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Experiments in Attempted Interspecific and Interpopulational Hybridzation of Grass Shrimp of the Genus Palaemonetes (Caridea Palaemonidae)

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ABSTRACT

EXPERIMENTS IN ATTEMPTED
INTERSPECIFIC AND INTERPOPULATIONAL HYBRIDIZATION
OF GRASS SHRIMP OF THE GENUS PALAEMONETES
(CARIDEA, PALAEMONIDAE)

Mark Andrew Boston
Old Dominion University, 1978
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The present study investigates the interfertility of *Palaemonetes* populations. Interspecific crosses were attempted between representatives of sympatric populations of *P. pugio*, *P. vulgaris*, and *P. intermedius*. Intraspecific crosses were undertaken between geographically separated populations of *P. pugio*. Laboratory conditions of 25°C, 25°/oo, and 14.5 hour photoperiod per day were maintained for all breeding attempts. Under these conditions interspecific hybridization of *Palaemonetes pugio* and *P. vulgaris* was not feasible. *P. intermedius* could not be hybridized with *P. pugio* and *P. vulgaris*. At 40 days of age *P. pugio* juveniles were significantly larger than *P. vulgaris* juveniles of the same age. In addition, *P. pugio* juveniles appeared to be fully developed in the adult characteristics investigated, whereas *P. vulgaris* juveniles were not. Neither species had developed complete sexual dimorphism at 40 days of age.

Intraspecific hybridization between representatives of *P. pugio* populations from Gulf Breeze, Florida, and Rudee Inlet, Virginia Beach, Virginia, was successful, producing larvae from all types of crosses attempted. Survival rates for interpopulational crosses involving Rudee Inlet females and Gulf Breeze males were significantly lower than the survival rates of the intrapopulational control crosses. Intrapopulational hatch sizes from Rudee Inlet

P. pugio were significantly larger than the hatch sizes of the other three mating types.

Extended larval development time resulted in increased length of post-larvae among *Palaemonetes pugio* and *P. vulgaris* progeny.

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INTRODUCTION

General Introduction

Personal observation of *Palaemonetes*, grass shrimp, of the lower Chesapeake Bay has revealed an amount of morphological variability so extreme that it obscures the identity of the species in some cases. Such variability may result from naturally occurring introgressive hybridization. This could be significant to the taxonomist, ecologist and assayist.

These species, *Palaemonetes pugio*, *P. vulgaris*, and *P. intermedius* are similar in general biology and ecology. Williams (1965) described the habitats of each as being in "estuarine waters, especially in beds of submerged vegetation". Bowler and Seidenberg (1971) reported that *P. pugio* and *P. vulgaris* "have very similar niche requirements". Since these species appear to occupy similar ecological niches, we would suspect them to be highly competitive when they occur sympatrically. Coexistence would not be expected unless there were at least some small difference between their respective niches. Habitat partitioning may occur between *Palaemonetes pugio* and *P. vulgaris* due to different salinity tolerances (Holthuis, 1952; Knowlton & Williams 1970; Bowler & Seidenberg, 1971). More recently, however, it was shown that in areas where the salinity remained above 3 $^{o}/oo$ there was no such partitioning effect (Thorp & Hoss, 1975). Thorp (1976) showed that *P. vulgaris* competitively displaces *P. pugio* from shell to mud bottoms at the onset of spring, the reproductive season. This has been the only evidence given for habitat partitioning between these two species. No niche differences have been described which might explain the coexistence of P. intermedius with P. pugio and P. vulgaris in medium and high salinities.

Since these species coexist under very similar niche requirements and their identities are sometimes obscured, there is a possibility that interspecific hybridization is occurring. The present study was undertaken to investigate the interfertility of Palaemonetes populations. Interspecific crosses were attempted between representatives of sympatric populations of P. pugio, P. vulgaris, and P. intermedius. Intraspecific crosses were undertaken between geographically separated populations of P. pugio which exist in different niches due to disparate environmental stresses.

Experimental Objectives and Theory

Hybridization has been shown to occur within the Decapoda under laboratory conditions (Carlberg et al., 1978; Lucas, 1970; Thakur, 1960; Tsukerzis, 1975). That hybridization could occur in the laboratory between closely related sympatric Palaemonetes species, therefore, is a distinct possibility. Data are presented to evaluate the feasibility of interspecific hybridization of these species under the controlled laboratory conditions of $25^{o}/oo$, $25^{o}C$, and 14.5/9.5 light-dark cycle.

This problem was approached by pairing females of each species with males of the other two species. Each pair of potentially breeding shrimp was isolated in a separate container. Three pairs of shrimp for each species combination were used in order to reduce the variability of results encountered by the use of only one pair. A large number of pairs for each mating type would have been more desirable, but was not possible due to limited facilities.

Data were collected to test also the theory that geographically separated populations of P. pugio are reproductively isolated. Representatives of the populations were interbred reciprocally for analysis of fertility of the cross and of developmental characteristics of the larvae. They were intrabred as an experiment control. As before, three pairs were set up for each mating combination attempted.

The importance of these combined experiments becomes evident when one considers the extent to which bioassays have been and are being conducted with Palaemonetes species. Some of these include a mirex toxicity study completed by Redmann (1973), an experiment of the effects of mercury on survival and development by Shealy and Sandifer (1975), an analysis of the effect on development of larvae reared from populations in kepone contaminated and uncontaminated sites by Provenzano et al. (1976), a nitrite and nitrate toxicity study by Hinsman (1977), an investigation into the effect of mirex on predator-prey interactions by Tagatz (1976), and an evaluation of the acute toxicity of chlorine by Roberts (1975).

The identity of these species may be clarified through experimentation and this may in turn help to determine the reliability of using these species in bioassay work.

PREVIOUS INVESTIGATIONS

Hybridization Research

In the past, hybridization experiments have produced more economical varieties of grain and livestock, yielding larger, healthier, and more prolific organisms. Hybridization research pertaining to these organisms is exhaustive. Crossbreeding experiments among crustaceans are few.

Hybridization experiments have been conducted on non-decapod crustaceans, including the Amphipoda, Branchiopoda, Copepoda, and Isopoda (Bowen, 1964; Bozic, 1955; Menzies, 1972; Roux and Goedmaker, 1975; Ueno, 1971). Most of these dealt with attempts to obtain offspring from crosses of geographically isolated populations of the same species. Some met with success. Others failed because of disparities in reproductive physiology, habitat related differences, and other barriers to reproduction.

The extent of research in this field among decapods is limited. Review of the literature, from 1947 - 1977, yielded only five works involving decapod hybridization. Thakür (1960) was apparently the first to cross species of decapods under laboratory conditions. He succeeded in breeding two geographically separated species of fresh-water prawns using a female *Caridina weberi* var. *sumatrensis* with a male *Caridina rajadhari*. No attempt was made to indicate the viability of the hybrid larvae. The importance of this experiment becomes evident in the conclusion of his report where he states; "Enquiries were made to Dr. L. B. Holthuis of the Leiden Museum and to Dr. I. Gordon of the British Museum about any previous notes on crossbreeding among decapod crustaceans. They have communicated that to their

For instance, the influence of temperature on numerous physical and physiological systems may be involved in cline formation. A species which extends over a wide range of temperature regimes is simultaneously influenced by different selection pressures in each habitat. As a result of a clinal situation, geographic variation of a character within the species occurs and in some cases may lead to geographic speciation. In fact, several authors have proposed the phenomenon of "speciation by distance over a continuously occupied range..." for many marine organisms (Kinne, 1975).

The amount of variation over such a range and hence the incidence of speciation is dependent in part on the frequency and amount of gene exchange. Therefore, infrequent occurrences of migratory adults or larvae between contiguous populations may result in reduced genetic cohesiveness. This, in turn, leads to the genetic differentiation of the populations and may eventually lead to speciation.

Biology of *Palaemonetes* Species

Distribution & Description

All three species used in this study may be found in "estuarine waters, especially in beds of submerged vegetation" from New York to Texas (Williams, 1965). The ranges of *Palaemonetes pugio* and *P. vulgaris* extend north to Massachusetts. In all three species, total body lengths range from approximately 20-50 millimeters, mature males are usually smaller than mature females, and all specimens are colorless, when alive (Williams, 1965).

General Biology

Background information on *Palaemonetes* reproductive life history was provided by Burkenroad (1947) and Knowlton and Williams (1970). Environmental optima for the species of interest, *Palaemonetes pugio*, *P. vulgaris*, and *P. intermedius*, were found in Bowler and Seidenberg, 1971; Broad and Hubschman, 1962; Knowlton, 1965; Sandifer, 1973; and Thorp and Hoss, 1975.

Adults of the three species can be distinguished by morphological characteristics (Broad, 1957; Fleming, 1969; Holthuis, 1952; Hubschman and Broad, 1974; and Williams, 1965).

Salinity above 20 $^{o}/oo$ apparently has little effect on the development of these species (Broad and Hubschman, 1962; Knowlton, 1965; and Sandifer, 1973). Little (1967) induced winter breeding of *P. pugio* achieving 100% egg deposition at 25°C and a 14.5/9.5 light-dark cycle.

Ecological Significance

Palaemonetes species are of paramount importance to the tidal marsh community. Welsh (1975) reported that *P. pugio* was a dominant species in the tidal marsh ecosystem of Bissel Cove, Narragansett Bay, Rhode Island. It is highly adapted to the stressful environment of a tidal marsh including an adaptation to low oxygen levels. This enhances the growth of the population because of the apparent reduction in predation and competition in such a habitat. *P. pugio* is important to the ecosystem primarily in rapidly breaking down detritus and converting it to dissolved organic matter, feces, and shrimp biomass which can be utilized at several different trophic levels (Welsh, 1975).

EXPERIMENTAL METHODS

Collection of Test Animals

Specimens of *Palaemonetes pugio* and *P. vulgaris* were collected from Rudee Inlet. *P. intermedius* were obtained from Broad Bay. Both locations are in Virginia Beach, Virginia (Figure 1). The Florida population of *P. pugio* was collected in the Gulf Breeze vicinity (Figure 2).

All Virginia specimens were gathered from the intertidal zone during mid to low tide with push net or dip nets. Salinity was measured to the nearest 1 $^{o}/oo$ with an American Optical T/C Refractometer. Temperatures were recorded to the nearest 1.0°C. Hydrographic conditions, species collected, and species present at the time of collections are summarized in Table 1.

Handling of Collected Specimens

Captured shrimp were gradually acclimated to experimental conditions over a 3 to 5 day period. Except for those shrimp collected in 1977, all specimens were maintained at these conditions two to three months prior to mating. Duration of the initial acclimation period was dependent on the degree of difference between the hydrographic conditions at the collection site and the laboratory environment.

Males of each species were distinguished from females according to the characteristics of the first two pairs of pleopods (Meehan, 1936). All males were placed together in a ten-gallon aquarium equipped with a bottom gravel filter at a salinity of 25$^{o}/oo$ and room temperature, 23 - 25°C.

Table 1. Collection sites including hydrographic data, dates of collection, species collected, and other species present at the time of collection.

	Gulf Breeze	Rudee Inlet	Rudee Inlet	Broad Bay
Date	9 IX 76	24 IX 76	19 III 77	30 IV 77
Temp.	31.5°C	24°C	16°C	23°C
Salinity	5 ‰	20 ‰	13 ‰	20 ‰
Collected	*P. pugio*	*P. pugio* *P. vulgaris*	*P. pugio* (males only)	*P. intermedius*
Others	unknown		*P. vulgaris*	*P. pugio* *P. vulgaris*

Figure 1. Location map of Virginia collection sites. Sample areas are indicated by rectangles. *Palaemonetes pugio* and *P. vulgaris* collected at Rudee Inlet. *P. intermedius* collected from Broad Bay.

