-sized males of the other two types.
    b) Female pumpkinseeds offered a choice of nesting-sized longear and pumpkinseed males also chose conspecifics. Again male size was implicated as female pumpkinseeds of various sizes chose males closest in size to their own when offered a choice unequal-sized pumpkinseed males.
    c) Male pumpkinseeds chose conspecifics but male longears did not choose when both were offered a choice of unequal-sized pumpkinseed and longear females.
2. Hybridization in the study area likely occurs between small female pumpkinseeds and male longears.
3. Hybrid males, artificially backcrossed to both longear and pumpkinseed females, produced a significantly lower percent hatch of eggs than either intraspecific cross.

4. Selection against the hybrids through their reduced fertility will act very slowly to reduce the incidence of hybridization in the study area as the female parental genotype (pumpkinseed) will be maintained through her subsequent matings with conspecific males, and the male parental genotype (longear) will also be maintained through previous or subsequent conspecific matings.

Appendix I Fork Lengths of Fish Used in Activity Box Experiments.

I-1. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 6. L, longear; P, pumpkinseed; N, fish dug nest in activity box gravel during experiment.

	Test fish	Stimulus fish	
	♀ L	♂ L	♂ P
1	7.0	8.3	9.5
2	7.8	8.3	11.0
3	8.6	8.5	10.3 (N)
4	7.5	8.5	11.1
5	7.5	8.4	10.7 (N)
6	7.5	7.9 (N)	10.3
7	7.2	9.1	11.2 (N)
8	7.7	8.8	11.3
9	8.0	9.1	11.3
10	6.6	8.2	11.0
Mean	7.54	8.51	10.77
S.D.	0.55	0.39	0.57

I-2. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 7. L, longear; P, pumpkinseed; N, fish dug nest in activity box gravel during experiment.

	Test fish	Stimulus fish		
	♀L	♂L	♂P	
1	7.8	9.5	12.5	
2	8.1	10.5	12.8	
3	8.7	10.5	12.8	
4	7.4	10.5	12.8	
5	7.5	10.5	16.0	(N)
6	7.5	10.5	16.0	(N)
7	7.8	8.5	16.5	(N)
8	8.5	9.4	12.5	
9	7.8	9.4	12.5	
10	9.0	9.5	12.5	
Mean	8.01	9.88	13.69	
S.D.	0.55	0.71	1.72	

I-3. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 9. L, longear; P, pumpkinseed.

	Test fish	Stimulus fish	
	♀ L	♂ L	♂ P
1	9.3	10.3	13.1
2	9.1	9.8	12.1
3	8.2	9.2	11.5
4	10.3	9.8	11.6
5	9.2	9.4	11.8
6	8.0	9.0	10.6
7	8.9	10.2	10.8
8	9.1	8.7	12.3
9	8.8	9.0	12.7
Mean	8.99	9.49	11.83
S.D.	0.66	0.57	0.82

I-4. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 10. L, longear; P, pumpkinseed; N, fish dug nest in activity box gravel during experiment.

	Test fish	Stimulus fish	
	♀ L	♂ L	♂ P
1	7.2	9.2	9.3
2	8.5	9.2	9.3
3	8.0	7.6 (N)	8.0
4	7.5	9.6	10.0
5	8.0	9.6	10.0
6	8.2	9.2	9.3 (N)
7	9.0	9.2	9.3
8	8.5	11.0	11.2
9	7.8	8.9	9.0
10	7.0	9.6	10.0
11	7.5	9.6	10.0
12	6.5	11.0 (N)	11.2
13	7.4	8.7	8.5
14	6.6	8.9	9.0
15	7.8	8.5	8.7
16	7.6	8.5	8.5
Mean	7.69	9.27	9.46
S.D.	0.68	0.86	0.91

I-5. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 11. L, longear; H, hybrid; N, fish dug nest in activity box gravel during experiment.

