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A study of hybridization and behavioral characters in *Percina notogramma* (Rany and Hubbs) and *Percina peltata* (Stauffer), family Percidae

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A Study of Hybridization and Behavioral Characters in Percina
notogramma (Raney and Hubbs) and Percina peltata (Stauffer),
Family Percidae

A Thesis

Presented to the Faculty of the Graduate School
of the University of Richmond
in Partial Fullfilment of the Requirements of the
Degree of Master of Science

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By

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Introduction

In view of the close phylogenetic relationship between the two sympatric species of darters, Percina notogramma (Raney and Hubbs) and Percina peltata (Stauffer), placed in the subgenus Alvordius by Bailey and Gosline (1955), it is of interest to compare them in more detail than has been done in the past. Percina notogramma occurs only in the Chesapeake Drainage in Virginia, Maryland and West Virginia whereas P. peltata ranges from Virginia to New York. Adequate comparisons of their external morphological characters have been presented by Raney and Hubbs (1948) in their original description of P. notogramma. Supplementary data were provided by Hogarth and Woolcott (1966) in a paper describing the subspecies, P. n. montuosa, from the James River. In the present study, some observations indicating introgressive hybridization and others on behavioral characters will be followed by an attempt to integrate the evidence each provides concerning the phylogenetic relationship of the two species.

Hybridization

Materials and Methods. Treatment of characters used in this study was the same as that used by Hogarth and Woolcott (1966), and some of their data were used to make comparisons between P. peltata and P. notogramma. Comparison of hybrids with parental stock was made with a 2-tail t-test. An hypothesis proposing introgressive hybridization between P. peltata and various populations of P. notogramma was tested using a 1-tail t-test. Differences having values with a probability less than five per cent were considered significant.

Locality data for fishes included in this study were given by Hogarth and Woolcott (1966). The data for collections mentioned specifically in the text are included here. The catalog number of the University of

Richmond Museum is followed by the number of fish in the sample (in parentheses). Localities for P. notogramma from the York River drainage, Va. were: UR 556 (2), Caroline Co., Matta R. UR 575 (8), Caroline Co., Matta R. UR 866 (3), Caroline Co., Matta R. UR 1292 (8), Louisa Co., Deep Cr. UR 1920 (7), Louisa Co., Deep Cr. UR 2038 (6), Louisa Co., South Anna R. UR 2039 (9), Orange Co., Madison Run. UR 2086 (10), Hanover Co., South Anna R. UR 2466 (15), Spotsylvania Co., Po R.

The locality for P. notogramma from the James River drainage, Va. was UR 2464 (1), Fluvanna Co., Rivanna R.

The localities for P. peltata were: UR 2085 (76), Hanover Co., South Anna R. (York R. drainage, Va.). UR 2465 (16), Fluvanna Co., Rivanna R. (James R. drainage, Va.).

UR 2464, UR 2465 and UR 2466 were collected subsequent to the study of Hogarth and Woolcott (1966).

A hybrid between P. notogramma and P. peltata, identified from UR 2085, was redesignated UR 2462 and another hybrid collected with UR 2464 and UR 2465 was designated UR 2463.

Results and Discussion. A natural male hybrid between P. peltata and P. notogramma was identified from specimens of the two species from the South Anna River (UR 2085 and UR 2086). The hybrid was compared with P. peltata and P. notogramma from the same collection and the data summarized in Table 1. No other species of Percina occurs in the South Anna. Of six meristic characters where P. peltata was found to differ significantly from P. notogramma, the hybrid differed significantly from P. peltata in having more belly scales (fig. 3) than specimens of the same sex and from P. notogramma in having fewer scales around the caudal peduncle and fewer dorsal spines. In addition, it differed significantly from both species in having an extra scale at the pelvic base anterior to the normal one (fig. 3) and a greater number of

dorsal rays. The hybrid, year class I, was significantly larger than P. peltata or P. notogramma of the same year class (fig. 1A-C), a difference attributed to heterosis.

Differences for a number of qualitative characters are summarized in Table 2, which was adapted from a table prepared by Raney and Hubbs (1948) for use in differentiating P. peltata and P. notogramma. Some of the criteria given by Raney and Hubbs (1948) either were modified or were not included as they did not describe accurately the parental populations involved. The hybrid was similar to P. peltata in two characters, to P. notogramma in five characters, and was intermediate in five characters (fig. 1A-C, 2A-C and 3A-C).

Further examination of this population of P. notogramma indicated that these fish did not conform to the species type, but approached P. peltata in a number of characters. The intermediacy of this population is most evident in the color pattern. The continuous pale dorso-lateral streak, for which the species was named, was broken by extensions from the dorsal blotches that enclosed light areas similar to those of P. peltata (fig. 2C). The number of breaks in the pale streak was significantly greater in fish from this locality than those in the headwaters of the North Anna River (UR 2039) (fig. 2D), which was without good habitats for P. peltata. Other localities in the South Anna River without P. peltata (UR 1292, UR 1920 and UR 2038) and more closely located to the one in which the hybrid was collected, were intermediate in this respect (fig. 8). To explain these differences, it is hypothesized that gene flow is occurring from P. peltata to P. notogramma in the South Anna River, and the hybrid identified from this locality is a back-cross between them. Percina notogramma (UR 556, UR 575, UR 866 and UR 2466) from tributaries of the Mattaponi River exhibited the same type of color pattern; thus, it appears that the effects of introgressive hybridization with P. peltata are exten-

sive within the York drainage.

Evidence linking hybridization with the color pattern of P. notogramma from the York drainage (pale streak lacking) was provided from an examination of another hybrid (UR 2463) from the Rivanna River (James drainage). This specimen was identified as a hybrid on the basis of qualitative characters (Table 3). In meristic characters it resembled P. notogramma and differed significantly from P. peltata in all three characters in which there were significant species differences. The pale streak was absent from this hybrid, but was present in P. notogramma from the James River system and other drainage systems.

If introgression had occurred in the York drainage, one would expect other characters to be affected as well. To determine possible effects on meristic characters, raw data collected by Hogarth and Woolcott (1966) were used. From their data a meristic index was computed which provided good separation of P. peltata and P. notogramma. The index was computed by adding the characters with the highest mean value for P. notogramma (dorsal spines, dorsal rays, lateral-line scales, scales around the caudal peduncle, scales above the lateral-line and scales below the lateral-line). From this sum the characters with the highest mean values for P. peltata (anal rays, total pectoral rays and vertebrae) were subtracted. The meristic index value was determined for each specimen of P. notogramma in a particular drainage system and the mean value for each drainage system (i.e. the Patuxent, Potomac, Rappahannock, York and Lower James) was computed. A similar value was calculated for P. peltata in the York drainage. These values were then used to compare P. peltata in the York drainage with P. notogramma in each drainage system (fig. 9). Percina notogramma from the York drainage system were also compared with P. notogramma from each of the other drainage systems. The mean value for P. peltata was significantly

lower than that of P. notogramma from any drainage system. Percina notogramma from the York drainage had a meristic index more closely approaching that of other P. notogramma (from which it differed significantly) than to P. peltata. However, the index was more like that of P. peltata than was the index of any of the others. Also P. notogramma from the York drainage was most similar to P. peltata in more meristic characters than P. notogramma from any other drainage system (Tables 4 and 5).

Opportunity for hybridization between these species is present. The locality in the South Anna River, where hybridization was most evident, was below a dam which created an ideal habitat for P. peltata from one that was marginal to begin with. Fish hybrids are most often found in areas where the habitat has been altered (Hubbs, 1955). Even though a hybrid was found in the Rivanna River there was no evidence of introgressive hybridization, apparently because the habitat had not been modified extensively. Gravid females and ripe males of both species occurred in close proximity in the South Anna River; although in nature spawning was not observed between P. notogramma and P. peltata, spawning attempts in an aquarium have been seen and photographed (fig. 4B).