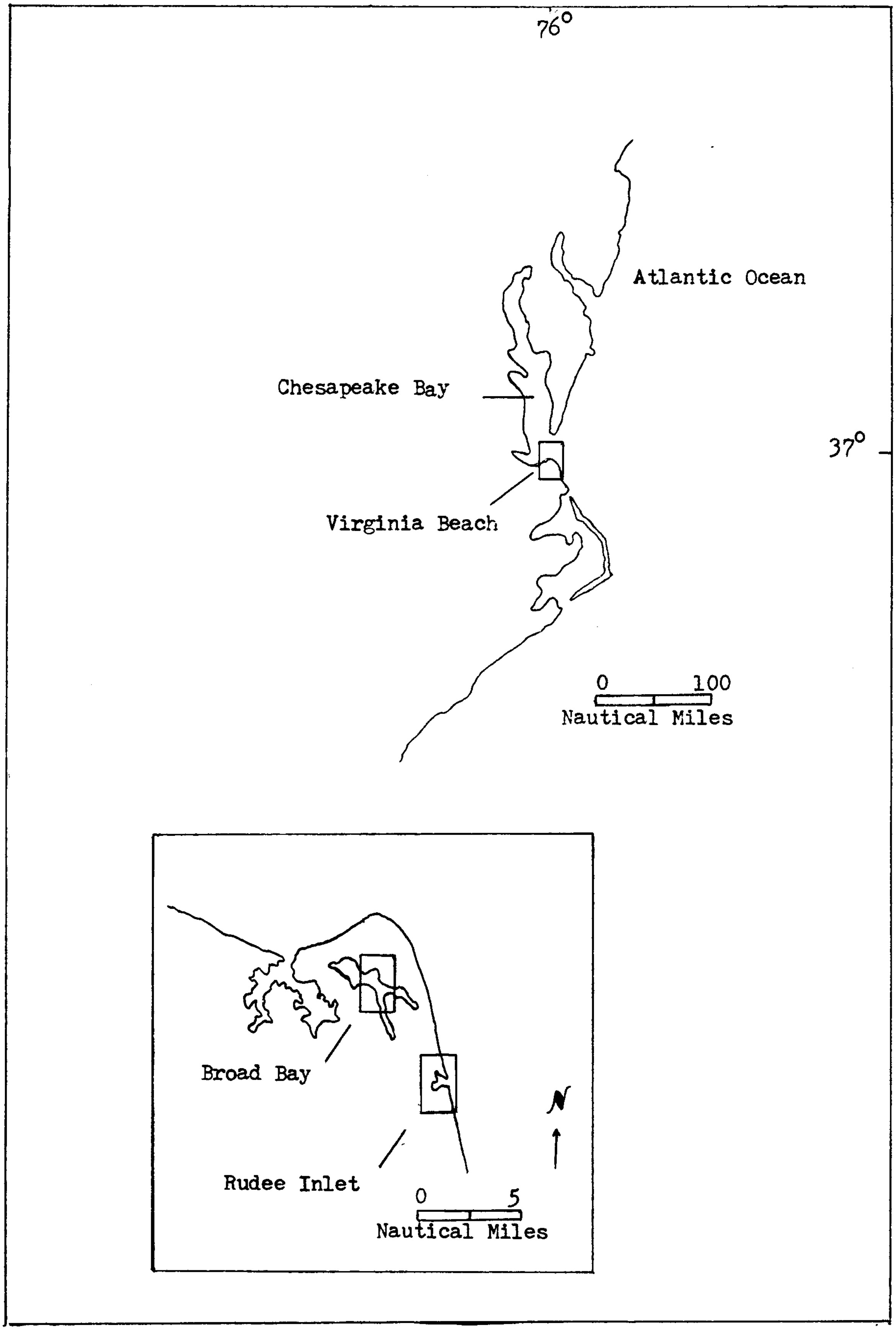

76°
Atlantic Ocean
Chesapeake Bay
37°
Virginia Beach
0 100
Nautical Miles
Broad Bay
N
Rudee Inlet
0 5
Nautical Miles

Figure 2. Location map of Florida collection site. Area of sampling indicated by rectangle. P. pugio collected from Gulf Breeze vicinity.

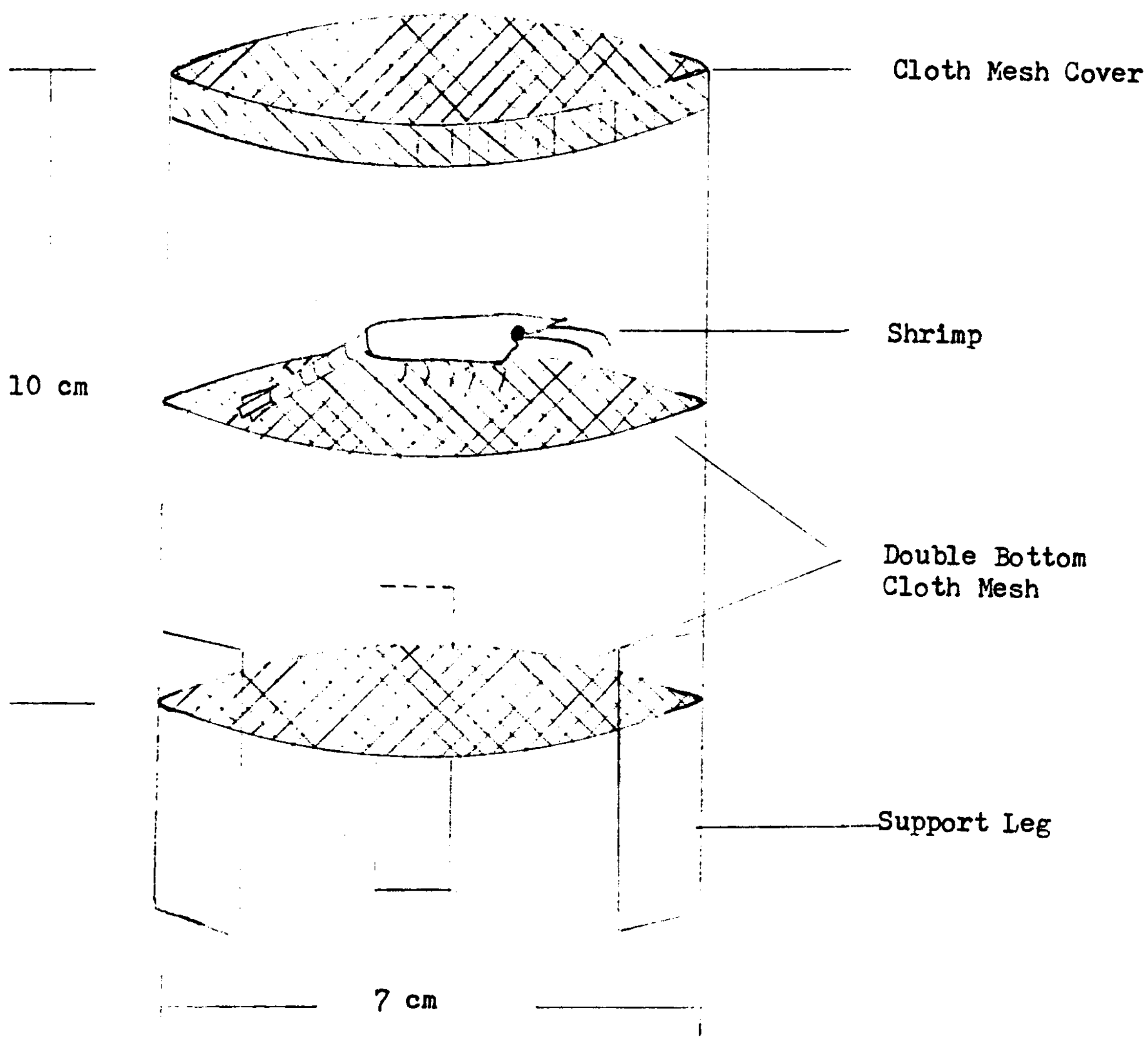
Females were placed in similar aquaria under identical conditions, but each female was maintained individually in a holding pen fabricated from a 10-cm length of 7-cm diameter translucent plastic tubing (Figure 3). A layer of nylon mesh netting (approx. 1/2 cm x 1/2 cm) was spread across the bottom of the tube and attached with silicone-base aquarium sealant. A second layer of netting was attached several centimeters above the first to support the female. This double layer later allowed the newly hatched zoeae an avenue of escape through the bottom of the tube, thereby reducing the number captured and eaten by the mother. A third layer of mesh was attached by rubber band to the top of the container to prevent escape of the adult.

The adults were fed daily on one of a variety of fish, dog food, squid flakes, brine shrimp, and detritus. Females remained in their holding pens until a molt was recovered, implying that they held no viable spermatozoa (Burkenroad, 1947). As an added precaution, each female was maintained until she had dropped or picked off an infertile batch of eggs. All molts, from both males and females, were preserved in 70% ethanol and labeled according to their assigned number.

Following the deposition of a molt and an infertile batch of eggs, each female was paired with an appropriate male. Interspecific matings were attempted reciprocally between P. pugio and P. vulgaris. Each cross was attempted using at least three pairs of shrimp. No individual was used in more than one pair. Intraspecific crosses run in triplicate for each species served as an experimental control. In addition, all individuals involved in interspecific crosses were bred intraspecifically to demonstrate their potential fertility.

Interspecific crosses involving P. intermedius also were attempted.

Figure 3. Holding pen-Hatching unit
Utilized to isolate females prior to mating attempts and following egg deposition.


Cloth Mesh Cover
Shrimp
10 cm
Double Bottom
Cloth Mesh
Support Leg
7 cm

All precautions and controls previously discussed also apply to these crosses. However, the number of unique breeding pairs for each cross varied and some of the individuals involved in interspecific breeding were not shown to be potentially fertile by intraspecific breeding.

Palaemonetes intermedius ♀ X *P. pugio* ♂
P. pugio ♀ X *P. intermedius* ♂
P. intermedius ♀ X *P. vulgaris* ♂
P. vulgaris ♀ X *P. intermedius* ♂

Control cross:

P. intermedius ♀ X *P. intermedius* ♂

Palaemonetes pugio interpopulational crosses included the following:

Gulf Breeze ♀ X Rudee Inlet ♂
Rudee Inlet ♀ X Gulf Breeze ♂

Each cross was run using three different mating pairs. Control runs consisted of intrapopulational crosses run in triplicate as follows:

Gulf Breeze ♀ X Gulf Breeze ♂
Rudee Inlet ♀ X Rudee Inlet ♂

An additional mating was performed using specimens of *P. pugio* from Rudee Inlet. Treatment of the adults and larvae for this cross was identical to that previously described, with the exception that progeny were raised one per compartment in plastic trays containing 18 compartments. Each compartment contained 25 ml of artificial seawater. The progeny were raised individually to 40 days of age to give an estimate of the relationship between total length of the juvenile and postmetamorphosis development time. This also allowed estimation of the relationship between postmetamorphosis growth in length and postmetamorphosis development time. A single hatch of larvae was used in estimating these phenomena.

All of the preceding crosses were attempted in (1-quart)plastic sherbert or Cool Whip containers at 25 $^{o}/oo$, 25°C, and 14.5 hour photoperiod per day. Temperature and photoperiod were controlled in a Percival model I-35L

EXPERIMENTS IN ATTEMPTED INTERSPECIFIC AND INTERPOPULATIONAL HYBRIDIZATION OF GRASS SHRIMP OF THE GENUS *PALAEMONETES* (CARIDEA, PALAEMONIDAE)

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Anthony J. Provenzano, Jr.
(Director)

incubator. Artificial seawater, used in all experiments, was prepared with Instant Ocean Synthetic Sea Salts (Aquarium Systems, Inc.) and tap water. The values for photoperiod and temperature were chosen because Little (1967) used them and succeeded in inducing breeding of P. pugio during the winter. Salinity was chosen arbitrarily, but was within the range favorable to these species (Broad and Hubschman, 1962; Sandifer, 1973).

Females which had deposited eggs were transferred to the holding pen-hatching units. These units were maintained in 3.5 liter aquaria at room temperature. The females remained there until they hatched larvae 11 to 15 days later, or until the eggs were picked off or dropped indicating that fertilization had not taken place (Burkenroad, 1947). Because Thakur discovered that multiple matings produced hybrids while single matings failed, females which yielded infertile eggs were placed back with the original male for another attempt. If eggs showed early development but failed to hatch on the second try, additional attempts were made until no further progress in embryological development was observed or until larvae were hatched.

Handling of Newly Hatched Larvae

Upon hatching, larvae were transferred to culture dishes 20 cm in diameter each containing 1 liter of artificial seawater. Larvae were transferred at random into two dishes. If the hatch exceeded 72 larvae, 36 per bowl, a third bowl was used in order to facilitate rearing the entire hatch. The number of larvae per hatch was recorded.

Seawater in each dish was replaced daily. Larvae were transferred by pipette as per Hinsman (1977) and were fed live *Artemia salina* nauplii (San Fransisco Bay Brand, Menlo Park, California) daily in the amount of

10 nauplii per ml of rearing water, shown by Provenzano et al. (1977) to be adequate. Larvae were reared at 25 $^{o}/oo$ and 25°C, on a 14.5/9.5 light-dark cycle. During rearing, percent survival, larval development time (days), and postlarval length were recorded. The length of live postlarvae (from the tip of the rostrum to the tip of the telson, omitting setae) was measured to the nearest 0.05 millimeters using calipers.