	Test fish	Stimulus fish	
	♀ L	♂ L	♂ H
1	8.8	8.0	10.5
2	8.3	8.0	10.5
3	7.7	8.4	11.5
4	7.4	8.5	12.1
5	8.3	8.0	10.5
6	7.1	8.4 (N)	11.5
7	6.6	8.4 (N)	11.5
8	7.4	8.3	10.4 (N)
9	8.5	8.3	10.4 (N)
10	7.8	8.7	11.6 (N)
11	8.5	8.3	12.0 (N)
12	7.3	8.4	11.3
Mean	7.81	8.31	11.15
S.D.	0.67	0.22	0.65

I-6. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 12. L, longear; H, hybrid; N, fish dug nest in activity box gravel during experiment.

	Test fish	Stimulus fish	
	♀L	♂L	♂H
1	8.6	10.7	10.5
2	9.5	10.3	10.2 (N)
3	8.4	10.9	11.1 (N)
4	9.2	10.7	10.3 (N)
5	8.1	10.7	10.3 (N)
6	9.3	10.0	9.8 (N)
7	8.7	10.0	9.8 (N)
8	7.9	10.7 (N)	10.5
9	9.1	10.7 (N)	10.5
10	8.0	10.3	10.2
11	8.0	10.3	10.2
12	8.5	10.9	11.1 (N)
13	7.9	10.9	11.1 (N)
14	8.2	10.7	10.3 (N)
15	9.0	10.0	9.8 (N)
Mean	8.56	10.52	10.38
S.D.	0.55	0.34	0.44

I-7. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 13. L, longear; P, pumpkinseed.

	Test fish	Stimulus fish	
	♀L	sm. ♂ P	lg. ♂ P
1	9.0	10.4	15.0
2	8.4	10.8	15.1
3	8.3	10.6	15.0
4	8.5	9.2	17.2
5	9.1	10.8	15.1
6	7.9	10.8	14.8
7	8.2	10.6	15.0
8	8.2	10.4	15.0
9	8.5	10.4	15.0
10	8.3	10.8	15.1
11	8.0	10.8	14.8
12	8.5	10.6	15.0
Mean	8.39	10.52	15.18
S.D.	0.66	0.45	0.65

I-8. Fork lengths (cm) of fish used in the experiment the results of which are shown in Figure 14. L, longear; P, pumpkinseed; N, fish dug nest in activity box gravel during experiment.

	Test fish	Stimulus fish	
	♀ P	♂ L	♂ P
1	9.5	8.3	9.3
2	- *	8.3	9.5
3	8.9	8.1	10.0
4	9.9	8.0	9.7
5	8.9	8.3	11.0
6	9.2	8.5	11.1
7	9.6	8.4	10.7 (N)
8	10.8	7.9	10.3
9	10.5	9.1 (N)	11.2
10	9.0	8.8	11.3
11	9.4	9.1	11.3
12	9.1	8.2	11.0
Mean	[illegible]3	8.42	10.53
S.D.	0.64	0.40	0.74

*died after experiment and discarded before fork length measured.

I-9. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 15. L, longear; P, pumpkinseed; N, fish dug nest in activity box gravel during experiment.

	Test fish	Stimulus fish	
	♀ P	♂ L	♂ P
1	12.2	9.4 (N)	14.5 (N)
2	12.5	9.8	14.2 (N)
3	12.5	9.8	14.2 (N)
4	12.2	9.0	14.0 (N)
5	11.7	10.2 (N)	14.2 (N)
6	12.3	10.4 (N)	13.9 (N)
7	13.3	10.4 (N)	13.9 (N)
8	13.0	9.4 (N)	14.5 (N)
9	14.6	9.4 (N)	14.5 (N)
10	11.2	9.8	14.2 (N)
11	10.6	9.0	14.0 (N)
12	10.3	10.2	14.2 (N)
13	10.3	10.4 (N)	13.9 (N)
14	9.8	10.4 (N)	13.9 (N)
Mean	11.89	9.83	14.15
S.D.	1.34	0.52	0.23

I-10. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 17. L, longear; P, pumpkinseed; N, fish dug nest in activity box gravel during experiment.

	Test fish	Stimulus fish	
	♀P	♂L	♂P
1	11.9	10.3	13.1
2	11.4	9.8	11.6
3	11.1	9.4	11.8
4	12.3	9.0	10.6
5	12.2	9.8	13.2
6	14.9	9.2	12.4
7	15.0	8.2	13.8 (N)
Mean	12.69	9.39	12.36
S.D.	1.60	0.68	1.11

I-11. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 18. L, longear; P, pumpkinseed.