In studies of 45 artificial hybrid combinations among etheostomatine fishes, Hubbs (1959) found that the mortality rate was no higher in the hybrids than in the controls, if the parents were in the same subfamily. Therefore, it is quite probable that P. notogramma x P. peltata hybrids could also survive. However, Hubbs (1961) found a decrease in the frequency of fertilization for sympatric populations of darters if fertilization was not immediate (i.e. if eggs were placed in water several seconds after the sperm). There was no decrease in the frequency of fertilization below that of controls if the populations of those species were allopatric.

Social Behavior

Winn (1958) showed the value of behavioral characters for determination of phylogenetic relationships among species of darters. Since New's study (1966) of the reproductive behavior of P. peltata, comparisons of the behavior of P. notogramma and P. peltata have been facilitated.

Males of both species became ripe before the females, and stayed ripe long after the eggs were laid (Table 6). Ripe females of P. notogramma have been observed at temperatures as low as 8 C in the Matta River; P. peltata females were gravid but not ripe at this temperature. Gravid females of P. notogramma were found in the South Anna River at 10 C, and females of the mountain subspecies, P. n. montuosa, have been found ripe at 13 C and 16 C in Partridge Creek where flooding of the spawning sites appeared to prevent earlier spawning. Spawning is assumed to occur in riffles, as all ripe or gravid P. notogramma were collected in water of about 1 foot in depth, over a rubble bottom and in moderate current. Percina peltata apparently differs from P. notogramma in preferred spawning sites. Relatively few ripe P. peltata were collected in shallow riffles with P. notogramma even in localities where P. peltata were common. New (1966) reported they spawn in water about 3 feet deep. No data were obtained for P. notogramma in slow sandy streams.

Gravid P. notogramma, brought into the laboratory, have been observed to spawn in a 2.5 gal aquarium, and attempt to spawn in a 10 gal aquarium at temperatures between 15 and 20 C. Due to the small size of the aquaria, it is probable that normal courtship behavior did not develop, as not enough room was available for males to establish territories. However, spawning positions were probably normal. Mounting of the female P. notogramma by the male was similar to that described by New (1966) for P. peltata from New York. Attempted spawning, with the male and female upright, and in horizontal position, occurred over sand (fig. 4A) and over an exposed

slate bottom. New (1966) reported that P. peltata spawned over gravel. Eggs of P. notogramma have been found following simultaneous quivering of male and female in the slate-bottomed aquaria, but no complete spawning act was observed over sand. Eggs were yellow and measured 1.2 mm in diameter; they had one large oil droplet 0.6 mm in diameter.

Fights similar to those described by New (1966) occurred commonly among P. peltata. During intense fighting, butting was almost continuous, without intervening display. The agonistic behavior of P. notogramma, however, was more ritualistic than that of P. peltata as the latter were never seen engaging in such bouts; instead, their fights conformed to a different pattern. As the aggressor fish approached, an attacked fish assumed a position perpendicular to the aggressor, rested on the bottom and often turned partially on its side, thus presenting the maximum lateral exposure to the attacking fish (fig. 5). Dorsal fins were fully erect, and during an intense display the attacked fish undulated. This display prevented butting, or at least inhibited it, for several seconds. If butting did occur, the attacked fish either fled or circled and assumed the role of the aggressor. Circling often occurred without any butting. This alternation of position sometimes continued as long as five minutes before a fish showed submission by fleeing with its dorsal fin depressed. Fights most often occurred after feeding, and seemed to be associated with a darkening of the spot at the base of the first dorsal fin and of the first lateral blotch immediately behind the opercle (fig. 5). This pattern was present in all fights observed during the study.

Fights between two P. peltata occasionally involved the perpendicular display described for P. notogramma; display always occurred in fights between P. peltata and P. notogramma. New (1966) did not describe the perpendicular display in his discussion of the agonistic behavior of P. peltata

from New York, nor did Winn (1958) mention it in his discussion of the behavior of 14 species of darters.

New (1966) noted that P. peltata formed aggregations in higher aquatic plants such as Potamogeton sp., Anacharis sp. and Vallisneria sp. during summer and autumn months; similar aggregations of P. peltata in Potamogeton sp. were seen in Virginia. When specimens of P. peltata were collected from among aquatic plants, they had a green background color; however, this color has been observed in areas free of vegetation. Percina notogramma, seldom found in aquatic vegetation, almost always had a tan background color regardless of the collection site.

In an aquarium with a white background and scattered dark areas, P. peltata showed a strong tendency to form aggregations over the dark areas, particularly when frightened (fig. 6); aggregations rarely were seen over a white background. Percina notogramma did not show a similar tendency. The fishes used in these experiments were from the South Anna, where introgressive hybridization is suspected, and from the Rivanna River. Few studies of substrate color selection in fishes have been made, but Kwain (1967) demonstrated that two-year-old Salmo gairdneri are most frequently found over a black substrate rather than a white one.

Another behavioral difference between the two species was noted when they were placed in a 50-gal tank with rocks at one end and a plain sand bottom at the other. When lights were off, P. peltata hid among the rocks; when the lights were on, they moved over the plain sand. Percina notogramma remained among the rocks at all times. After swimming about during the day a given individual always returned to the same rock at night.

Individual Behavior at Different Water Temperatures

Because of the more northern distribution of P. peltata, Hubbs and Raney (1948) suggested that this species might be adapted better to cold temperatures than P. notogramma. Also, in the James drainage system P. peltata occurs at higher elevations than does the nominal subspecies of P. notogramma. Their suggestions led to observations on behavioral characters at different temperatures.

Materials and Methods. Each fish was kept in a separate 2.5 gal aquarium to prevent social interactions, but a constant flow of water through plexiglass siphon tubes between the aquaria assured that all fish were exposed to essentially the same conditions. Entrance by the fish into the siphon tubes was prevented by a net. Each siphon emptied into a jar in the adjoining aquarium, the overflow from which filled the adjacent aquarium (fig. 7). The water level in an aquarium was thus prevented from dropping below the level of the top of the jar in the following aquarium. All aquaria were aerated. The volume of water in each aquarium was maintained at about 1.5 gal. The rate of flow between each of the aquaria was approximately one gal per minute. The last aquarium in the series, which did not contain fish, had two heaters that were used to maintain the desired temperature in all of the aquaria. The water flowed from the aquaria into a container provided with a standpipe connected to a water pump. The water pump had a brass impeller, but no toxic effects on the fishes were observed.

The water was cooled by pumping it through a bed of tygon tubing (ID 1 mm) in the freezing compartment of a refrigerator. The tubing was divided into 20 lengths, each 15 ft long. From the refrigerator, the water flowed back to the first aquarium in the series. No fish were placed in the first aquarium because of the force and irregularities of flow. Insulation was provided by a large styrofoam box that enclosed the aquaria.

Each aquarium had a glass window placed in front; another window of plastic was located in the top; two 4 ft, 30 watt fluorescent lights were placed 15 inches above the water level.

Four P. notogramma and four P. peltata were placed in separate aquaria in an alternating fashion (i.e. one specimen of P. notogramma in the second aquarium, one P. peltata in the third, one P. notogramma in the fourth, etc.) so that any changes in water quality affected both species equally. Fishes used in this experiment were collected from the South Anna River (Table 7). Fishes from the Rivanna River were used in a preliminary study.

Observations were made at temperatures of 30 C, 23 C and 12 C. The highest temperature approached that which preliminary studies had shown to be lethal; the intermediate slightly above ambient temperature; and the lowest near the limit for the cooling capacity of the equipment. Observations were begun after a four-day acclimation period at 30 C. During the observation periods, which were at 2300 and 0600 hours, the room was kept dark; only the lights over the aquaria were on. Attempts were made to avoid distracting the fish. The time of the observation periods were chosen so that there would be no activity in the laboratory. The morning observations were made one-half hour after the lights were turned on; evening observations, one hour before the lights were turned off. During each observation period, measurements were made first on the amount of time the fishes swam with the pelvic fins off the bottom during a 5 min period; the second measurement was the length of time required for 20 opercular beats. Next, a 1 ml volume of frozen brine shrimp that was thawed in water was added to each aquarium. The amount of time required for each fish to "nip" at the food ten times was recorded. Experience showed that if a fish had not nipped at the food ten times within a 5 min period, a much longer time might be required; in that case, feeding time was recorded as over 5 minutes.