Larvae were maintained until the brood had reached an age of 40 days. At 40 days, juveniles were examined for sex, length, rostral armature, relative lengths of segments of the second pereiopod, and tooth configuration on the chela of the second pereiopod (Table 2).

Statistical Methods

Simple bivariate regression analyses were calculated on the Dec system 10 computer as per Nie et al. (1970).

Two and three-way nested analyses of variance and covariance with repeated measures (Dixon, 1975) were also calculated on this computer system.

Chi-square analyses, t-tests for unequal sample sizes, and comparisons of the standard errors of means (Sokal & Rohlf, 1969) were calculated on a desk calculator. T-test analyses varied in method according to the relative equality of the sample variances.

Table 2. Morphological characteristics examined in 40-day-old juveniles of *Palaemonetes pugio* and *P. vulgaris* and descriptions of each as given by Holthuis (1952). Sex, length (mm), and the number of dorsal rostral spines were also recorded.

Characteristic	*P. pugio*	*P. vulgaris*
Rostrum Dorsal	tip naked	spines up to tip
Rostrum Ventral	generally 2 or 3 spines	generally 4 or 5 spines
Dorsal rostral spines on carapace	1	2
Carpus second pereiopod of female	longer than palm, as long as merus	shorter than palm, shorter than merus
Carpus second pereiopod of male	almost as long as chela, as long as merus	longer than palm, shorter than merus
Teeth of chela of second pereiopod $\frac{\text{dactylus}}{\text{fixed finger}}$	$\frac{0}{0}$	$\frac{2}{1}$

RESULTS

The developmental history for all progeny used in the following analyses is tabulated in Appendices A to J. The data are tabulated by mating type, female and culture dish.

Interspecific Breeding

Larvae could not be produced from interspecific crosses attempted in this study. One to four breeding attempts were completed for each cross. Eggs were deposited by each female from one to four times.

Egg masses produced in reciprocal crosses of Palaemonetes pugio and P. vulgaris were dropped or picked off within a period of 1 to 3 days, indicating that no fertilization had taken place (Burkenroad, 1947). In some cases ovaries ripened within the female were retained 2 to 3 weeks, then vanished overnight. In such instances the ovaries yellowed prior to disappearing. The history of each of these crosses is illustrated in Table 3.

All males and females involved were shown to be potentially fertile in matings with members of their own species. In all such intraspecific crosses larvae were produced.

Neither the retention time of the egg mass nor the condition of the ovaries was recorded for crosses involving P. intermedius. No hybrid larvae were produced. A summary of these hybridization attempts is given in Table 4.

Females of all three species, when bred intraspecifically, bore young. Results of these, including time (days) required for eggs to mature and hatch size data, where available, for P. pugio and P. vulgaris are compiled in

Table 3. Synopsis of interspecific reciprocal crosses involving P. pugio and P. vulgaris. Dates for the events of egg deposition, egg miscarriage, disappearance of ripe ovaries, and the retention time (days) of infertile eggs are given.

P. pugio ♀ X P. vulgaris ♂	Egg Deposition	Egg Miscarriage	Egg Retention (days)	Disappearance of Ripe Ovaries
1	5 II 77	6 II 77	1	
	13 II 77	14 II 77	1	
				4 IV 77
				20 V 77
	6 VI 77	7 VI 77	1	
2				9 III 77
				11 IV 77
	21 IV 77	23 IV 77	2	
	4 V 77	6 V 77	2	
3	5 VII 77	7 VII 77	2	
	19 VII 77	20 VII 77	1	
P. vulgaris ♀ X P. pugio ♂				
1	24 XI 76	26 XI 76	2	
	8 XII 76	10 XII 76	2	
	21 XII 76	23 XII 76	2	
	4 I 77	5 I 77	1	
	1 II 77	3 II 77	2	
	13 II 77	15 II 77	2	
2	4 II 77	6 II 77	2	
	17 II 77	18 II 77	1	
	15 III 77	17 III 77	2	
	28 III 77	31 III 77	3	
	10 IV 77	12 IV 77	2	
3	8 I 77	10 I 77	2	
	22 I 77	24 I 77	2	
	5 II 77	7 II 77	2	
	15 III 77	16 III 77	1	

Table 4. Summary of *Palaemonetes intermedius* interspecific breeding attempts. All males had demonstrated their fertility.

Breeding Pair	Number of Attempts yielding infertile eggs: Female proven fertile	Number of Attempts yielding infertile eggs: Female not proven fertile
P. pugio ♀ X *P. intermedius* ♂		
Female A	2	
Female B		2
P. intermedius ♀ X *P. pugio* ♂		
Female A	2	
Female B	2	
Female C	1	
P. vulgaris ♀ X *P. intermedius* ♂		
Female A	2	
Female B		1
P. intermedius ♀ X *P. vulgaris* ♂		
Female A	2	
Female B	2	
Female C	3	
Female D		2

Table 5. Similar information was not recorded for P. intermedius intraspecific crosses.

Relationship of Postlarval Length to Duration of Larval Development

Larvae which metamorphosed latest were generally largest. Regression coefficients were calculated to evaluate the portion of the variance in postlarval length explained by the duration of larval development. The significance of the regression coefficients were estimated by calculation of F ratios which were computed by the following method (Nie et al., 1970):

$$F = \frac{SS_{reg}/1}{SS_{res}/N-2}$$

where 1 and N equal the degrees of freedom for SS_{reg} and SS_{res} respectively.

The regression coefficients 0.032 and 0.042 for the intraspecific cross data of P. pugio and P. vulgaris, respectively, indicate a small amount of variance in postlarval length explained by the duration of larval development. Both coefficients, however, are highly significant at the 0.001 level (Table 6).

Effects of Population Density on Larval Development

The number of larvae initially placed in a culture dish may have exerted some effect on survival, postlarval length, and larval development time. For instance, lower survival may occur in dishes with greater population densities because of increased competition for food, lower oxygen levels, and higher metabolic waste concentrations. Such an effect would necessarily influence the results of anova statistics computed on these parameters. Therefore, regression analysis estimating the amount of variance in each of the three developmental parameters explained by the number of larvae initially placed

Table 5. Summary of the breeding history of intraspecific crosses for *P. pugio* and *P. vulgaris*. An *a* designation indicates the production of a second hatch of larvae from a particular breeding pair.

Intraspecific Mating Types *P. pugio*	Start of Cross	Date Eggs were Laid	Date of Hatch	Egg Dev. Time (days)	Hatch Size
1	26 XI 76	17 XII 76	30 XII 76	13	125
2	26 XII 76	8 I 77	21 I 77	13	42
3	21 I 77	22 I 77	4 II 77	13	150
4	12 I 77	14 I 77	27 I 77	13	42
4a	27 I 77	11 II 77	26 II 77	15	102
P. vulgaris					
1	8 XII 76	12 XII 76	23 XII 76	11	26
1a	23 XII 76	3 I 77	16 I 77	13	111
2	13 XII 76	2 I 77	14 I 77	12	118
3	26 XII 76	31 XII 76	12 I 77	13	40
3a	12 I 77	17 I 77	28 I 77	11	91

Table 6. Regression analysis of the relationship of postlarval length to larval development time for P. *pugio* and P. *vulgaris* intraspecific control crosses.

B = regression coefficient

	Analysis of Variance	Degrees of Freedom	Sum of Squares	Computed F
P. *pugio*	Regression	1	2.29	19.79***
B = 0.032	Residual	90	10.17	
P. *vulgaris*	Regression	1	1.55	16.37***
B = 0.042	Residual	75	6.92	

*** Significant at 0.1% level

in the culture dish was computed. In every instance, the regression coefficient was insignificant at the 5% level (Table 7).

Relationship of Juvenile Length to Postmetamorphosis Development Time

Computation of the variance in total length of juvenile shrimp at 40 days of age explained by postmetamorphosis development time yielded a 0.138 regression coefficient, significant at the 5% level. Calculation of the variance in postmetamorphosis growth explained by postmetamorphosis development time yielded a 0.222 regression coefficient. This is highly significant at the 0.1% level (Table 8). Data used to compute these coefficients are compiled in Appendix K. Postmetamorphosis development time ranged from 5-27 days in *P. pugio* and from 9-25 days in *P. vulgaris*.

Comparison of Larval and Postlarval Development of *P. pugio* and *P. vulgaris*

Analysis of variance (Dixon, 1975) was performed on postlarval length, larval development time (days), arcsine transformation of percent survival, and juvenile length at 40 days of age to compare *P. pugio* and *P. vulgaris* young. F ratios were computed using expected MS values according to methods described in Sokal & Rohlf, 1969, for nested anovas with unequal sample sizes.

Postlarval Length

Differences in the length of *P. pugio* and *P. vulgaris* postlarvae were not significant at the 5% level. However, differences among the postlarvae hatched from different females within these species were significant at the 5% level. A significant difference in length resulted from the culture dish in which a group of larvae were raised. This was significant at the 0.1%

Table 7. Summary of the regression analyses evaluating the portion of variance in percent survival, postlarval length, and larval development time (days) which is explained by the number of larvae initially placed in a culture dish for *P. pugio* and *P. vulgaris* intraspecific control crosses.

B = regression coefficient

	Analysis of Variance	Degrees of Freedom	Sum of Squares	Computed F
P. pugio percent survival B = 0.162	Regression	1	78.36	0.58 ns
	Residual	11	1222.62	
P. pugio postlarval length B = 0.003	Regression	1	0.02	0.40 ns
	Residual	11	0.49	
P. pugio development time B = - 0.025	Regression	1	1.87	0.11 ns
	Residual	11	156.16	
P. vulgaris percent survival B = - 0.678	Regression	1	395.44	3.09 ns
	Residual	10	1023.29	
P. vulgaris postlarval length B = - 0.005	Regression	1	0.02	0.48 ns
	Residual	10	0.33	
P. vulgaris development time B = 0.039	Regression	1	1.28	0.36 ns
	Residual	10	28.54	

ns Not significant at 5% level

Table 8. Regression analyses estimating the variance in total juvenile length at 40 days of age explained by postmetamorphosis development time (days) and estimating the variance in postmetamorphosis growth in length explained by postmetamorphosis development time. Both calculations are based on a single hatch of P. pugio larvae.

B = regression coefficient

	Analysis of Variance	Degrees of Freedom	Sum of Squares	Computed F
Total juvenile length	Regression	1	6.19	6.63*
B = 0.138	Residual	55	49.45	
Postmetamorphosis growth in length	Regression	1	15.95	20.93***
B = 0.222	Residual	55	40.38	

* Significant at 5% level
*** Significant at 0.1% level

level (Table 9). The ranges and means for postlarval length were 6.2 to 8.2 (mean 7.1mm) and 6.0 to 7.6 (mean 6.8mm) for P. pugio and P. vulgaris progeny, respectively.

Larval Development Time (days)

Differences in the duration of larval development between these species were not significant at the 5% level. The differences in larval development time attributable to parentage and rearing dish were significant at 5% and 0.1% levels, respectively (Table 9). Larval development time ranged from 13 to 35 days with an approximate mean of 19 days for P. pugio, while P. vulgaris development ranged from 14 to 30 days with an approximate mean of 20 days.

Survival

Survival data did not differ significantly between these species or between females within species (Table 10). Mean survival approximated 83% for P. pugio larvae and 76% for the P. vulgaris larvae.

Juvenile Length at 40 Days of Age

The only significant difference (5% level) between these species was in the lengths of juvenile shrimp. A pictorial representation of the foregoing is presented in Figure 4, which is based on the data catalogued in Appendix R. Differences due to parentage and rearing dish were significant, 1% and 0.1% levels, respectively (Table 9). P. pugio had a mean length of 12.4 mm and a range of 8.2 to 15.1 mm, whereas P. vulgaris specimens were much smaller ranging from 7.6 to 12.2 mm with a mean of 10.3 mm.