	Test fish	Stimulus fish	
	♀P	sm. ♂P	lg. ♂P
1	10.3	10.4	15.0
2	8.5	10.8	15.1
3	10.2	10.8	15.1
4	9.0	10.8	14.8
5	8.5	9.2	17.2
6	9.5	10.4	15.0
7	9.4	10.4	15.0
8	9.8	10.8	15.1
9	9.4	10.8	15.1
10	9.0	10.8	14.8
Mean	9.34	10.52	15.22
S.D.	0.66	0.50	0.71

I-12. Fork lengths (cm) of fish used in the experiments the results of which are shown in figure 19. L, longear; P, pumpkinseed; N, fish dug nest in activity box gravel during experiment.

	Test fish	Stimulus fish	
	♀ P	sm. ♂ P	lg. ♂ P
1	12.6	10.4	15.0 (N)
2	12.4	9.4	17.2 (N)
3	12.2	9.4	17.2 (N)
4	12.7	10.4	15.0 (N)
5	11.8	10.4	15.0 (N)
6	11.0	10.8	15.1 (N)
7	11.0	10.8	14.8 (N)
8	12.2	10.8	14.8 (N)
9	11.5	10.6	15.0 (N)
10	11.2	10.6	15.0 (N)
11	12.4	10.4	15.0 (N)
12	12.0	10.4	15.0 (N)
13	12.1	10.8	15.1
14	11.4	10.8	15.1
15	12.4	10.8	14.8
16	12.0	10.8	14.8
17	11.0	10.6	15.0 (N)
18	12.6	10.6	15.0 (N)
Mean	11.92	10.49	15.22
S.D.	0.52	0.43	0.73

I-13. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 20. L, longears, P, pumpkinseed; N, fish dug nest in activity box gravel during experiment.

	Test fish	Stimulus fish	
	♀P	sm. ♂P	lg. ♂P
1	16.4	10.4	15.0
2	13.0	10.8	15.1
3	14.7	10.8	14.8
4	13.3	10.6	15.0 (N)
5	13.8	9.4	17.2 (N)
6	14.6	10.8	15.1 (N)
7	13.5	10.6	15.0 (N)
8	15.5	9.2	17.2 (N)
9	14.8	10.8	15.1 (N)
10	15.0	10.8	14.8 (N)
11	13.4	10.6	15.0 (N)
12	14.5	10.4	15.0
Mean	14.38	10.43	15.36
S.D.	1.01	0.55	0.87

I-14. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 21. L, longear; P, pumpkinseed; H, hybrid; N, fish dug nest in activity box gravel during experiment.

	Test fish	Stimulus fish	
	♀ P	♂ L	♂ H
1	10.6	9.5	13.8 (N)
2	11.0	9.5	13.8 (N)
3	11.8	9.5	13.2 (N)
4	11.6	9.5	13.2 (N)
5	11.5	9.7	13.8 (N)
6	10.6	9.7	13.8 (N)
7	10.4	9.1	13.9 (N)
8	11.5	9.4	12.6 (N)
9	12.6	9.4	12.6 (N)
10	10.4	9.5	13.8 (N)
11	12.1	9.5	13.2
12	11.7	9.7	13.8 (N)
13	10.4	9.7	13.8 (N)
14	10.6	9.1	13.9
15	11.2	9.4	12.6 (N)
16	11.1	9.4	12.6 (N)
Mean	11.19	9.48	13.40
S.D.	0.67	0.19	0.54

I-15. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 22. L, longear; P, pumpkinseed.

	Test fish	Stimulus fish	
	♂ L	♀ L	♀ P
1	8.4	7.4	11.0
2	8.6	6.8	8.5
3	7.9	8.1	8.4
4	7.9	6.7	8.5
5	8.1	6.7	9.9
6	9.4	7.6	9.9
7	7.9	7.4	9.9
8	7.1	7.6	10.6
9	7.7	7.0	9.2
10	8.5	7.8	11.4
11	9.4	7.5	12.2
12	9.3	7.7	9.0
13	8.5	7.5	9.1
14	8.1	6.7	9.0
15	8.3	7.5	10.4
16	9.0	7.8	10.3
17	10.5	7.5	9.2
18	8.4	5.9	12.0
19	8.9	6.1	– *
20	– *	– *	12.3
Mean	8.55	7.28	10.04
S.D.	0.77	0.59	1.27

*Fork lengths not recorded

I-16. Fork lengths (cm) of fish used in the experiment the results of which are shown in figure 23. L, longear; P, pumpkinseed.