Uneaten food particles were carried away with the water flow, and were trapped in the container at the end of the series. The fishes were observed for 5 days at each temperature. For observations at the lower temperatures a 6-day acclimation period was used.

Water temperatures and dissolved O_2 concentrations were determined at each observation period. Temperatures in the first aquarium for the nominal 30 C observations had a mean value of 30.0 C, and ranged from 29.8 C to 30.3 C. As the water flowed through the aquaria it cooled and temperatures in the last aquarium averaged 0.5 C cooler. Temperatures for the nominal 23 C observations had a mean value of 22.7 C and ranged from 21.2 C to 24.0 C in the first aquarium; and temperatures in the last aquarium were 0.2 C cooler. Temperatures at the nominal 12 C observations had a mean value of 12.4 C and ranged from 10.8 C to 13.2 C in the first aquarium; and those in the last aquarium were 0.6 C warmer. Dissolved oxygen concentrations were close to saturation values at each temperature.

As the behavior of the fishes seemed to be different in the morning than in the evening, the effect of the time of day was included in the analysis. All observations were made on the same fishes, so a three-factor analysis of variance with repeated measures of time and temperature factors was appropriate. As feeding times were occasionally exceptionally long, (positively skewing the data), it was necessary to perform a reciprocal transformation of all measurements. Measurements that were originally recorded as "over five minutes" were rounded off to zero on the transformed scale; after transformation, the data appeared to be normally distributed, and the F_{max} test indicated that the variance was homogeneous. Statistical procedures used were those of Winer (1962).

Data on swimming time were also positively skewed. In this case a square root transformation produced a distribution best suited to quantitative analysis. From data on the opercular time, opercular rates were

computed and these data appeared normally distributed.

Results and Discussion. Temperature had a significant effect on the length of feeding time but there was no significant species effect (Table 8). A test of the main effects showed that observation time had no significant effect, but as there was a significant interaction between observation time and temperature, a test of the simple main effects was appropriate. This test showed that observation time had a significant effect at 30 C. At that temperature the fish fed faster at 0600 (Table 9).

The results show that temperature and time had significant effects on swimming activity (Table 10), and swimming time was greater for P. notogramma than for P. peltata (Table 11). The difference in swimming activity is probably related to the different habitats in which these species occur. Percina notogramma, with a significantly lower density ($\bar{x} = 1.01$), is often found in slow streams where less energy is required to maintain its position. Percina peltata (density $\bar{x} = 1.04$), on the other hand, generally occurs in fast streams, and were it to swim as much as P. notogramma, more energy would be required to maintain its position (Table 7). Specimens of adult P. notogramma were able to maintain a position off the bottom by use of their fins only, whereas P. peltata of the same size required body movements to maintain position. It should be pointed out, however, that juvenile P. peltata were more buoyant than adults and that had younger fishes been used, differences between the species would not have been as large.

Temperature, species and interaction between temperature and time of day had significant effects (Table 12). As time of day did not have a significant effect a test of the simple effects was appropriate. This analysis showed that at 12 C there was a significant effect. At that temperature, opercular rates were higher at 2300 hours (Table 13). As

temperature decreased there was a corresponding decrease in opercular rate.

Percina notogramma had an opercular rate consistently lower than that of P. peltata.

Times of maximum swimming and feeding activity and maximum opercular rates did not always occur at the same time of day (Tables 9, 11 and 13). Similarly Ali (1964) found, in experiments with Salmo salar (Atlantic Salmon) that were not run concurrently, that maxima of oxygen consumption, locomotor and feeding activity did not occur at the same time.

The results obtained from the experimental study where fishes from the South Anna River were used agreed with those obtained in the preliminary study on fishes from the Rivanna River.

Discussion

There have been a number of reports of natural intrageneric, and even intergeneric, hybrids among the darters (Hubbs, 1955). However, introgression is rare, but has been reported between the sibling species, Etheostoma olmstedi and Etheostoma nigrum (Cole, 1965). No reports were found of introgression in darters that were not sibling species. Because of its rarity in darters, introgression can be regarded as an indicator of close phylogenetic relationship, and its occurrence between P. notogramma and P. peltata provides supporting evidence for their placement in the subgenus Alvordius by Bailey and Gosline (1955).

The population of P. notogramma from the South Anna River, where introgression was most evident, was much more similar to the typical P. notogramma than to P. peltata in morphological (figs. 1, 2, 3 and 8) and behavioral characters. The most probable explanation is that P. notogramma is the recurrent parental species as defined by Anderson (1962), and

that backcrosses with P. peltata are rare. Another possible explanation is that the more intermediate forms have been unable to compete successfully with P. peltata and that selection has tended to eliminate them. Brown and Wilson (1956), in a general paper on character displacement, proposed that such a mechanism caused Micropterus punctulatus (Spotted Bass) and M. dolomieu (Smallmouth Bass) to be more distinct where they occur sympatrically than in localities where they were allopatric.

In view of the close relation between P. notogramma and P. peltata, it was surprising to find striking differences in their social behavior. Differences in ritualization of agonistic behavior is particularly interesting because Winn (1958) proposed that this character was useful in separating primitive and advanced darters. Based on this criterion P. peltata would be classified as primitive, as indicated by New (1966), and P. notogramma as more advanced. Differences in the tendency to form aggregations is probably related to adaptation to the different microhabitats utilized by these species. The limited observations of reproductive behavior indicated that the two species were alike in basic spawning position. However, differences in preferred spawning site probably serve as an isolating mechanism. The yellow color of the eggs of P. notogramma is like that described by Winn (1958) for more advanced darters. Winn found that primitive species such as Percina caprodes and Percina maculatus had clear eggs as did New (1966) for P. peltata. Lower density and more active swimming by P. notogramma are primitive characters according to Winn (1958), but are interpreted here as being related to a secondary invasion of slow streams. The mountain subspecies of P. notogramma in the James River is ecologically more similar to P. peltata, as Hogarth and Woolcott (1966) reported that this form lives in faster water than does P. n. notogramma.

That these two closely related species should differ in a number of

characters used to differentiate primitive and advanced forms indicates that these criteria are not as distinct as formerly thought. As Winn (1958) studied only three species of Percina, it may be that variation within the genus is greater than that shown in those species.

Analysis of variance for the results of the behavioral studies of P. notogramma and P. peltata at different temperatures indicated that there was no significant interaction between species and temperature. It thus appears that the reaction of both species to temperature was similar. However, further studies should be conducted before a decision can be made on this point.

Data on the spawning temperatures of P. n. notogramma from the Matta River, and of P. n. montuosa from Partridge Creek, provide evidence concerning their subspecific designation by Hogarth and Woolcott (1966). One of the key characters used to differentiate the two subspecies was the larger number of vertebrae in P. n. montuosa. Bailey and Gosline (1955) had previously attributed that character to cooler temperatures during development. As the spawning temperatures for the fish from Partridge Creek were actually warmer, the hypothesis of Bailey and Gosline seems unlikely. However, a careful study in which temperatures are monitored during development is needed before a final conclusion can be reached.

Summary

1. One hybrid between P. notogramma and P. peltata was identified from the York drainage in the South Anna River and another from the James drainage in the Rivanna River.
2. Introgression from P. peltata to P. notogramma in the York drainage was observed in color pattern and in meristic characters. Introgression was not evident in other drainage systems.

3. Although the effects of introgression were extensive in the York drainage, a locality in the South Anna River is believed to have been the site of more intensive hybridization. Its population of P. notogramma had a color pattern more nearly similar to P. peltata. This condition was probably induced by alteration of the habitat through the construction of a dam.

4. It is believed that this is the first reported case of introgression between darters that were not sibling species. Because of its rarity in darters, this introgression may indicate close phylogenetic relationship between the two species.