Juvenile Sex Ratio Analysis

Chi-square analysis of observed sex ratios in P. pugio and P. vulgaris juveniles indicates that there is much less than a 0.5% chance that the

Table 9. Three-way nested analysis of variance and covariance with repeated measures comparing P. pugio and P. vulgaris progeny. Variance is analyzed among species, among females within species, and among culture dishes within females for postlarval length, larval development time (days) and juvenile length at 40 days of age.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Postlarval Length				
Groups (among species)	123718.22	1	123718.22	5.04 ns
Subgroups (among females)	112742.73	5	22548.55	4.05 *
Subsubgroups (among dishes)	92662.88	18	5174.94	6.08 ***
Error (within dishes)	541431.42	640	845.99	

Expected $MS'_{subgroups}$ = 24568.69

Expected $MS'_{subsubgroups}$ = 5561.70

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Larval Development Time				
Groups(among species)	211.15	1	211.15	0.44 ns
Subgroups (among females)	2232.53	5	446.51	3.23 *
Subsubgroups (among dishes)	2278.89	18	126.61	19.44 ***
Error (within dishes)	6.51	641		

Expected $MS'_{subgroups}$ = 484.86

Expected $MS'_{subsubgroups}$ = 138.88

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Juvenile Length				
Groups (among species)	5820205.31	1	5820205.31	19.26 *
Subgroups (among females)	1230370.19	4	307592.55	9.10 **
Subsubgroups (among dishes)	387841.88	12	32320.16	3.64 ***
Error (within dishes)	4602160.56	518		

Expected $MS'_{subgroups}$ = 302167.56

Expected $MS'_{subsubgroups}$ = 33791.58

ns Not significant at 5% level
* Significant at 5% level
** Significant at 1% level
*** Significant at 0.1% level

Figure 4. Mean, range, and standard deviation of juvenile lengths in millimeters for *P. pugio* and *P. vulgaris* progeny at 40 days of age. Horizontal lines indicate the mean juvenile length for each species, vertical lines the range and vertically oriented boxes represent ± one standard deviation unit around the means. Numbers located above each unit depict the sample size on which these statistics are based.

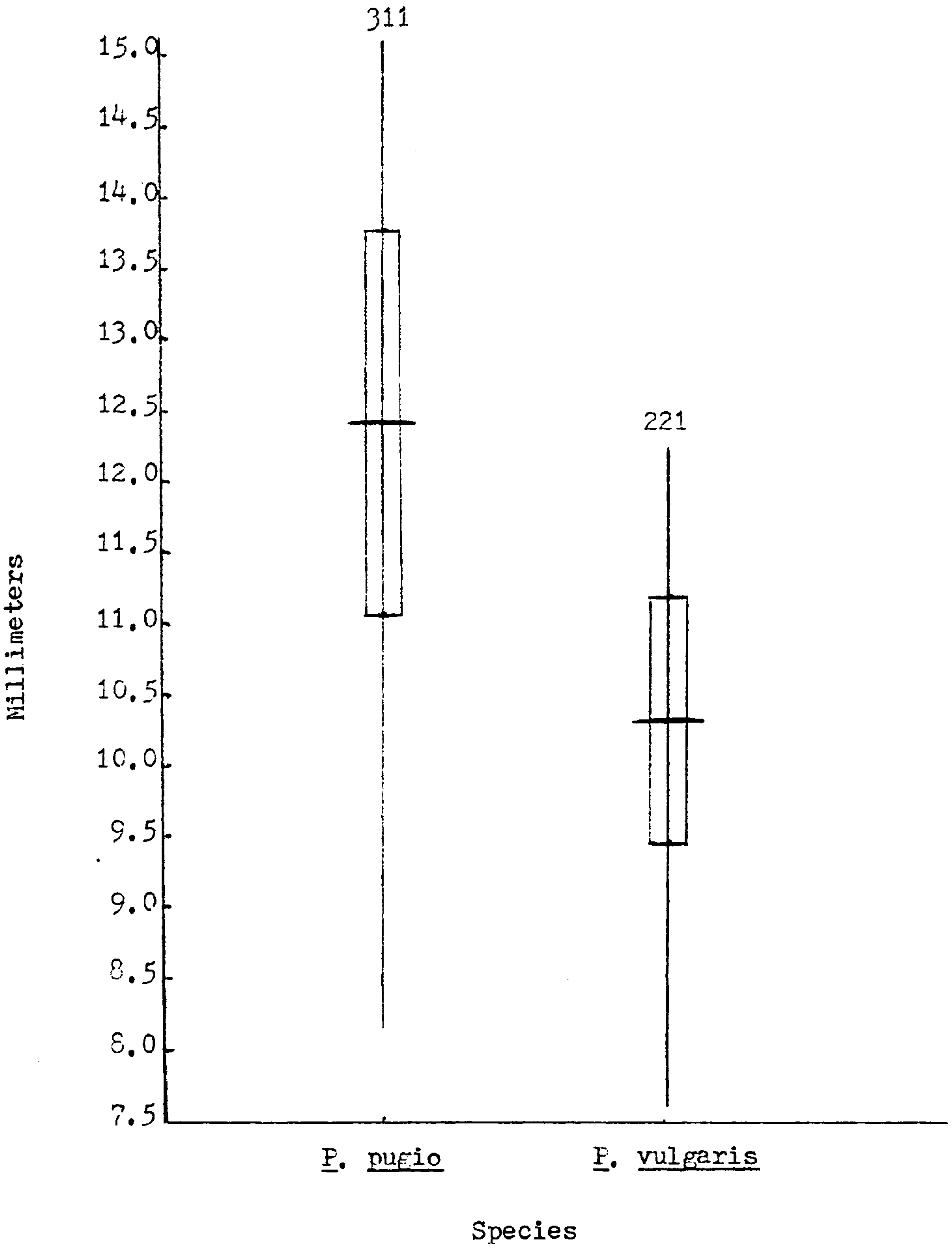

311
221
15.0
14.5
14.0
13.5
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
Millimeters
P. pugio
P. vulgaris
Species

Table 10. Two-way nested analysis of variance and covariance with repeated measures comparing the arcsine transformations of percent survival for P. pugio and P. vulgaris progeny. Variance is analyzed among species and among females within species.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Groups (among species)	1805061.88	1	1805061.88	2.30 ns
Subgroups (among females)	3806029.63	5	761205.92	1.32 ns
Error (within females)	10352673.60	18	575148.53	

Expected $MS'_{subgroups} = 784809.47$

ns Not significant at 5% level

observed deviation from a 1:1 sex ratio is a chance deviation (Table 11).

Juvenile Rostral Armature

A final statistical consideration involved the comparison of rostral armature (number of spines) between the juveniles of these two species. In observing the data accumulated, no real difference was evident. Therefore, an abbreviated analysis was completed to determine the need for computing more complicated anova statistics. This involved the calculation of sample means and the standard errors thereof (Sokal & Rohlf, 1969).

The two parameters investigated were the numbers of dorsal and ventral spines on the rostrum.

In both analyses, the standard error of the mean of one species overlapped the mean of the opposing species (Appendix L). Such results indicate that no significant difference would ensue from anova statistics comparing the rostral armature of these two species.

Consequently, no analysis of variance was computed for these characteristics.

Juvenile Morphology

P. intermedius, as its name implies, is morphologically intermediate between P. pugio and P. vulgaris. This relationship is represented in Figure 5, where Holthuis' (1952) summary of the distinguishing characteristics of these species is tabulated and diagramed to show similarities of each shrimp. Although close examination of the species facilitates identification in most cases, there are instances in which they are difficult to distinguish. This is especially true among juveniles (Holthuis, 1952). The present study of P. pugio and P. vulgaris progeny substantiates his observation.

Table 11. Chi-square analysis for goodness of fit assuming a 1:1 sex ratio for P. pugio and P. vulgaris juveniles.

A. P. pugio juveniles

Sex	Observed Frequency	Expected Frequency	X^2
Male ♂	41	158	173.28
Female ♀	275	158	

$X^2_{0.005}(1) = 7.88$

Probability < 0.005

B. P. vulgaris juveniles

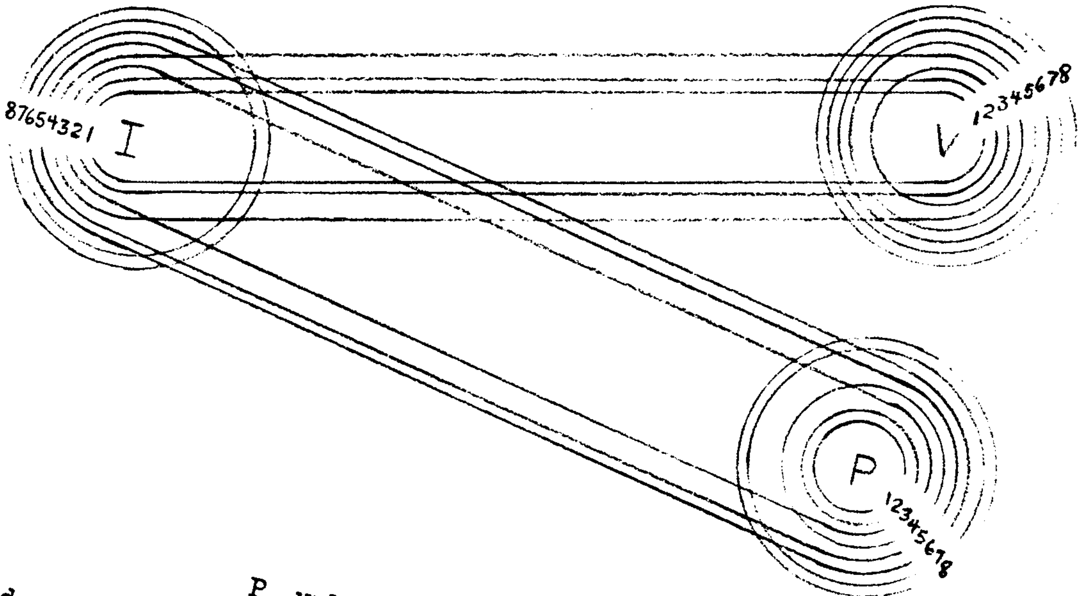
Sex	Observed Frequency	Expected Frequency	X^2
Male ♂	25	111	133.30
Female ♀	197	111	

$X^2_{0.005}(1) = 7.88$

Probability < 0.005

Figure 5. A pictorial association of the distinguishing characteristics of three *Palaemonetes* species. Numbered lines correspond to the numbered characteristics of the table (Holthuis, 1952). Lines encircling more than one species, therefore, denote characteristics common to the species encircled.

P = P. pugio
V = P. vulgaris
I = P. intermedius



	P. vulgaris	P. intermedius	P. pugio
1. Rostrum dorsal	teeth up to tip	teeth up to tip	tip naked
2. Rostrum ventral	generally 4 or 5 teeth	generally 4 or 5 teeth	generally 2 or 3 teeth
3. Dorsal rostral teeth on carapace	2	1	1
4. Scaphocerite $\frac{\text{length}}{\text{breadth}}$	about 3	about 3	about 2.5
5. Carpus second legs female	shorter than palm, 0.7 times merus	distinctly longer than palm, as long as merus	distinctly longer than palm, as long as merus
6. Carpus second legs male	1.1 times as long as palm, 0.8 times merus	almost as long as chela, as long as merus	almost as long as chela, as long as merus
7. Teeth of chela of second legs $\frac{\text{dactylus}}{\text{fixed finger}}$	$\frac{2}{1}$	$\frac{1}{0}$	$\frac{0}{0}$
8. Pleura of fifth abdominal segment	rounded or rectangular	rounded	generally pointed

The examination of 316 P. pugio juveniles from three different females showed that these juveniles, almost without exception, conform to Holthuis' description in the characters analyzed. There were only two instances in which these characters were deviant. In both cases, a single shrimp from each of two females had developed with one and one half dorsal rostral spines on the carapace rather than one.

P. vulgaris juveniles showed a much wider range of morphological expression, making species identification extremely difficult, if not impossible, for many individuals. A total of 221 specimens from three different females were examined.

Normal P. vulgaris adults have a spine located on the dorsal tip of the rostrum. All of the juveniles examined displayed a naked dorsal rostral tip, which is diagnostic of P. pugio. These naked tips or platforms, were, in general, not as large as those exhibited in P. pugio juveniles.

Another distinguishing characteristic among adults pertains to the number of dorsal rostral teeth or spines on the carapace. P. pugio adults characteristically have one spine while P. vulgaris adults have two. In examination of juveniles, if the anterior base of a spine was behind the posterior margin of the base of the eyestalk the spine was considered to be located on the carapace. Those P. vulgaris juveniles examined showed three separate forms of this characteristic. Only five specimens exhibited two dorsal rostral spines on the carapace, diagnostic of the adult form. One hundred and fifty-nine (159) individuals showed an intermediate case of one and one half spines and the remaining 57 juveniles had only one dorsal rostral spine on the carapace which is characteristic of P. pugio. Nevertheless, a rather obscure difference does exist between normal P. pugio juveniles and P. vulgaris juveniles bearing only one dorsal rostral spine on the carapace. Among P. pugio juveniles the carapace spine occurs after an interval which is similar in length to the spaces between the other dorsal rostral spines. When a single spine was present on the carpace of P. vulgaris juveniles, it occurred after an interval somewhat larger than the spaces between the other dorsal rostral spines (Figure 6).