	Test fish	Stimulus fish	
	♂P	♀ P	♀ L
1	11.0	11.0	7.4
2	9.6	8.4	8.1
3	10.5 (N)	9.9	7.7
4	11.1	11.3	6.7
5	10.2	10.8	7.1
6	12.3	9.9	7.6
7	11.0 (N)	9.9	7.4
8	10.6 (N)	10.6	7.6
9	11.2	9.2	7.0
10	11.4	11.4	7.8
11	12.8	12.2	7.5
12	11.5	9.0	7.7
13	12.4	9.1	7.5
14	11.6	10.4	7.5
15	11.8	10.3	7.8
16	12.0	9.2	7.5
17	10.1	9.5	7.0
18	11.2	9.5	6.8
19	11.9	12.0	5.9
20	10.6	— *	6.1
Mean	11.24	10.19	7.29
S.D.	0.83	1.06	0.56

*Fork length not recorded

Appendix II Egg Ripeness and Order of Extrusion.

II-1. LXL Crosses

	Plexiglass Container Number										
	1	2	3	4	5	6	7	8	9	10	11
# Dead	178	84	39	18	79	55	59	72	35	36	58
# Hatch	50	50	125	91	67	68	104	78	45	19	39
Total	228	134	164	109	146	103	163	150	80	55	97
% Hatch	22	37	76	84	46	66	64	52	56	35	40
# Dead	30	19	46	45	122	59	117	92	115	90	61
# Hatch	113	142	108	216	208	182	126	71	145	111	179
Total	143	161	154	261	420	241	243	163	260	201	240
% Hatch	29	88	70	83	71	76	42	44	56	53	75
# Dead	170	140	155	56	94	35	16				
# Hatch	70	65	72	140	123	129	41				
Total	240	205	227	196	217	164	57				
% Hatch	29	32	32	71	57	79	72				
# Dead	81	155	117	82	75	81	50				
# Hatch	177	80	90	36	75	62	54				
Total	258	235	207	118	150	143	84				
% Hatch	69	34	43	31	50	43	41				
# Dead	58	73	75	65	56	62	32				
# Hatch	87	137	50	85	47	31	7				
Total	145	210	125	150	103	93	39				
% Hatch	60	65	40	57	46	33	18				
# Dead	50	115	121	95	74	36					
# Hatch	121	86	99	11	46	11					
Total	171	201	220	106	120	47					
% Hatch	71	43	45	10	38	23					

II-2. PxP Crosses

	Plexiglass Container Number											
	1	2	3	4	5	6	7	8	9	10	11	12
# Dead	74	96	90	73	111	107	157	129	174	163	188	
# Hatch	154	175	113	114	179	175	177	152	155	121	60	
Total	228	271	203	187	290	282	334	281	329	284	248	
% Hatch	67	64	56	60	61	62	52	54	47	42	24	
# Dead	67	43	46	39	44	41	41	71	67	91	155	
# Hatch	74	110	98	84	53	117	94	78	97	37	210	
Total	141	153	144	123	97	158	135	149	164	128	365	
% Hatch	53	72	68	68	55	74	70	52	59	29	58	
# Dead	60	19	16	31	33	45	29	69	62	83	64	
# Hatch	51	50	60	118	99	130	151	96	72	69	79	
Total	111	69	76	149	132	175	180	165	134	152	143	
% Hatch	46	73	79	79	75	74	84	58	54	45	55	
# Dead	464	240	349	285	166	113	137	112	113	96	120	142
# Hatch	36	104	138	227	247	285	210	263	333	287	263	174
Total	500	344	487	512	413	398	347	375	446	383	383	316
% Hatch	7	30	28	44	60	72	61	70	74	74	69	55
# Dead	25	10	16	11	15	24	50	59	22	31		
# Hatch	210	224	251	215	235	274	260	325	245	198		
Total	235	234	267	226	250	298	310	384	267	229		
% Hatch	89	96	94	95	94	92	84	85	91	86		
# Dead	41	39	76	71	82	104	60	81	97	95	94	
# Hatch	310	380	215	210	265	225	254	190	185	175	215	
Total	351	380	291	281	347	329	314	271	282	270	309	
% Hatch	88	91	74	75	76	68	81	70	66	65	70	

Appendix III Measurements of Egg Diameters.