5. The population in which introgression was most evident was more nearly similar to P. notogramma than to P. peltata. It is proposed that backcrossing with P. peltata is rare, or that the intermediate forms have been eliminated because they were unable successfully to compete with P. peltata.

6. Differences in preferred spawning habitat appear to be an important isolating mechanism; however, a few gravid and ripe specimens of P. peltata were found with spawning populations of P. notogramma in the Matta and South Anna rivers. Attempted spawnings between P. notogramma and P. peltata have been observed in an aquarium.

7. Spawning in P. notogramma was observed in an aquarium over a slate bottom. Mounting was similar to that described by New (1966) for P. peltata.

8. Males of both species were ripe early in March, and stayed ripe until the end of May.

9. Ripe females of P. n. notogramma were collected at cooler temperatures than were females of P. n. montuosa from Partridge Creek. Flooding of the spawning sites may have prevented earlier spawning in Partridge Creek. The higher number of vertebrae in P. n. montuosa has been attributed to cooler temperatures during development (Bailey and Gosline, 1955) but that hypothesis now seems unlikely.

10. Ripe and gravid females of P. notogramma and ripe males were collected in rubble riffles in water about 1 foot deep and in moderate current; the spawning site of P. notogramma in slow sandy streams is not known.

11. The agonistic behavior of P. notogramma was more ritualistic than that of P. peltata.

12. Temperature and time of observation had a significant effect on locomotor and feeding activities and on opercular rate of both species. In general, fish were more active later in the day and had a higher opercular rate at that time.

13. Percina peltata had a stronger tendency to form aggregations in the field and in the laboratory.

14. Percina notogramma swam off the bottom more than did P. peltata. The adaptive value of this difference was discussed in relation to the different habitats utilized by these species.

15. The opercular rate of P. notogramma was slower than that of P. peltata.

16. Percina notogramma appeared to be more advanced than P. peltata if criteria proposed by Winn (1958) were used.

Literature Cited

Ali, M. A. 1964. Diurnal rhythm in rates of oxygen consumption, locomotor and feeding activity of yearling Atlantic salmon (Salmo salar) under various light conditions. Proc. Indian Acad. Sci. 60: 249-263.

Anderson, E. 1962. The role of hybridization in evolution, p. 287-314. In W. H. Johnson and W. C. Streeters, (ed.), This is life: essays in modern biology. Holt, Rinehart and Winston, New York.

Bailey, R. M. and W. A. Gosline. 1955. Variation and systematic significance of vertebral counts in the American fishes of the family Percidae. Misc. Publ. Mus. Zool. Univ. Mich. (93): 1-44.

Brown, W. L., Jr. and E. O. Wilson. 1956. Character displacement. Syst. Zool. 5: 49-64.

Cole, C. F. 1965. Additional evidence for the separation of Etheostoma olmstedi Storer from Etheostoma nigrum Rafinesque. Copeia 1965 (1): 8-13.

Hogarth, W. T. and W. S. Woolcott. 1966. The mountain stripeback darter, Percina notogramma montuosa, n. ssp. Chesapeake Sci. 7: 101-109.

Hubbs, C. 1959. Laboratory hybrid combinations among etheostomatine fishes. Texas Jour. Sci. ¹¹/₂: 49-56.

Hubbs, C. 1961. Differences between gamete compatibility of Etheostoma lepidium sperm and eggs of related percoid fishes from allopatric and sympatric populations. Texas Jour. Sci. 8: 427-433.

Hubbs, C. L. 1955. Natural hybridization in fishes. Syst. Zool. 4: 1-20.

Kwain, Wen-Hwa and H. McCrimmon. 1967. The behavior and bottom color selection of rainbow trout, Salmo gairdneri Richardson, exposed to different light intensities. Anim. Behav. 15: 79-81.

New, J. G. 1966. Reproductive behavior of the shield darter, Percina peltata, in New York. Copeia 1966 (1): 20-28.

Raney, E. C. and C. L. Hubbs. 1948. Hadropterus notogrammus, a new percid fish from Maryland, Virginia, and West Virginia. Occ. Pap. Mus. Zool. Univ. Mich. (512): 1-26.

Winer, B. J. 1962. Statistical principals in experimental design. McGraw-Hill Book Co., Inc., New York. 672 p.

Winn, H. E. 1958. Comparative reproductive behavior and ecology of fourteen species of darters (Pisces-Percidae). Ecol. Monogr. 28: 155-191.

Table 1. Comparison of meristic characters of P. notogramma (UR 2086) and P. peltata (UR 2085) with hybrid, P. notogramma x P. peltata (UR 2462), from South Anna R.*

Character	<u>P. notogramma</u> n = 10 Mean (SD)	Hybrid	<u>P. peltata</u> n = 20 Mean (SD)
Number of:			
Lateral line scales	57.5 (3.1)	52	53.6 (8.8)
Scales below lateral line	9.9 (1.3)	9	8.4 (0.9)
Scales around caudal peduncle	*21.4 (0.8)	19	19.0 (0.8)
Belly scales on males	9.0 (1.0)	7	*4.8 (0.5)
Dorsal spines	*13.6 (0.8)	11	12.4 (0.8)
Anal rays	9.7 (0.8)	10	9.2 (0.5)

* Means differing significantly at 0.5% level from values for hybrid.

Table 2. Comparison of qualitative characters of P. notogramma (UR 2086) and P. peltata (UR 2085) with hybrid, P. notogramma x P. peltata (UR 2462), from South Anna R.*

Character	<u>P. notogramma</u>	Hybrid	<u>P. peltata</u>
Color marking along midline of back	Tan blotches connect irregularly to tan stripe along upper side, not separated from latter by continuous pale streak.	Like <u>P. notogramma</u>	Blackish saddles interconnect before and behind by oblique extension, enclose oval light areas and form a zigzag black line on upper back.
Dark nuchal blotch	May partially enclose a light area.	Intermediate, completely encloses poorly developed light area.	Encloses near its center a light oval spot that often has a small spot in its center.
Stripe behind chin	Absent	Intermediate, pale	Dark

Table 2. Continued

Character	<u>P. notogramma</u>	Hybrid	<u>P. peltata</u>
Spot on breast	Pale and diffuse	Intermediate, dark and diffuse	Dark and discrete
Shape of markings on first dorsal fin	Diffuse	Intermediate, discrete markings but not crescents	Discrete crescents
Location of pigment concentration on first dorsal fin	Anterior base	Like <u>P. peltata</u>	Markings in a row
Dark caudal spot	Median	Like <u>P. peltata</u>	Below middle of tail base
Dark blotches along side	Oval	Like <u>P. notogramma</u>	Quadrate

Table 2. Continued

Character	<u>P. notogramma</u>	Hybrid	<u>P. peltata</u>
Shape of head	Pointed	Like <u>P. notogramma</u>	Blunt
Squamation:			
Cheek	Scaled	Intermediate	Scaleless
Opercle	Scales exposed	Intermediate	Scales imbedded
Subopercle	Scaled	Like <u>P. notogramma</u>	Scaleless
Ventral scales	Oval or slightly longer than wide	Like <u>P. notogramma</u>	Much longer than wide
Base of pelvic fins	Narrow	Like <u>P. peltata</u>	Wide

* Adapted from table prepared by Raney and Hubbs (1948) to compare P. notogramma with P. peltata

Table 3. Qualitative characters of hybrid, P. notogramma x P. peltata
(UR 2463), from Rivanna R.*

Character	Hybrid
Color marking along back	Like <u>P. notogramma</u> from York drainage but intermediate between <u>P. peltata</u> and <u>P. notogramma</u> elsewhere, tan blotches connect irregularly to zigzag tan stripe along upper side, not separated by continuous pale streak.
Dark nuchal blotch	Like <u>P. peltata</u>
Stripe behind chin	Intermediate, pale
Spot on breast	Like <u>P. peltata</u>
Shape of markings on first dorsal fin	Intermediate, discrete but not crescent shaped
Location of pigment concentration on first dorsal fin	Like <u>P. peltata</u>
Dark caudal spot	Like <u>P. peltata</u>
Dark blotches along side	Intermediate, quadrate but less so than <u>P. peltata</u>

Table 3. Continued

Character	Hybrid
Shape of head	Like <u>P. notogramma</u>
Squamation:	
Cheek	Like <u>P. notogramma</u>
Opercle	Intermediate, scales partially imbedded.
Subopercle	Like <u>P. notogramma</u>
Base of pelvic fins	Like <u>P. notogramma</u>
Background color in field	Like <u>P. notogramma</u> , tan, <u>P. peltata</u> collected at same time were green.