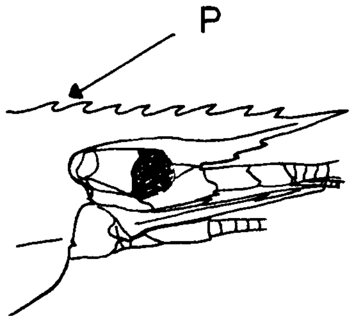
A study of the relative lengths of the segments of the second pereiopod showed only one major discrepancy from Holthuis' description of adult characteristics. In all P. vulgaris juveniles observed, the carpus was slightly longer than the palm of the second pereiopod. In normal P. vulgaris adults the carpus is shorter than the palm. This character varies with sex in P. pugio.

The tooth ratio for the dactylus and fixed finger of the second pereiopod also exhibited substantial deviation from the norm of the species. P. pugio adults characteristically have a $\frac{0}{0}$ ratio or no teeth on the inner margins of the chela, whereas P. vulgaris normally has a 2/1 tooth ratio. P. vulgaris juveniles examined in this study showed five variations of this ratio. Only 22 individuals had tooth ratios characteristic of the species (2/1). One specimen had a $\frac{0}{1}$ ratio, five a $\frac{1}{1}$ ratio, and 41 individuals had a $\frac{1}{0}$ ratio. The remaining 153 juveniles displayed a $\frac{0}{0}$ tooth ratio which is characteristic of P. pugio adults. The preceding results are tabulated with respect to species, female, and dish in Appendices P and Q.

Intrapopulational and Reciprocal Interpopulational Breeding of Palaemonetes pugio

Interpopulational breeding between geographically separated populations of P. pugio was successful. Larvae were produced in all types of crosses attempted, including control crosses. The history of each mating pair is compiled in Table 12.

Figure 6. Representation of the anterior sections of *P. pugio* and *P. vulgaris* juveniles. Intervals between the single carapace spine and the dorsal rostral spines of *P. pugio* and *P. vulgaris* juveniles are designated P and V, respectively.


P

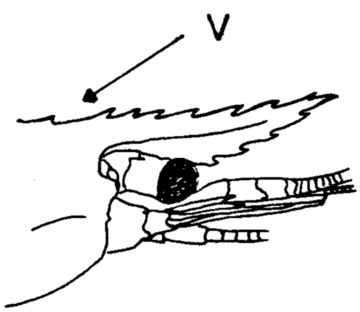

V

Table 12. Summary of inter- and intrapopulational crosses of <u>Palaemonetes pugio</u> from Rudee Inlet, Virginia, and Gulf Breeze, Florida.

RI = Rudee Inlet <u>P. pugio</u>
GB = Gulf Breeze <u>P. pugio</u>

RI ♀ X GB ♂	Start of Cross	Egg Deposition	Egg Miscarriage	Eggs Hatched	Egg Retention (days)	Hatch Size
1	21 XII 76	9 I 77		24 I 77	15	36
2	3 I 77	26 I 77	29 I 77			
2a	3 II 77	9 III 77		23 III 77	14	48
3	9 I 77	21 I 77		5 II 77	15	14
3a	7 II 77	9 II 77	10 II 77			
3b	17 II 77	21 II 77		8 III 77	15	9
GB ♀ X RI ♂						
1	19 X 76	20 XII 76		30 XII 76	10	20
1a	31 XII 76	9 I 77		20 I 77	11	29
2	20 X 76	9 XI 76	11 XI 76			
2a	11 XI 76	23 XII 76		8 I 77	16	15
2b	12 I 77	9 II 77		23 II 77	14	27
3	21 X 76	20 XII 76		30 XII 76	10	25
3a	31 XII 76	12 I 77		24 I 77	12	11
GB ♀ X GB ♂						
1	28 X 76	26 XI 76		10 XI 76	14	38
2	9 XI 76	10 XII 76		22 XII 76	12	75
3	14 XI 76	5 XII 76		20 XII 76	15	40
3a	3 I 77	15 I 77		28 I 77	13	46
RI ♀ X RI ♂						
1	26 XI 76	17 XII 76		30 XII 76	13	125
2	26 XII 76	8 I 77		21 I 77	13	42
3	21 I 77	22 I 77		4 II 77	13	150
4	12 I 77	14 I 77		27 I 77	13	42
4a	27 I 77	11 II 77		26 II 77	15	102

Relationship of Postlarval Length to Duration of Larval Development

Regression coefficients were computed to estimate the amount of variance in postlarval length which can be explained by the duration of larval development. F ratios were computed by the methods previously described (Nie et al., 1970).

Regression coefficients were small for all types of crosses and for the combined data, ranging from 0.032 - 0.065. However, in all cases the coefficient was highly significant at the 0.1% level (Table 13).

Effects of Population Density of Larval Development

The amount of variance in postlarval length, larval development time, and percent survival which can be explained by the initial dish population density is also computed by regression analysis (Nie et al., 1970).

These calculations, without exception yielded small regression coefficients which were not significant at the 5% level (Tables 14, 15, and 16).

Larval and Postlarval Comparisons

Analysis of variance, two and three-way, (Dixon 1975) was computed on postlarval length, duration of larval development (days), arcsine transformations of percent survival, and juvenile length at 40 days of age. These tests were completed to evaluate possible differences in the larvae, postlarvae, and juveniles reared from intrapopulational and reciprocal interpopulational crosses of *Palaemonetes pugio*.

Survival data was computed on premetamorphosis offspring. Data were not recorded for postlarval and juvenile survival. Larvae which were accidently killed in the course of the experiment were eliminated from the data pool.

Table 13. Regression analysis of the relationship of postlarval length to larval development time for P. pugio. Progeny from intrapopulational crosses and reciprocal interpopulational crosses for Gulf Breeze and Rudee Inlet populations are analyzed.

B = regression coefficient
RI= Rudee Inlet P. pugio
GB= Gulf Breeze P. pugio

	Analysis of Variance	Degrees of Freedom	Sum of Squares	Computed F
RI ♀ X GB ♂	Regression	1	4.75	47.36***
B = 0.065	Residual	35	3.31	
GB ♀ X RI ♂	Regression	1	11.77	106.23***
B = 0.051	Residual	52	5.54	
GB ♀ X GB ♂	Regression	1	4.62	70.89***
B = 0.058	Residual	62	3.91	
RI ♀ X RI ♂	Regression	1	2.29	19.79***
B = 0.032	Residual	90	10.17	
Combined data	Regression	1	31.25	247.45***
of all matings	Residual	245	30.69	
B = 0.055				

*** Significant at 0.1% level

Table 14. Summary of regression analyses evaluating the portion of variance in postlarval length, which can be explained by the number of larvae initially placed in a culture dish. Computations are included for intrapopulational and reciprocal interpopulational crosses of *P. pugio* from Rudee Inlet and Gulf Breeze.

B = regression coefficient
RI = Rudee Inlet *P. pugio*
GB = Gulf Breeze *P. pugio*

	Analysis of Variance	Degrees of Freedom	Sum of Squares	Computed F
RI ♀ X GB ♂	Regression	1	0.00034	0.0025 ns
B = 0.001	Residual	4	0.27046	
GB ♀ X RI ♂	Regression	1	0.04	0.26 ns
B = 0.012	Residual	4	0.31	
GB ♀ X GB ♂	Regression	1	0.00	0.00 ns
B = - 0.000	Residual	6	0.13	
RI ♀ X RI ♂	Regression	1	0.02	0.40 ns
B = 0.003	Residual	11	0.49	
Combined data	Regression	1	0.05	0.625 ns
of all matings	Residual	31	2.33	
B = 0.003				

ns Not significant at 5% level

Table 15. Summary of regression analyses evaluating the amount of variance in larval development time (days) which can be explained by culture dish population density. Computations are included for intrapopulational crosses and reciprocal interpopulational crosses of *P. pugio* from Rudee Inlet and Gulf Breeze.

B = regression coefficient
RI = Rudee Inlet *P. pugio*
GB = Gulf Breeze *P. pugio*

	Analysis of Variance	Degrees of Freedom	Sum of Squares	Computed F
RI ♀ X GB ♂	Regression	1	3.62	0.07 ns
B = - 0.146	Residual	4	103.18	
GB ♀ X RI ♂	Regression	1	2.92	0.12 ns
B = 0.107	Residual	4	48.84	
GB ♀ X GB ♂	Regression	1	2.59	0.17 ns
B = - 0.073	Residual	6	62.32	
RI ♀ X RI ♂	Regression	1	1.87	0.11 ns
B = - 0.025	Residual	11	156.16	
Combined data of all matings	Regression	1	6.28	0.44 ns
B = - 0.034	Residual	31	416.45	

ns Not significant at 5% level

Table 16. Summary of regression analyses evaluating the amount of variance in percent survival which can be explained by culture dish population density. Computations are included for intrapopulational crosses and reciprocal interpopulational crosses of P. pugio from Rudee Inlet and Gulf Breeze.

B = regression coefficient
RI = Rudee Inlet P. pugio
GB = Gulf Breeze P. pugio

	Analysis of Variance	Degrees of Freedom	Sum of Squares	Computed F
RI ♀ X GB ♂	Regression	1	53.86	0.17 ns
B = - 0.565	Residual	4	624.58	
GB ♀ X RI ♂	Regression	1	117.38	0.87 ns
B = - 0.681	Residual	4	270.77	
GB ♀ X GB ♂	Regression	1	2.75	0.02 ns
B = 0.075	Residual	6	495.63	
RI ♀ X RI ♂	Regression	1	78.36	0.58 ns
B = 0.162	Residual	11	1222.62	
Combined data	Regression	1	155.36	0.92 ns
of all matings	Residual	31	4891.22	
B = 0.167				

ns Not significant at 5% level

Jumpers or shrimp stranded on the sides of the culture dish, were occasionally encountered. These were tabulated and compared between mating types prior to in depth analysis of the data. Jumpers, as a percent of the total mortality, are recorded in Table 17. These data indicated that death caused by stranding may have been more prevalent in the progeny of some mating types than in others, so these were left in the data pool and computed into percent survival statistics. A summary of the significance levels of the analyses of variance which follow may be found in Table 30.

Gulf Breeze ♀ X Gulf Breeze ♂ Progeny vs.
Rudee Inlet ♀ X Rudee Inlet ♂ Progeny

Intrapopulational crosses of Gulf Breeze and Rudee Inlet *P. pugio* produced offspring which were not significantly different in duration of larval development, arcsine transformations of percent survival, postlarval length, or juvenile length (Table 18 and 19). However, deviations in these characteristics attributable to parentage were significant at the 5%, 5%, 0.1% and 1% levels, respectively. An even more dramatic difference was revealed among culture dishes within females. Here, significance was at the 0.1% level for all parameters measured. Survival data was computed with a two-way anova. Therefore, differences between culture dishes were not analyzed.

Gulf Breeze ♀ X Gulf Breeze ♂ Progeny vs.
Gulf Breeze ♀ X Rudee Inlet ♂ Progeny

Comparisons of developmental characteristics between the progeny of these crosses (among types) again yielded no significant differences. Among female differences within the mating types were significant at the 0.1% level for postlarval length and larval development time. However, survival and juvenile length variations among females were not significant.

Table 17. Jumpers or larvae stranded, expressed as a percent of the total mortality within mating types.