III-1 Diameters (mm) of eggs from eight female longears.

	1	2	3	4	5	6	7	8
	1.31	1.31	1.35	1.38	1.40	1.28	1.28	1.35
	1.28	1.35	1.42	1.44	1.33	1.31	1.31	1.44
	1.30	1.30	1.45	1.49	1.35	1.28	1.33	1.33
	1.40	1.28	1.31	1.45	1.30	1.23	1.42	1.35
	1.37	1.33	1.35	1.47	1.33	1.38	1.31	1.38
	1.31	1.35	1.33	1.61	1.28	1.28	1.44	1.33
	1.31	1.31	1.49	1.31	1.40	1.25	1.28	1.38
	1.45	1.33	1.40	1.31	1.25	1.25	1.49	1.38
	1.31	1.35	1.35	1.31	1.35	1.25	1.38	1.31
	1.31	1.31	1.56	1.38	1.28	1.21	1.37	1.37
	1.33	1.31	1.45	1.44	1.25	1.18	1.31	1.38
	1.44	1.33	1.37	1.56	1.31	1.12	1.31	1.35
	1.42	1.30	1.59	1.35	1.33	1.25	1.31	1.33
	1.31	1.28	1.42	1.49	1.40	1.28	1.30	1.37
	1.37	1.37	1.31	1.47	1.28	1.18	1.35	1.38
	1.40	1.37	1.40	1.45	1.38	1.28	1.31	1.38
	1.38	1.31	1.35	1.42	1.30	1.21	1.37	1.35
	1.38	1.38	1.30	1.45	1.35	1.26	1.35	1.35
	1.38	1.35	1.44	1.45	1.28	1.10	1.38	1.42
	1.33	1.38	1.35	1.59	1.35	1.30	1.31	1.35
Mean	1.35	1.33	1.40	1.44	1.33	1.25	1.35	1.36
S.D.	0.05	0.03	0.08	0.09	0.05	0.06	0.06	0.03

Total Mean 1.35

S.D. 0.08

III-2 Diameter (mm) of eggs from five female pumpkinseeds.

	1	2	3	4	5
	1.11	1.11	1.14	1.21	1.11
	1.06	1.12	1.23	1.21	1.14
	1.09	1.11	1.19	1.21	1.11
	1.14	1.14	1.18	1.12	1.14
	1.11	1.11	1.21	1.23	1.11
	1.00	1.12	1.18	1.21	1.21
	1.14	1.11	1.18	1.25	1.11
	1.21	1.11	1.18	1.21	1.16
	1.14	1.18	1.14	1.18	1.11
	1.28	1.18	1.21	1.14	1.21
	1.25	1.16	1.18	1.21	1.11
	1.12	1.14	1.14	1.18	1.14
	1.26	1.14	1.18	1.21	1.18
	1.14	1.16	1.18	1.19	1.14
	1.16	1.09	1.16	1.16	1.07
	1.23	1.09	1.21	1.21	1.11
	1.14	1.11	1.21	1.28	1.14
	1.07	1.16	1.28	1.18	1.07
	1.21	1.14	1.14	1.19	1.18
	1.18	1.09	1.18	1.21	1.02
Mean	1.15	1.13	1.19	1.20	1.13
S.D.	0.07	0.03	0.03	0.03	0.05

Total Mean 1.16

S.D. 0.05

Appendix IV Total Eggs Stripped, Numbers of Eggs Hatched and Percent Hatch for Artificial Crossing Experiments.

IV-1. Experiments using stripping method A. Summer 1.
P, pumpkinseed; L, longear; H, hybrid.
Numbers in brackets indicate the order in which males were stripped.