* P. notogramma and P. peltata from Rivanna R. as described in Table 2 except where indicated.

Table 4. Comparison of scale and vertebral counts of P. notogramma from various drainage systems with P. peltata from the York drainage.*

	Number of scales and vertebrae									
	Lateral line		Above lateral line		Below lateral line		Around caudal peduncle		Vertebrae	
	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
<u>P. notogramma</u>										
Patuxent	7	59.0 (2.0)	6	6.2 (0.6)	7	9.8 (0.6)	7	21.1 (1.7)	2	40.5 (0.6)
Potomac	4	56.2 (3.8)	4	6.2 (1.3)	4	10.0 (0.7)	4	22.2 (1.5)	4	41.0 (0.7)
Rappahannock	14	58.4 (3.2)	14	6.6 (0.9)	14	10.3 (0.8)	14	21.1 (0.8)	14	41.3 (0.8)
York	95	<u>54.7</u> (2.0)**	95	<u>6.1</u> (0.8)	95	<u>9.4</u> (1.1)	95	<u>20.5</u> (1.4)	95	41.4 (1.5)
Lower James	74	56.7 (3.2)	75	6.4 (1.0)	75	9.5 (0.8)	74	20.9 (1.6)	53	<u>42.7</u> (1.8)
<u>P. peltata</u>	16	54.1 (2.6)	16	5.8 (0.7)	16	8.6 (0.4)	16	19.1 (1.0)	14	43.1 (1.5)

* Adapted from data of Hogarth and Woolcott (1966).

** Mean values most similar to those of P. peltata are underlined.

Table 5. Comparison of fin ray counts of P. notogramma from various drainage systems with P. peltata from the York drainage.*

No.	Number of rays and spines								
	Dorsal spines		Dorsal rays		Anal rays		Total pectoral rays		
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	
<u>P. notogramma</u>									
Patuxent	7	13.3	(0.3)	13.3	(0.4)	10.1	(1.0)	27.7	(1.0)
Potomac	4	<u>13.0</u> **	(0.7)	13.2	(1.3)	<u>10.0</u>		26.5	(0.9)
Rappahannock	14	13.8	(0.4)	13.1	(0.4)	10.1	(1.0)	27.9	(1.7)
York	95	13.6	(1.1)	12.7	(0.7)	9.7	(0.6)	<u>28.0</u>	(1.3)
Lower James	75	14.2	(0.7)	<u>13.0</u>	(0.7)	9.9	(0.8)	27.7	(1.1)
<u>P. peltata</u>	16	12.7	(0.3)	12.9	(1.2)	10.0	(0.4)	28.8	(1.4)

* Adapted from data of Hogarth and Woolcott (1966).

** Mean values most similar to those of P. peltata are underlined.

Table 6. Dates, water temperatures and sexual condition when P. n. notogramma, P. n. montuosa and P. peltata were collected.

Matta R., Caroline Co.: 27 March 67, 7 C, P. n. notogramma males, ripe; P. n. notogramma females, gravid. 25 March 67, 8 C, P. n. notogramma males and females, ripe. 29 March 67, 12 C, P. n. notogramma males, ripe; P. peltata females, ripe.

South Anna R., Hanover Co.: 30 March 67, 10 C, P. n. notogramma males, ripe; P. n. notogramma females, gravid; P. peltata males, ripe; P. peltata females, gravid. 19 April 67, 16 C, P. n. notogramma males, ripe. 27 April 67, 11 C, P. n. notogramma females, not ripe; P. peltata males, ripe. 1 June 67, 16 C, P. n. notogramma males, ripe; P. peltata males, ripe. 28 June 67, P. n. notogramma males, not ripe; P. peltata males, not ripe.

Partridge Cr., Amherst Co.: 15 April 64, 13 C, P. n. montuosa females, ripe. 27 March 67, 9 C, P. n. montuosa males ripe. 6 April 67, 16 C, P. n. montuosa males and females, ripe.

Table 7. Standard length, weight and density of P. notogramma and P. peltata from South Anna R. used for observations of individual behavior.

	St Lg (mm)	Weight (g)	Density (g/cc)
<u>P. notogramma</u>			
1	6.4	2.1	1.01
2	6.5	2.3	1.01
3	6.9	2.6	1.02
4	6.3	2.1	1.01
<u>P. peltata</u>			
1	7.0	2.4	1.04
2	6.8	2.1	1.05
3	7.0	2.2	1.02
4	6.6	1.9	1.04

Table 8. Analysis of variance for the effects of species, temperature, and time of day on the amount of time required for P. peltata and P. notogramma to nip at food ten times.

Source	Degrees of freedom	Mean square	F ratio
Between subjects			
A (species)	1	1.5	0.8
Error (a)	6	2.0	
Within subjects			
B (time of day)	1	0.00	
AB	1	0.05	0.5
Error (b)	6	0.10	
C (temperature)	2	8.2	20.0*
AC	2	0.12	0.3
Error (c)	12	0.41	
BC	2	0.44	5.7*
ABC	2	0.15	1.8
Error (bc)	12	0.08	

* F ratio significant at 0.5% level.

Table 9. Mean reciprocal of feeding time (amount of time required to nip at food ten times divided into 100) at different temperatures and different times of day for pooled specimens of P. notogramma and P. peltata.

Time of day	Temperature		
	30 C	23 C	12 C
0600 hours	2.2	1.6	0.6
2300 hours	1.9	1.9	0.7

Table 10. Analysis of variance for the effects of species, temperature, and time of day on the amount of time spent swimming by P. peltata and P. notogramma during a five min period.

Source	Degrees of freedom	Mean square	F ratio
Between subjects			
A (species)	1	49.0	13.1*
Error (a)	6	3.7	
Within subjects			
B (time of day)	1	27.8	6.2*
C (temperature)	2	17.7	3.9*
AB	1	0.6	0.1
AC	2	10.8	2.4
BC	2	9.7	2.1
ABC	2	0.52	0.1
Pooled error	30	4.5	

* F ratio significant at 0.5% level.

Table 11. Mean square root of swimming time (amount of time spent swimming during a five min interval) of P. notogramma and P. peltata at different temperatures and at different times of day.

Species	Time of day					
	0600 hours			2300 hours		
	Temperature			Temperature		
	30 C	23 C	12 C	30 C	23 C	12 C
<u>P. notogramma</u>	4.0	1.4	0.7	5.0	5.3	1.1
<u>P. peltata</u>	0.2	0.2	0.4	1.3	2.8	0.5

Table 12. Analysis of variance for the effects of species, temperature, and time of day on the opercular rate of P. peltata and P. notogramma.

Source	Degrees of freedom	Mean square	F ratio
Between subjects			
A (species)	1	3120	17.9*
Error (a)	6	174	
Within subjects			
B (time of day)	1	20	0.5
C (temperature)	2	78165	187*
AB	1	4	0.1
AC	2	44	1.0
BC	2	211	5.1*
ABC	2	86	2.1
Pooled error	30	42	

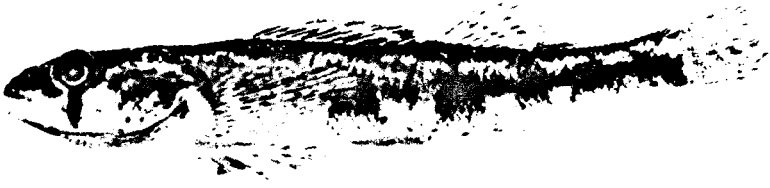
* F ratio significant at 0.5% level.