RI = Rudee Inlet *P. pugio*
GB = Gulf Breeze *P. pugio*

		RI ♀ X GB ♂	GB ♀ X RI ♂	GB ♀ X GB ♂	RI ♀ X RI ♂
Jumpers		9	6	5	5
Total Mortality		38	20	19	78
Jumpers / Total Mortality	X 100	23.68%	30.00%	26.30%	6.41%

Table 18. Three-way nested analysis of variance and covariance with repeated measures comparing the larvae of intrapopulational crosses of Gulf Breeze and Rudee Inlet P. pugio. Variance is analyzed among mating types, among females within types, and among dishes within females for postlarval length, larval development time (days), and juvenile length at 40 days of age.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Postlarval Length				
Groups (among types)	159053.57	1	159053.57	6.54 ns*
Subgroups (among females)	125473.66	5	25094.73	9.47 ***
Subsubgroups (among dishes)	33890.48	13	2606.96	2.78 ***
Error (within dishes)	487902.70	521	936.47	

Expected $MS'_{subgroups}$ = 24334.50

Expected $MS'_{subsubgroups}$ = 2650.92

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Larval Development Time				
Groups (among types)	170.77	1	170.77	0.27 ns
Subgroups (among females)	3254.38	5	650.88	4.48 *
Subsubgroups (among dishes)	1844.68	13	141.90	18.71 ***
Error (within dishes)	3950.39	521	7.58	

Expected $MS'_{subgroups}$ = 636.36

Expected $MS'_{subsubgroups}$ = 145.43

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Juvenile Length				
Groups (among types)	792188.25	1	792188.25	2.60 ns
Subgroups (among females)	1371961.38	4	342990.34	11.60 **
Subsubgroups (among dishes)	259124.88	9	28791.65	3.10 ***
Error (within dishes)	4001462.25	431	9284.14	

Expected $MS'_{subgroups}$ = 304687.78

Expected $MS'_{subsubgroups}$ = 29510.43

ns Not significant at 5% level
* Significant at 5% level
** Significant at 1% level
*** Significant at 0.1% level
ns* Not significant at 5% level, significant at 10% level (refer to Discussion)

Table 19. Two-way nested analysis of variance and covariance with repeated measures comparing larvae of intrapopulational crosses of Gulf Breeze and Rudee Inlet *P. pugio*. Variance is analyzed among mating types and among females within types for the arcsine transformation of percent survival.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Groups (among types)	1571905.13	1	1571905.13	2.76 ns
Subgroups (among females)	1608210.25	5	321642.05	0.47 ns
Error (within females)	8879160.25	13	683012.33	

Expected $MS'_{subgroups}$ = 570076.19

ns Not significant at 5% level

Deviations due to the rearing dish in which larvae were raised were not significant at the 5% level for the duration of larval development or for the length of the postlarvae. They were highly significant (0.1%), however, for the length of the juveniles. Anova statistics concerned with postlarval lengths, larval development time, and juvenile lengths are compiled in Table 20. Arcsine transformation of percent survival analysis is found in Table 21.

Gulf Breeze ♀ X Gulf Breeze ♂ Progeny vs.
Rudee Inlet ♀ X Gulf Breeze ♂ Progeny

Comparison of the progeny of these crosses yielded the first significant difference (5%) of this study between two mating types. This arose in the analysis of variance for arcsine transformations of percent survival (Table 23). This relationship is illustrated in Figure 7, based on the data tabulated in Appendix S. The remaining three developmental parameters investigated (postlarval length, larval development time, and juvenile length) were not significantly different between mating types (Table 22).

Conversely, deviations due to parentage for these three characteristics were significant but were not significant for the survival data.

As in the previous analysis, deviations attributed to the culture dish in which larvae were reared were significant at the 5% level for postlarval length and larval development time, but were not significant when the length of the juveniles was considered.

Rudee Inlet ♀ X Rudee Inlet ♂ Progeny vs.
Gulf Breeze ♀ X Rudee Inlet ♂ Progeny

None of the developmental characters analyzed were significantly different in the comparison of these two mating types.

Table 20. Three-way nested analysis of variance and covariance with repeated measures comparing the larvae of Gulf Breeze intrapopulational crosses and interpopulational crosses involving Gulf Breeze females and Rudee Inlet males. Variance is analyzed among mating types, among females within types, and among dishes within females for postlarval length, larval development time (days), and juvenile length at 40 days of age.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Postlarval Length				
Groups (among types)	185831.73	1	185831.73	7.57 ns
Subgroups (among females)	96649.18	4	24162.29	23.34 ***
Subsubgroups (among dishes)	7463.02	7	1066.15	0.70 ns
Error (within dishes)	387477.48	253	1531.53	

Expected $MS'_{subgroups}$ = 24543.70

Expected $MS'_{subsubgroups}$ = 1035.33

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Larval Development Time				
Groups (among types)	1027.03	1	1027.03	1.45 ns
Subgroups (among females)	2781.74	4	695.44	26.41 ***
Subsubgroups (among dishes)	182.27	7	26.04	1.20 ns
Error (within dishes)	5469.69	253	21.62	

Expected $MS'_{subgroups}$ = 706.85

Expected $MS'_{subsubgroups}$ = 26.33

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Juvenile Length				
Groups (among types)	10644.44	1	10644.44	0.07 ns
Subgroups (among females)	578620.09	4	144655.02	4.14 ns
Subsubgruops (among dishes)	204717.56	6	34119.59	3.77 ***
Error (within dishes)	2011150.38	222	9059.24	

Expected $MS'_{subgroups}$ = 157437.85

Expected $MS'_{subsubgroups}$ = 34925.58

ns Not significant at 5% level

*** Significant at 0.1% level

Table 21. Two-way nested analysis of variance and covariance with repeated measures comparing larvae of Gulf Breeze intrapopulational crosses and interpopulational crosses involving Gulf Breeze females and Rudee Inlet males. Variance is analyzed among mating types and among females for the arcsine transformation of percent survival.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Groups (among types)	142613.69	1	142613.69	1.83 ns
Subgroups (among females)	3386961.06	4	846740.27	1.07 ns
Error (within females)	5530459.81	7	790065.69	

Expected $MS'_{subgroups} = 848194.95$

ns Not significant at 5% level

Table 22. Three-way nested analysis of variance and covariance with repeated measures comparing the larvae of Gulf Breeze intrapopulational crosses and interpopulational crosses involving Rudee Inlet females and Gulf Breeze males. Variance is analyzed among mating types, among females within types, and among dishes within females for postlarval length, larval development time (days), and juvenile length at 40 days of age.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Postlarval Length				
Groups (among types)	62686.89	1	62686.89	6.07 ns
Subgroups (among females)	43344.51	4	10836.13	7.72 *
Subsubgroups (among dishes)	9832.88	7	1404.70	1.04 ns
Error (within dishes)	290811.73	215	1352.61	

Expected $MS'_{subgroups}$ = 10326.50

Expected $MS'_{subsubgroups}$ = 1403.31

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Larval Development Time				
Groups (among types)	194.26	1	194.26	0.36 ns
Subgroups (among females)	2279.71	4	569.93	30.05 ***
Subsubgroups (among dishes)	134.43	7	19.20	1.87 ns
Error (within dishes)	2204.24	215	10.25	

Expected $MS'_{subgroups}$ = 539.99

Expected $MS'_{subsubgroups}$ = 18.96

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Juvenile Length				
Groups (among types)	427862.66	1	427862.66	1.34 ns
Subgroups (among females)	1216329.31	4	304082.33	15.91 **
Subsubgroups (among dishes)	117476.03	6	19579.34	2.15 *
Error (within dishes)	1702613.25	187	9104.88	

Expected $MS'_{subgroups}$ = 320206.19

Expected $MS'_{subsubgroups}$ = 19117.98

ns Not significant at 5% level
* Significant at 5% level
** Significant at 1% level
*** Significant at 0.1% level

Table 23. Two-way nested analysis of variance and covariance with repeated measures comparing larvae of Gulf Breeze intrapopulational crosses and interpopulational crosses involving Rudee Inlet females and Gulf Breeze males. Variance is analyzed among mating types and among females for arcsine transformations of percent survival.

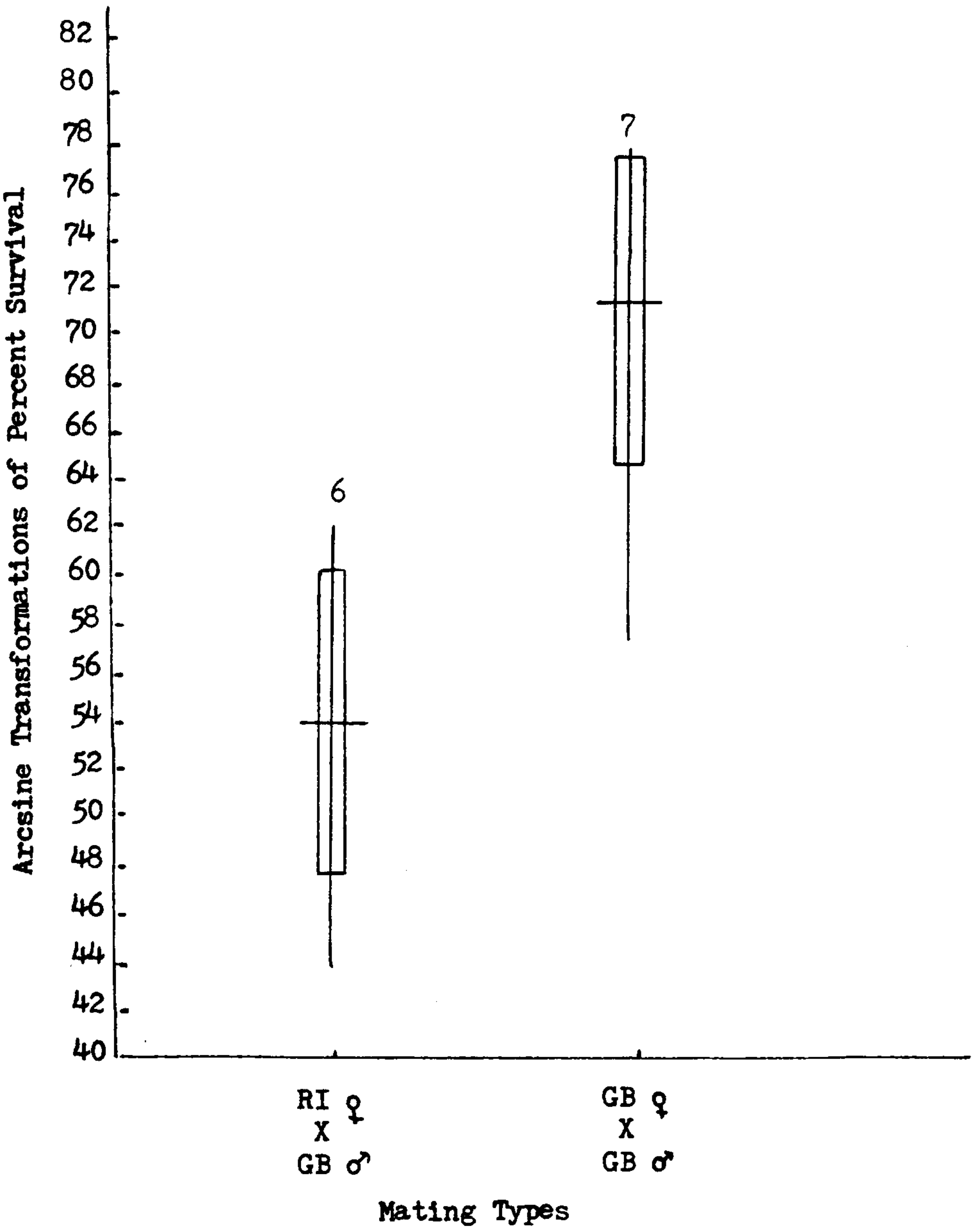
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Groups (among types)	9976568.50	1	9976568.50	18.60 *
Subgroups (among females)	2141921.06	4	535480.27	1.07 ns
Error (within females)	3499653.75	7	499950.54	

Expected $MS'_{subgroups} = 563721.76$

ns Not significant at 5% level
* Significant at 5% level

Figure 7. Mean, range, and standard deviation of the arcsine transformations of percent survival for Gulf Breeze intrapopulational crosses and interpopulational crosses involving Rudee Inlet females and Gulf Breeze males. Horizontal lines represent the mean, vertical lines the range, and vertically oriented boxes depict ± one standard deviation unit around the mean. Numbers above each of these boxes indicate the size of the sample on which these statistics were computed.

RI = Rudee Inlet *P. pugio*
GB = Gulf Breeze *P. pugio*


Arcsine Transformations of Percent Survival
82
80
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52
50
48
46
44
42
40
6
7
RI ♀
X
GB ♂
GB ♀
X
GB ♂
Mating Types

Differences due to parentage for arcsine transformations of percent survival were not significant either (Table 25). The effect of ancestry was evident for the other three parameters analyzed yielding significant differences at levels of 0.1%, 5%, and 1% for postlarval length, larval development time, and juvenile length, respectively. Deviations among dishes within females were significant at various levels for all parameters investigated (Table 24).

Rudee Inlet ♀ X Rudee Inlet ♂ Progeny vs.
Rudee Inlet ♀ X Gulf Breeze ♂ Progeny

The only characteristic showing significant difference between these two mating types was survival (Table 27). We observed an identical result when comparing intrapopulational Gulf Breeze progeny with the progeny of the foregoing interpopulational mating type. This relationship is illustrated in Figure 8 based on data tabulated in Appendix T.

Survival deviations were not significant among females, whereas postlarval length, duration of larval development, and juvenile length did show significant differences here.