		♂P				♂L				♂H			
		Total	# Hatch	%		Total	# Hatch	%		Total	# Hatch	%	
1.	♀P	123	25	20.3	(3)	157	59	37.6	(1)	167	58	34.7	(2)
	♀L	112	8	7.1		138	61	44.2		124	30	14.0	
2.	♀P	268	1	0.4	(1)	386	220	57.0	(2)	356	155	43.5	(3)
	♀L	200	0	0.0		223	77	34.5		328	83	25.3	
3.	♀P	467	330	70.7	(3)	251	242	96.8	(1)	258	186	72.1	(2)
	♀L	266	13	4.9		221	136	61.5		191	17	8.9	
4.	♀P	224	25	11.2	(2)	238	23	9.7	(3)	219	49	22.4	(1)
	♀L	301	23	7.6		227	5	2.2		295	12	4.1	
5.	♀P	244	156	63.9	(1)	204	99	48.5	(2)	274	118	43.1	(3)
	♀L	140	8	5.7		119	0	0.0		140	0	0.0	
6.	♀P	289	170	58.8	(3)	277	250	90.3	(1)	313	152	48.6	(2)
	♀L	124	0	0.0		100	5	5.0		132	0	0.0	
7.	♀P	206	171	83.0	(2)	267	146	54.7	(3)	292	264	90.4	(1)
	♀L	159	45	28.3		142	21	14.8		159	42	26.4	
8.	♀P	491	404	82.3	(1)	532	403	75.8	(2)	552	369	66.8	(3)
	♀L	204	20	9.8		222	6	2.7		209	7	3.3	
9.	♀P	210	79	25.5	(3)	364	195	53.6	(1)	398	185	46.5	(2)
	♀L	275	52	18.9		214	65	20.7		272	34	12.5	
10.	♀P	278	105	37.8	(2)	339	96	28.3	(3)	315	149	46.3	(1)
	♀L	340	204	60.0		487	234	47.9		328	195	59.5	
Total	♀P	2900	1466	50.6		3015	1733	57.5		3144	1685	53.6	
	♀L	2121	373	17.6		2093	610	29.1		2178	420	19.3	

IV-2. Experiments using stripping method B. Replicates 1-10, Summer 1; replicates 11-15, Summer 2.

		♂P			♂L			♂H		
		Total	# Hatch	%	Total	# Hatch	%	Total	# Hatch	%
1.	♀P	605	515	85.1	352	329	93.5	827	607	73.4
	♀L	118	108	91.5	107	101	94.4	108	74	68.5
2.	♀P	361	319	88.4	268	252	94.0	145	126	86.9
	♀L	193	87	45.1	143	117	82.4	90	20	22.2
3.	♀P	357	331	92.7	368	310	84.2	370	286	77.3
	♀L	376	102	28.2	422	249	59.0	362	27	7.5
4.	♀P	299	224	74.9	335	282	84.2	244	177	72.4
	♀L	149	49	32.9	226	133	54.8	226	54	23.9
5.	♀P	239	187	78.2	282	270	95.8	215	182	84.7
	♀L	110	36	32.8	124	117	94.4	89	67	75.3
6.	♀P	180	169	93.9	165	143	86.7	205	140	69.8
	♀L	108	96	88.9	96	77	80.2	79	45	57.0
7.	♀P	492	484	98.4	522	468	89.7	435	425	97.7
	♀L	386	285	73.8	227	214	94.3	222	174	78.4
8.	♀P	402	393	97.8	463	442	95.5	309	212	68.6
	♀L	199	144	72.4	265	211	79.6	314	184	58.6
9.	♀P	277	228	82.3	464	366	78.9	422	357	84.6
	♀L	124	11	8.9	78	53	67.9	230	40	17.4
10.	♀P	135	92	68.1	320	129	55.9	326	34	10.2
	♀L	181	146	80.7	346	230	66.5	245	164	66.9
11.	♀P	389	374	96.1	428	360	84.1	474	382	81.6
	♀L	327	205	62.7	447	318	71.1	490	262	53.5
12.	♀P	327	182	55.7	342	325	95.0	336	238	70.8
	♀L	500	376	75.3	321	290	90.3	362	292	80.7
13.	♀P	349	284	81.4	351	268	76.4	390	65	16.7
	♀L	324	287	88.6	439	285	64.9	315	54	17.1
14.	♀P	183	174	95.1	174	170	97.7	71	18	25.4
	♀L	267	198	74.3	226	211	93.4	248	173	69.8
15.	♀P	280	268	95.7	213	205	96.2	415	132	31.8
	♀L	423	310	73.3	293	286	97.6	245	93	38.0
Total	♀P	4875	4224	86.6	5047	4369	86.6	5184	3381	65.2
	♀L	3785	2440	64.4	3760	2892	76.9	3625	1723	47.5

Appendix V . Analysis of Variance Tables.