Table 13. Mean number of opercular beats per min of P. notogramma and P. peltata at different temperatures and different times of day.

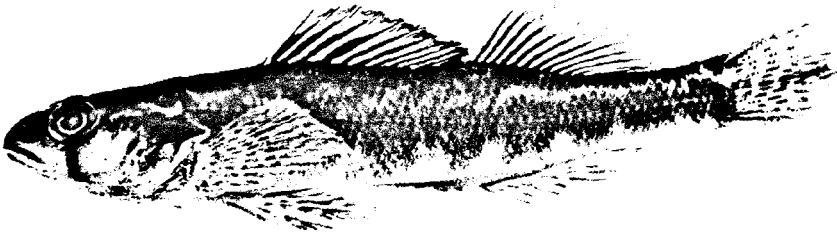
Species	Time of day					
	0600 hours			2300 hours		
	Temperature			Temperature		
	30 C	23 C	12 C	30 C	23 C	12 C
<u>P. notogramma</u>	66	35	14	61	41	19
<u>P. peltata</u>	83	52	31	76	51	44

Fig. 1. A. Lateral view of P. peltata, UR 2085, male, age class I, 51 mm SL, Hanover Co., Va., South Anna R. (York R. drainage). B. Hybrid, P. notogramma x P. peltata, UR 2462, male age class I, 57 mm SL, Hanover Co., Va., South Anna R. (York R. drainage). C. P. notogramma, UR 2086, male, age class I, 42 mm SL, Hanover Co., Va., South Anna R. (York R. drainage). D. P. notogramma, UR 2039, female, 57 mm SL, Orange Co., Va., Madison Run, trib of North Anna R. (York R. drainage). E. Hybrid, P. notogramma x P. peltata, UR 2463, female, 73 mm SL, Fluvanna Co., Va., Rivanna R. (James R. drainage).

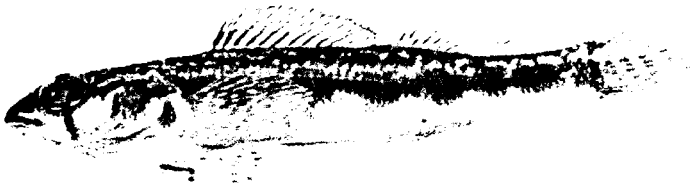
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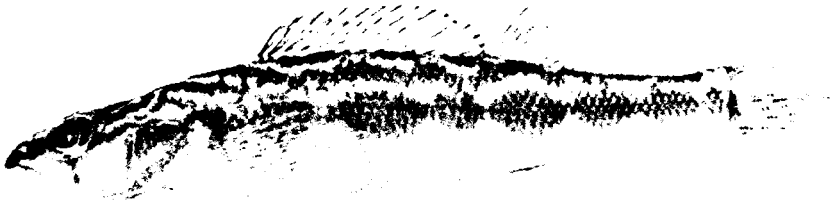
B



C



D



E

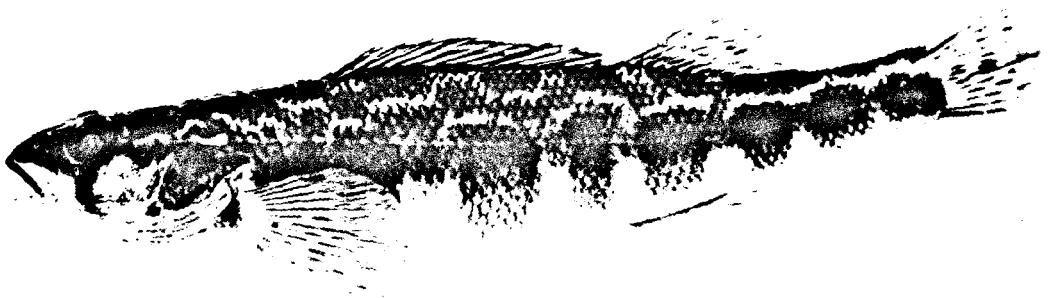
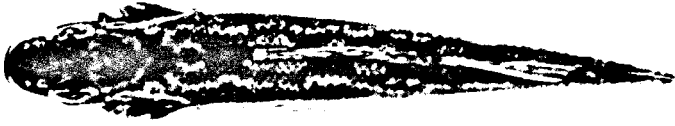
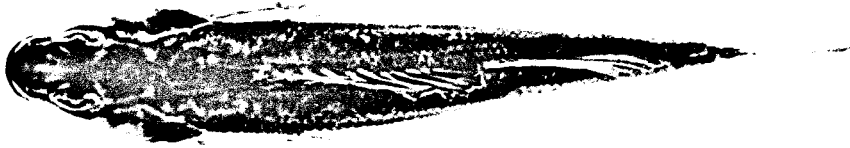


Fig. 2. Dorsal view of fishes shown in fig. 1 arranged in same order.

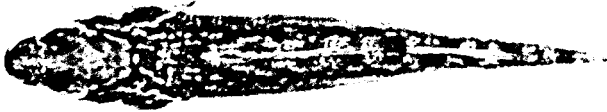
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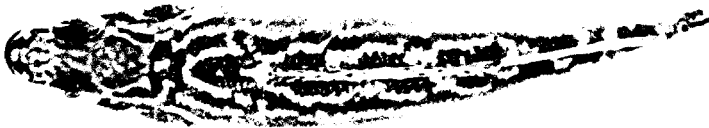
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C



D



E

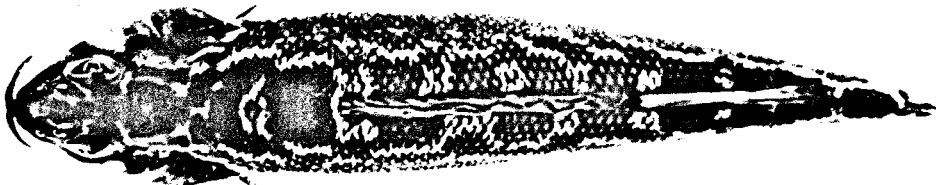
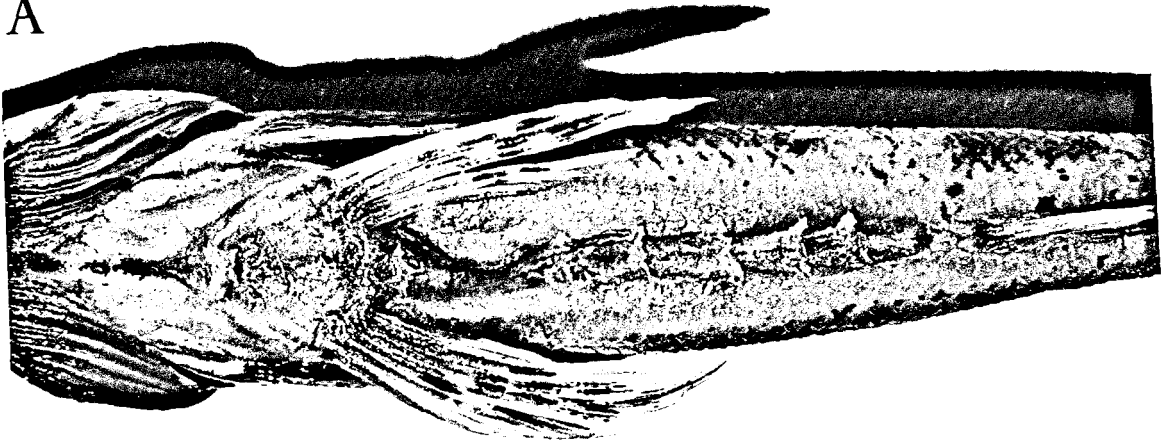
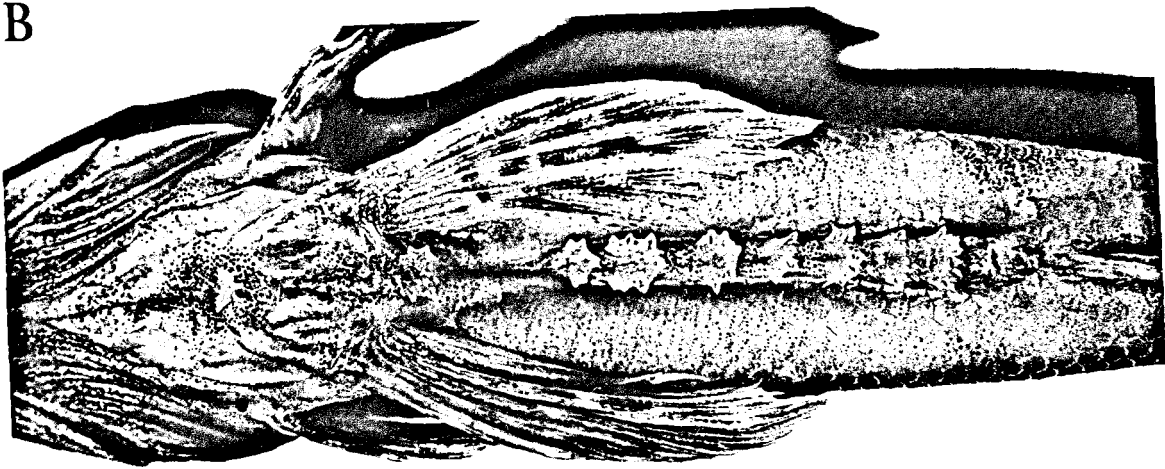