Highly significant differences were recorded among dishes within females for all parameters analyzed (Table 26).

Gulf Breeze ♀ X Rudee Inlet ♂ Progeny vs.
Rudee Inlet ♀ X Gulf Breeze ♂ Progeny

No significant differences were detected in the analyses performed to compare the progeny of the two interpopulational mating types. In addition, analysis of survival data among females showed no significant deviations (Table 29). By contrast, significant differences were disclosed, due to parentage, for postlarval length, larval development time, and juvenile length (Table 28).

Table 24. Three-way nested analysis of variance and covariance with repeated measures comparing the larvae of Rudee Inlet intrapopulational crosses and interpopulational crosses involving Gulf Breeze females and Rudee Inlet males. Variance is analyzed among mating types, among females within mating types, and among dishes within females for postlarval length, larval development time (days), and juvenile length at 40 days of age.

Source of Variation	Sum of Square	Degrees of Freedom	Mean Square	Computed F
Postlarval Length				
Groups (among types)	16452.26	1	16452.26	0.58 ns
Subgroups (among females)	184040.27	5	36808.05	13.45 ***
Subsubgroups (among dishes)	32669.09	12	2722.42	2.42 **
Error (within dishes)	527978.75	470	1123.36	

Expected $MS'_{subgroups}$ = 36805.94

Expected $MS'_{subsubgroups}$ = 2736.37

Source of Variation	Sum of Square	Degrees of Freedom	Mean Square	Computed F
Larval Development Time				
Groups (among types)	576.12	1	576.12	1.00 ns
Subgroups (among females)	3640.35	5	728.07	4.41 *
Subsubgroups (among dishes)	1963.77	12	163.65	11.32 ***
Error (within dishes)	6796.17	470	14.46	

Expected $MS'_{subgroups}$ = 575.87

Expected $MS'_{subsubgroups}$ = 164.95

Source of Variation	Sum of Square	Degrees of Freedom	Mean Square	Computed F
Juvenile Length				
Groups (among types)	501275.19	1	501275.19	1.70 ns
Subgroups (among females)	1530865.81	4	382716.45	7.87 **
Subsubgroups (among dishes)	444351.06	9	49372.34	4.53 ***
Error (within dishes)	4394649.31	403	10904.84	

Expected $MS'_{subgroups}$ = 295041.30

Expected $MS'_{subsubgroups}$ = 48644.47

ns Not significant at 5% level
* Significant at 5% level
** Significant at 1% level
*** Significant at 0.1% level

Table 25. Two-way nested analysis of variance and covariance with repeated measures comparing larvae of Rudee Inlet intrapopulational crosses and interpopulational crosses involving Gulf Breeze females and Rudee Inlet males. Variance is analyzed among mating types and among females for the arcsine transformations of percent survival.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Groups (among types)	615916.38	1	615916.38	0.71 ns
Subgroups (among females)	4402917.88	5	880583.58	1.23 ns
Error (within females)	8579774.50	12	714981.21	

Expected $MS'_{subgroups}$ = 870264.73

ns Not significant at 5% level

Table 26. Three-way nested analysis of variance and covariance with repeated measures comparing the larvae of Rudee Inlet intrapopulational crosses and interpopulational crosses involving Rudee Inlet females and Gulf Breeze males. Variance is analyzed among mating types, among females within types, and among dishes within females for postlarval length, larval development time (days), and juvenile length at 40 days of age.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Postlarval Length				
Groups (among types)	517.06	1	517.06	0.03 ns
Subgroups (among females)	130735.60	5	26147.12	8.95 ***
Subsubgroups (among dishes)	35038.94	12	2919.91	2.92 ***
Error (within dishes)	431312.98	432	998.41	

Expected $MS'_{subgroups}$ = 16679.44

Expected $MS'_{subsubgroups}$ = 2786.90

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Larval Development Time				
Groups (among types)	29.34	1	29.34	0.07 ns
Subgroups (among females)	3138.31	5	627.66	4.21 *
Subsubgroups (among dishes)	1915.94	12	159.66	19.54 ***
Error (within dishes)	3530.72	432	8.17	

Expected $MS'_{subgroups}$ = 398.70

Expected $MS'_{subsubgroups}$ = 149.18

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Juvenile Length				
Groups (among types)	1473.72	1	1473.72	0.004 ns
Subgroups (among females)	2168575.09	4	542143.77	14.68 ***
Subsubgroups (among dishes)	357109.41	9	39678.82	3.57 ***
Error (within dishes)	4086111.78	368	11103.56	

Expected $MS'_{subgroups}$ = 342618.58

Expected $MS'_{subsubgroups}$ = 36919.12

ns Not significant at 5% level
* Significant at 5% level
*** Significant at 0.1% level

Table 27. Two-way nested analysis of variance and covariance with repeated measures comparing larvae of Rudee Inlet intrapopulational crosses and interpopulational crosses involving Rudee Inlet females and Gulf Breeze males. Variance is analyzed among mating types and among females for arcsine transformations of percent survival.

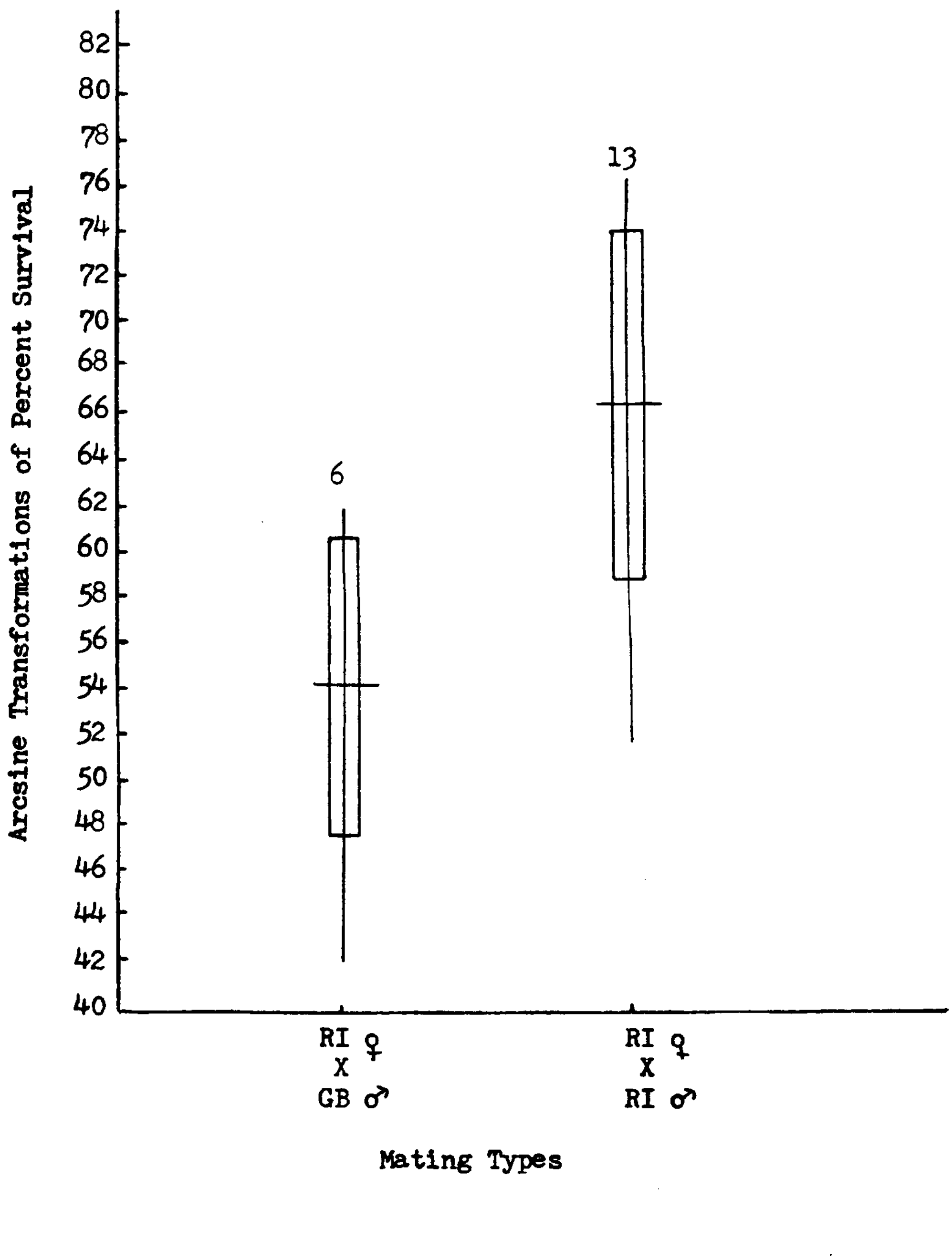
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Groups (among types)	5396055.38	1	5396055.38	8.62 *
Subgroups (among females)	3157877.75	5	631575.55	1.16 ns
Error (within females)	6548968.75	12	545747.40	

$$\text{Expected MS'}_{\text{subgroups}} = 626227.52$$

ns Not significant at 5% level
* Significant at 5% level

Figure 8. Mean, range, and standard deviation of the arcsine transformations of percent survival for Rudee Inlet intrapopulational crosses and interpopulational crosses involving Rudee Inlet females and Gulf Breeze males. Horizontal lines depict the mean, vertical lines the range, and vertical boxes represent ± one standard deviation unit around the mean. Numbers above each of these boxes indicate the sample size on which these statistics were computed.

RI = Rudee Inlet *P. pugio*
GB = Gulf Breeze *P. pugio*


Arcsine Transformations of Percent Survival
82
80
78
76
74
72
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66
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50
48
46
44
42
40
6
13
RI ♀ X GB ♂
RI ♀ X RI ♂
Mating Types

Table 28. Three-way nested analysis of variance and covariance with repeated measures comparing the larvae of the two types of interpopulational crosses. Variance analyzed among mating types, among females within types, and among dishes within females for postlarval length, duration of larval development (days), and juvenile length at 40 days of age.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Postlarval Length				
Groups (among types)	12835.07	1	12835.07	0.49 ns
Subgroups (among females)	101911.14	4	25477.78	18.40 **
Subsubgroups (among dishes)	8611.49	6	1435.25	0.71 ns
Error (within dishes)	330887.75	164	2017.61	

Expected $MS'_{subgroups}$ = 26053.16
Expected $MS'_{subsubgroups}$ = 1384.34

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Larval Development Time				
Groups (among types)	163.61	1	163.61	0.24 ns
Subgroups (among females)	2665.68	4	666.42	15.40 **
Subsubgroups (among dishes)	253.53	6	42.25	1.37 ns
Error (within dishes)	5050.02	164	30.79	

Expected $MS'_{subgroups}$ = 681.64
Expected $MS'_{subsubgroups}$ = 43.27

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Juvenile Length				
Groups (among types)	295532.47	1	295532.47	0.84 ns
Subgroups (among females)	1375233.88	4	343808.47	6.42 *
Subsubgroups (among dishes)	302702.16	6	50450.36	3.83 ***
Error (within dishes)	2095799.16	159	13181.13	

Expected $MS'_{subgroups}$ = 351933.98
Expected $MS'_{subsubgroups}$ = 53542.38

ns Not significant at 5% level
* Significant at 5% level
** Significant at 1% level
*** Significant at 0.1% level

Table 29. Two-way nested analysis of variance and covariance with repeated measures comparing the larvae of the two types of interpopulational crosses. Variance is analyzed among mating types and among females for arcsine transformations of percent survival.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	Computed F
Groups (among types)	7303920.31	1	7303920.31	5.92 ns
Subgroups (among females)	4936628.63	4	1234157.16	2.31 ns
Error (within females)	3200268.19	6	533378.03	

Expected $MS'_{subgroups} = 1234157.10$

ns Not significant at 5% level

Table 30. Summary of the significance levels obtained at each level of analysis for anovas comparing postlarval length, larval development time, arcsine transformations of percent survival, and juvenile length of each mating type.