V-1 Analysis of variance of cement tank data (XO data not included)

Source		df	MS	F	P
Type of Fish	(Fixed)	3	1.0	<1	ns[1]
Behaviour	(Fixed)	6	1845.6	462.6	<0.001
Type-Behaviour		18	7.9	1.97	<0.025
Fish	(Random)	76	2.3		
Behaviour - Fish		456	4.0		
Experimental Conditions	(Fixed)	80	3.9		
Behaviour - E.C.		480	4.6		

Since the Type-Behaviour interaction is significant, detailed investigations among behaviours may be pursued.

Source	df	MS	F	P
1. PC - PH in C				
♀ P	1	24.80	6.22	<0.025
♀ L	1	4.88	1.22	>0.10
♂ P	1	0.00	<1	ns
♂ L	1	0.58	<1	ns
2. PC - PH in E				
♀ P	1	1.30	<1	ns
♀ L	1	69.54	17.43	<0.001
♂ P	1	5.58	1.40	>0.10
♂ L	1	0.13	<1	ns
3. NC - NH in E				
♀ p	1	1.69	<1	ns
♀ L	1	57.14	14.32	<0.001
♂ p	1	1.81	<1	ns
♂ L	1	0.02	<1	ns
4. FC - FH in E				
♀ p	1	0.24	<1	ns
♀ L	1	4.46	1.12	>0.25
♂ p	1	0.50	<1	ns
♂ L	1	0.28	<1	ns

Source	df	MS	F	p
5. (FC, NC, PC) - (PH, NH, FH) in C				
♀ P	1	18.12	2.04	>0.05
♀ L	1	1.97	<1	ns
♂ P	1	0.04	<1	ns
♂ L	1	0.04	<1	ns
6. (FC, NC, PC) - (PH, NH, FH) in E				
♀ P	1	0.14	<1	ns
♀ L	1	108.12	27.10	<0.001
♂ P	1	2.99	<1	ns
♂ L	1	0.34	<1	ns

[1] ns - not significant

V-2 Analysis of variance of cement tank XO data

Source	df	MS	F.	P
Type of Fish (Fixed)	3	6.61	3.70	< 0.05
Fish	76	1.78		
Experimental Condition(Fixed)	1	77.92	102.35	< 0.001
Type - E.C. Interaction	3	0.73	<1	
Fish - E.C. Interaction	76	0.76		
(E-C) x (♀P - ♀L)	1	0.04	<1	ns
(E-C) x (♂P - ♂L)	1	0.30	<1	ns
(E-C) x (♀ - ♂)	1	1.85	2.43	>0.05
♀P - ♀L	1	3.50	1.96	>0.05
♂P - ♂L	1	3.90	2.19	>0.05
♀ - ♂	1	12.41	6.95	<0.025

V - 3 Analysis of variance (two-way with unequal but proportional subclass numbers) to determine if the 10 Method B crosses made in one year differed from the 5 Method B crosses made in a second year.

Source	df	MS	F	P
Subgroups	11	908.3		
Years (Random)	1	19.7	< 1	ns
Crosses (Fixed)	5	1689.7	8.9	< 0.025
Years x Crosses	5	304.7	1.7	ns
Error	78	187.4		
Total	89			