Fig. 3. A. Belly of P. peltata shown in fig. 1A. B. Hybrid, P. notogramma x P. peltata shown in fig. 1B. C. P. notogramma shown in fig. 1C.

A



B



C

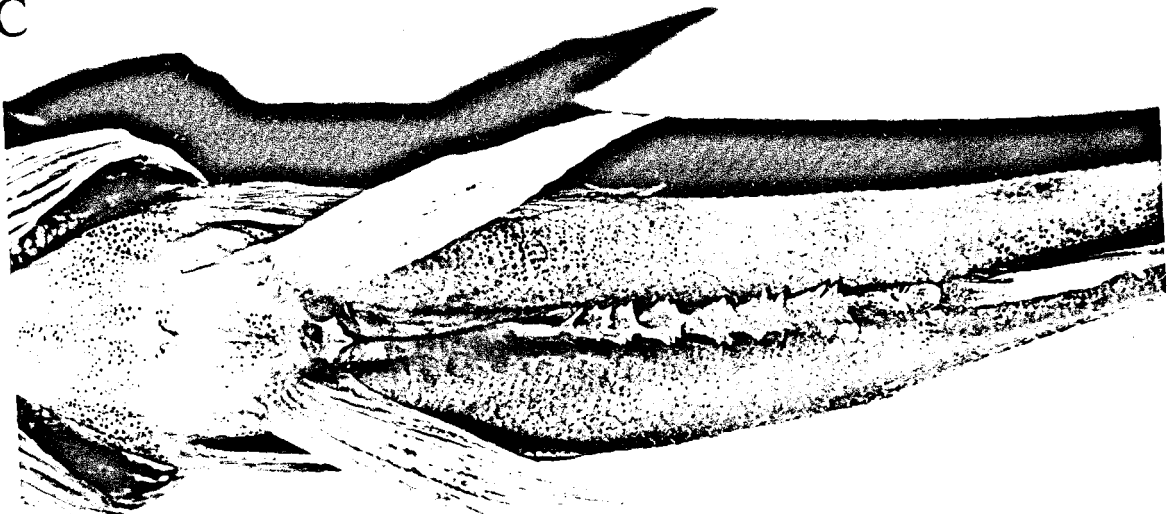


Fig. 4. A. Mounting position of P. notogramma. B. Mounting of P. peltata by P. notogramma. (Enlarged 2X)

A



B



Fig. 5. Agonistic display of P. notogramma. (Enlarged 2X)



Fig. 6. Aggregation of five P. peltata over black spot in a 50 gal aquarium. Five P. notogramma, also in aquarium, are not aggregated. (Reduced 3X)

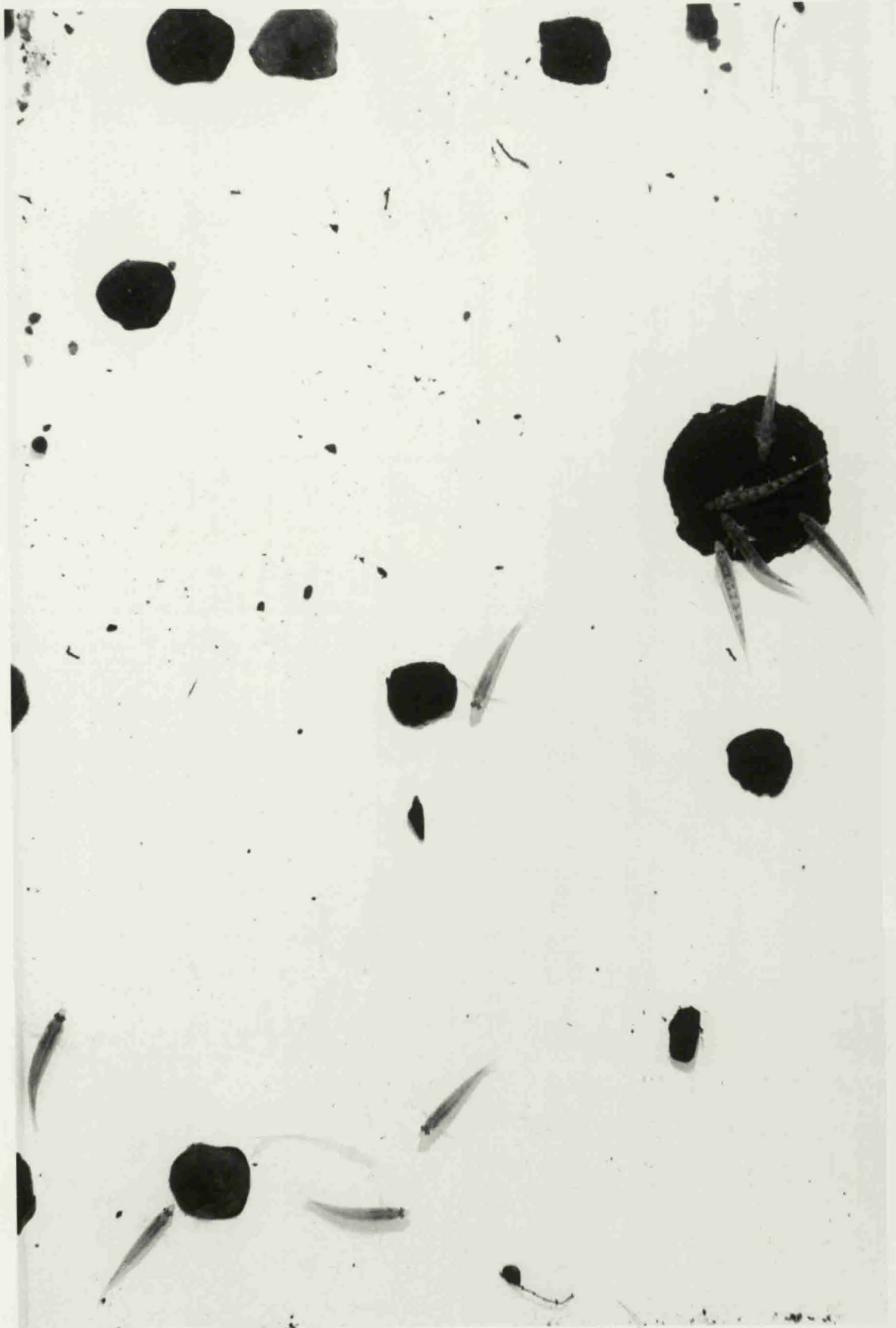
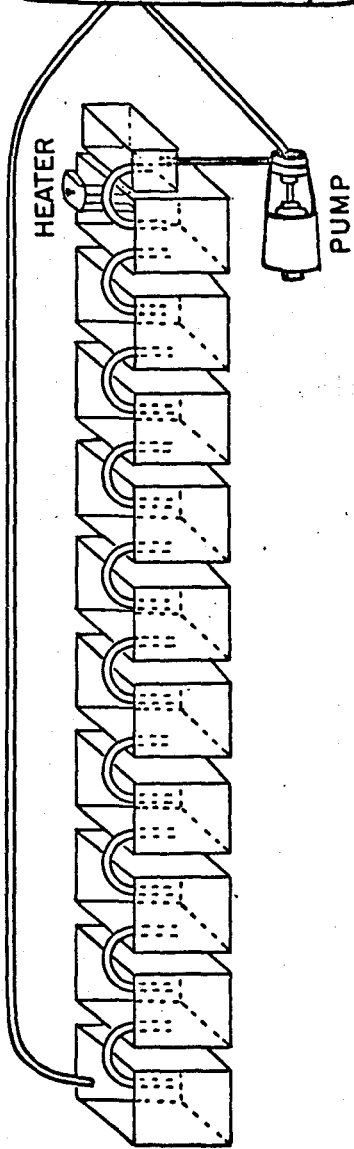
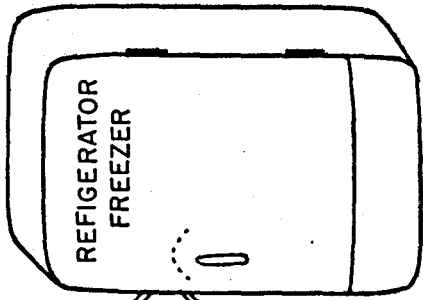
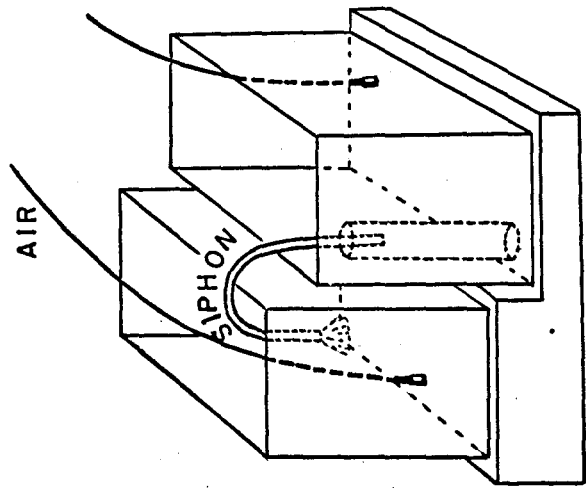