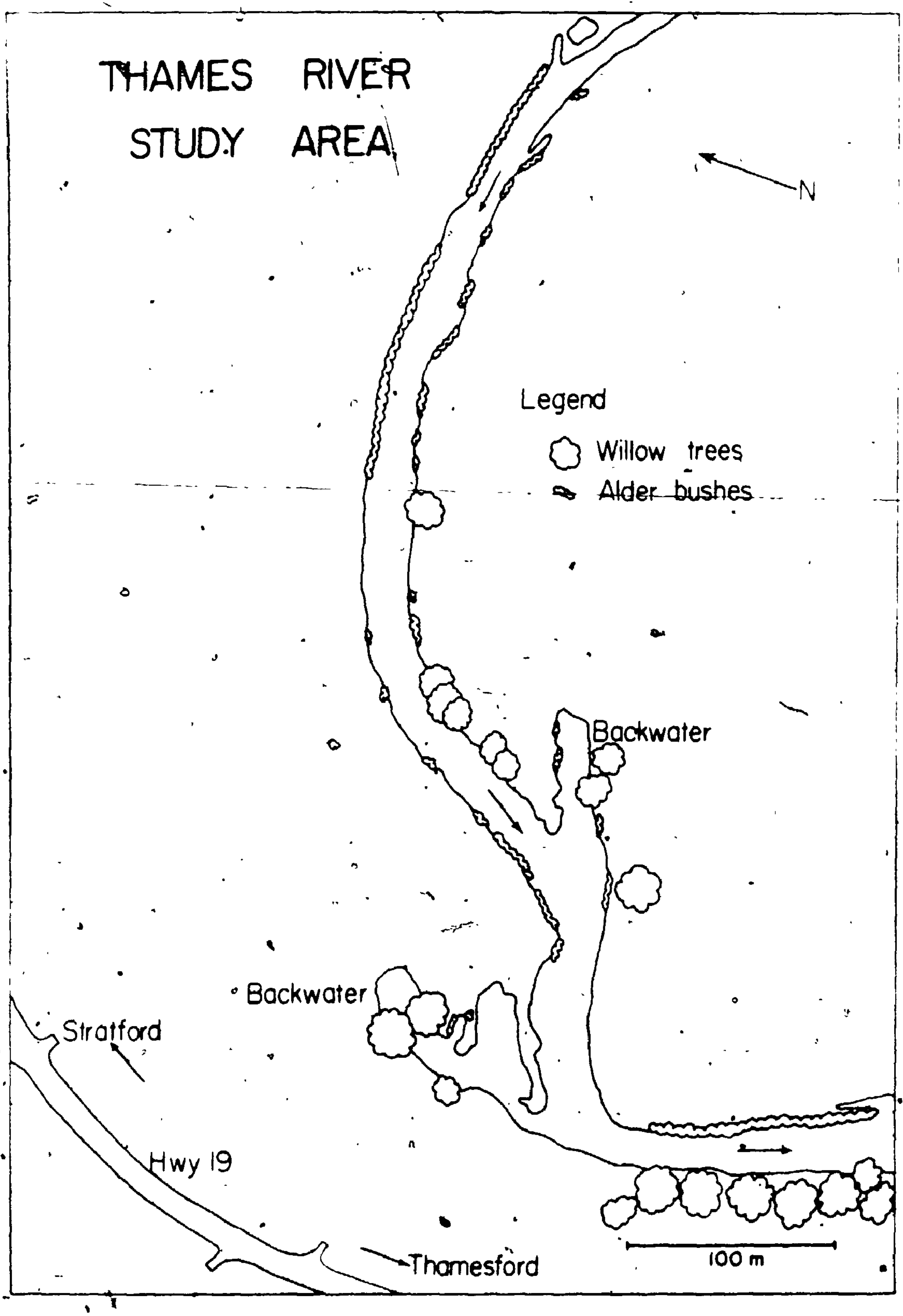
V - 4 Analysis of variance (two-way with unequal but proportional subclass numbers) to determine if Method A crosses differed from Method B crosses.

Source	df	MS	F	P
Subgroups	11	3,816.5	16.8	< 0.001
Methods (Fixed)	1	24,135.4	106.4	< 0.001
Crosses (Fixed)	5	2,839.2	12.5	< 0.001
Methods x Crosses	5	730.1	3.2	ns
Error	138	226.8		
Total	149			

V - 5 Analysis of variance to determine if differences exist among the six Method B crosses.

Source	df	MS	F	P
Crosses	5	1682.7	8.78	< 0.001
Error	84	192.4		
Total	89			

Appendix VI Map of the Thames River Study Area


THAMES RIVER
STUDY AREA
N
Legend
Willow trees
Alder bushes
Backwater
Backwater
Stratford
Hwy 19
Thamesford
100 m

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