Fig. 7. A. Schematic diagram of flow system. B. Two aquaria in series.



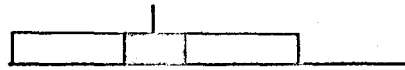
A.



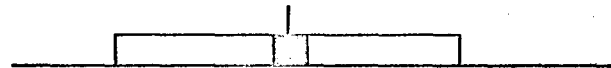
B.

Fig. 8. Comparison of the number of breaks in the dorso-lateral pale streak of P. notogramma from several localities within the York R. drainage. Range is indicated by a horizontal line; twice the standard error on each side of the mean, by a black bar; and one standard deviation on each side of mean, by black bar plus white bar at end.

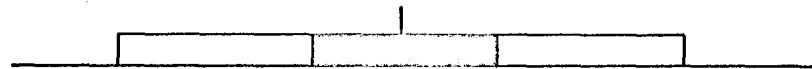
MADISON RUN (9)



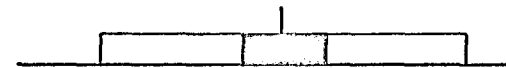
DEEP CREEK (15)



SOUTH ANNA R. (6)
(Not W. P. Peltata)



SOUTH ANNA R. (10)
(W. P. Peltata)



3 6 9 12 15 18 21

NUMBER OF BREAKS IN PALE STREAK

Fig. 9. Comparison of meristic index of P. notogramma from various drainage systems with P. peltata from the York R. drainage. Graphic methods same as fig. 8. Prepared from data of Hogarth and Woolcott (1966).

P. NOTOGRAMMA

PATUXENT (2)

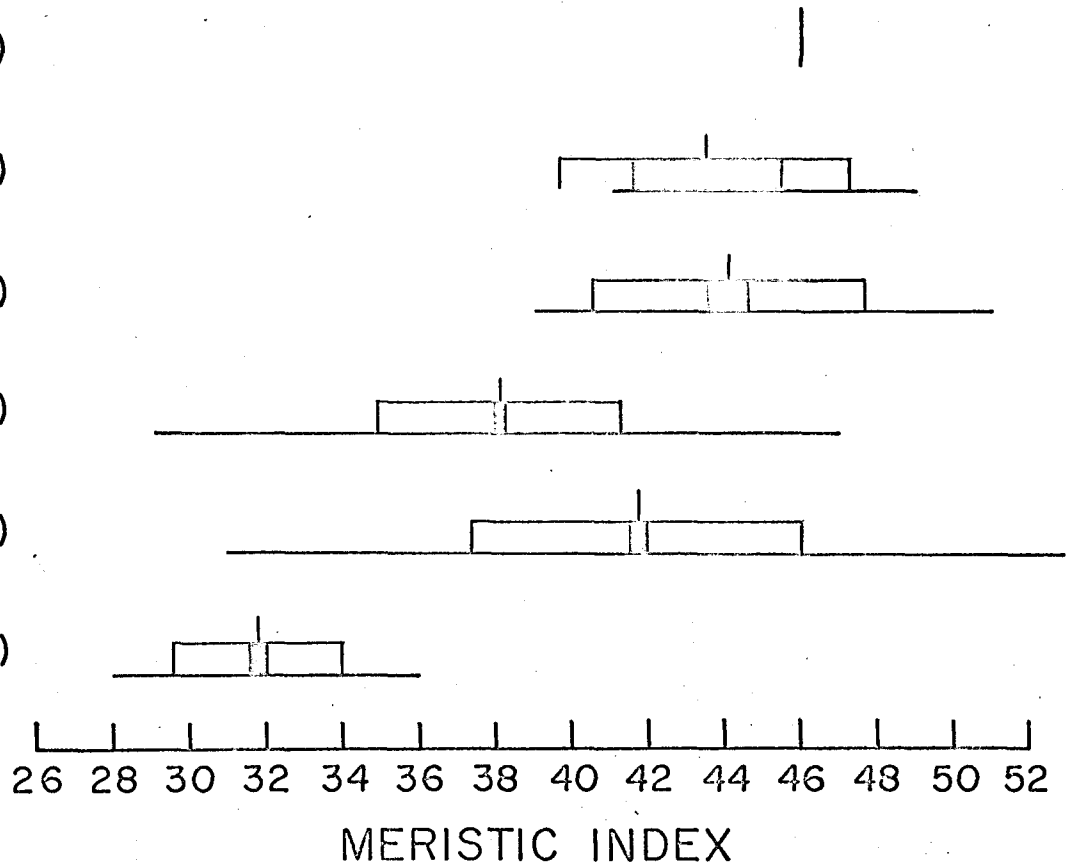
POTOMAC (4)

RAPPAHANNOCK (14)

YORK (94)

LOWER JAMES (50)

P. PELTATA (13)



Vita

Jules Junker Loos was born on December 12, 1941 in Philadelphia, Pennsylvania where he attended parochial elementary school and high school. He obtained a Bachelor of Science degree from The Pennsylvania State University in June 1963 with a major in Agricultural and Biological Science. After graduation he was employed as a biologist in the Limnology Department by the Academy of Natural Sciences of Philadelphia. He married the former Miss Helen Wu on November 18, 1964. In September 1966 he entered the University of Richmond. He is a member of the American Fisheries Society and Beta Beta Beta Honorary Fraternity. He will report for active duty in the United States Army on September 29, 1967 but plans to complete the requirements of the degree of Master of Science after separation from the military.