RI = Rudee Inlet *P. pugio*
GB = Gulf Breeze *P. pugio*
ns = Not significant at 5% level

Crosses Compared	Characteristics	Levels of Analysis: Among Types	Among Females	Among Dishes
GB ♀ X GB ♂ vs. RI ♀ X RI ♂	Postlarval length	ns	0.1%	0.1%
	Larval development time	ns	5 %	0.1%
	Juvenile length	ns	1 %	0.1%
	Survival	ns	5 %	
GB ♀ X GB ♂ vs. GB ♀ X RI ♂	Postlarval length	ns	0.1%	ns
	Larval development time	ns	0.1%	ns
	Juvenile length	ns	ns	0.1%
	Survival	ns	ns	
GB ♀ X GB ♂ vs. RI ♀ X GB ♂	Postlarval length	ns	5 %	5 %
	Larval development time	ns	0.1%	5 %
	Juvenile length	ns	1 %	ns
	Survival	5%	ns	
RI ♀ X RI ♂ vs. GB ♀ X RI ♂	Postlarval length	ns	0.1%	1 %
	Larval development time	ns	5 %	0.1%
	Juvenile length	ns	1 %	0.1%
	Survival	ns	ns	
RI ♀ X RI ♂ vs. RI ♀ X GB ♂	Postlarval length	ns	0.1%	0.1%
	Larval development time	ns	5 %	0.1%
	Juvenile length	ns	0.1%	0.1%
	Survival	5%	ns	
GB ♀ X RI ♂ vs. RI ♀ X GB ♂	Postlarval length	ns	1 %	ns
	Larval development time	ns	1 %	ns
	Juvenile length	ns	5 %	0.1%
	Survival	ns	ns	

Only juvenile lengths demonstrated differences which were significant among rearing dishes.

Juvenile Sex Ratio Analysis

Chi-square analysis of sex ratios in juveniles from our samples indicates that there is less than a 5% chance that the observed deviation from a 1:1 sex ratio is a chance deviation. In most cases this is a conservative probability (Table 31).

Juvenile Rostral Armature

Analysis of the rostral spination of juveniles between the different mating types was computed in the same manner as that for comparison of *P. pugio* and *P. vulgaris* juveniles. Results were also identical, in that the standard error of the mean for each mating type was of sufficient magnitude to overlap the means of the other mating types (Appendix L). The indication here, as before, is that none of the sample means would be significantly different upon completion of anova statistics. Therefore, such analyses were not computed.

Juvenile Morphology

Juvenile shrimp reared from the combined mating types showed no difference in morphology. They invariably conformed to the description of *P. pugio* adults presented by Holthuis (1952). These results are compiled by mating type, female, and culture dish in Appendices M to P.

Hatch Size Analysis

Statistical computations were also completed to compare the number of

Table 31. Chi-square analysis for goodness of fit assuming a 1:1 sex ratio for juveniles reared from all interpopulational and intrapopulational crosses of *Palaemonetes pugio*.

RI = Rudee Inlet *P. pugio*
GB = Gulf Breeze *P. pugio*

Cross	Sex	Observed Frequency	Expected Frequency	X^2
RI ♀ X GB ♂	Male ♂	25	34	4.76
	Female ♀	43	34	

$F_{.05}(1) = 3.84$

$F_{.025}(1) = 5.02$

$0.025 < \text{Probability} < 0.05$

Cross	Sex	Observed Frequency	Expected Frequency	X^2
GB ♀ X RI ♂	Male ♂	32	51	14.02
	Female ♀	71	52	

$F_{.005}(1) = 7.88$

$\text{Probability} < 0.005$

Cross	Sex	Observed Frequency	Expected Frequency	X^2
GB ♀ X GB ♂	Male ♂	34	66	31.0
	Female ♀	98	66	

$F_{.005}(1) = 7.88$

Probability 0.005

Cross	Sex	Observed Frequency	Expected Frequency	X^2
RI ♀ X RI ♂	Male ♂	59	186	172.95
	Female ♀	314	187	

$F_{0.005}(1) = 7.88$

$\text{Probability} < 0.005$

larvae produced per hatch of intrapopulational and reciprocal interpopulational crosses of P. pugio from Gulf Breeze and Rudee Inlet.

A t-test was applied here due to the small amount of data available. Table 32 summarized t-tests where sample variances were assumed to be the same, i.e. they were not significantly different at the 5% level. T-test analysis revealed that the mean hatch sizes of these three mating types were not significantly different at the 5% level.

Table 33 summarized t-tests conducted on the premise that sample variances were not the same, i.e. they were significantly different at the 5% level. Results of these t-tests showed significant differences between the sample means tested at the 5% level.

Intrapopulational crosses of Rudee Inlet shrimp apparently result in larger hatches when compared to the hatches of the other mating types attempted (Figure 9). Hatch size data are tabulated in Appendix U.

Table 32. T-test for unequal sample sizes of the hypothesis that two sample means come from the same population. Assumes that variances in the populations compared are not significantly different at the 5% level.

GB = Gulf Breeze *P. pugio*
RI = Rudee Inlet *P. pugio*

Mating Type	Mean Hatch Size ($\bar{Y}$)	Variance (s^2)	Sample Size (n)
#1 (RI ♀ X GB ♂)	$\bar{Y}_1 = 34$	373.39	10
#2 (GB ♀ X RI ♂)	$\bar{Y}_2 = 39$	899.15	12
#3 (GB ♀ X GB ♂)	$\bar{Y}_3 = 51$	419.11	10

T-tests	$t'_{.05}$	t_s
$\bar{Y}_1$ vs $\bar{Y}_2$	2.09	0.45 ns
$\bar{Y}_1$ vs $\bar{Y}_3$	2.09	1.07 ns
$\bar{Y}_2$ vs $\bar{Y}_3$	2.10	1.91 ns

ns Not significant at the 5% level

Table 33. T-test for unequal sample sizes of the hypothesis that two sample means come from the same population. Assumes that variances in the populations compared are significantly different at the 5% level.

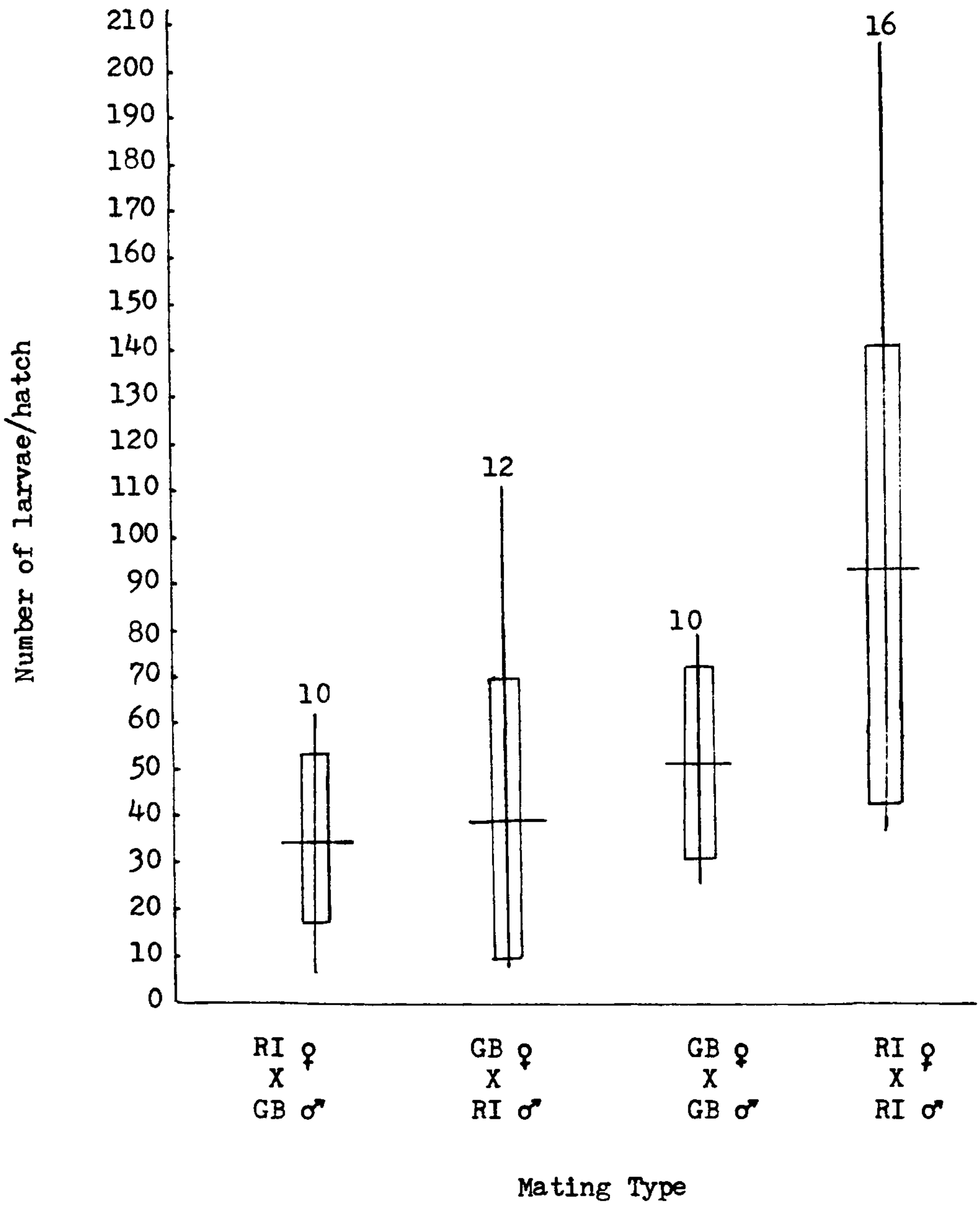
RI = Rudee Inlet <u>P. pugio</u>
GB = Gulf Breeze <u>P. pugio</u>

Mating Type	Mean Hatch Size ($\bar{Y}$)	Variance (s^2)	Sample Size (n)
#1 (RI ♀ X GB ♂)	$\bar{Y}_1 = 34$	373.39	10
#2 (GB ♀ X RI ♂)	$\bar{Y}_2 = 39$	899.15	12
#3 (GB ♀ X GB ♂)	$\bar{Y}_3 = 51$	419.11	10
#4 (RI ♀ X RI ♂)	$\bar{Y}_4 = 92$	2457.07	16

T-tests	$t'_{.05}$	t_s
$\bar{Y}_1$ vs $\bar{Y}_4$	2.16	4.19 *
$\bar{Y}_2$ vs $\bar{Y}_4$	2.15	3.50 *
$\bar{Y}_3$ vs $\bar{Y}_4$	2.16	2.93 *

* Significant at the 5% level

Figure 9. Mean, range, and standard deviation of hatch size (number of larvae per hatch) for intrapopulational and reciprocal interpopulational crosses. Vertical lines indicate range in number of larvae per hatch for each mating type. Horizontal lines denote mean number of larvae per hatch. Vertically oriented boxes represent ± one standard deviation unit around the mean. Numbers at the top of each unit denote the sample size (number of hatches) for each mating type.



210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Number of larvae/hatch
10
12
10
16
RI ♀ X GB ♂
GB ♀ X RI ♂
GB ♀ X GB ♂
RI ♀ X RI ♂
Mating Type

DISCUSSION

Interspecific Hybridization

In the present study, interspecific breeding between *Palaemonetes pugio* and *P. vulgaris* was not successful. Eggs were not retained and no larvae were produced. Females extruded eggs in what appeared to be a normal fashion, but retained them no longer than three days. Usually, eggs were dropped within two days. Therefore, under the laboratory conditions described, interspecific breeding of these species does not appear to be feasible.

Several experimental precautions and controls support this summation. First, interspecific attempts were conducted using three unique pairs of shrimp to reduce the variation inherent in using only one pair. Second, all individuals involved in these crosses were shown to be potentially fertile by intraspecific breeding. This eliminated the rather remote possibility that at least one member of each interspecific pair was sterile. Third, the salinity and temperature regimes maintained during breeding attempts have been shown to be in the desirable range for the larval development of these species (Broad and Hubschman, 1962; Knowlton, 1965; Knowlton and Williams, 1970; Sandifer, 1973; and Thorp and Hoss, 1975). In addition, Little (1968) achieved 100% egg deposition among *Palaemonetes pugio* with the temperature and photoperiod used in this research. Finally, intraspecific control crosses run in triplicate invariably yielded larvae giving evidence to support the validity of the method in which adult shrimp were handled throughout the study.

An overview of the current study shows that the sympatric Palaemonetes species, P. pugio and P. vulgaris would not successfully interbreed in the laboratory. Although this does not preclude the possibility of natural hybridization or hybridization under different laboratory conditions, the evidence available indicates that these species do not interbreed and that they are distinct species. In addition to the current research, Thorp (1976) has presented evidence for habitat partitioning of these closely related sympatric species. Future research should be directed at determining what prevented hybridization in the present study. Possibilities include aspects of behavioral or physical incompatability. Research to determine whether mating or attempted mating occurs between these species may help to answer questions of compatability by comparing interspecific mating behavior with intraspecific mating behavior. At the same time, sperm release could be observed and the fate of the spermatazoa recorded.

Interspecific breeding attempts involving P. intermedius also failed to yield mature eggs or larvae. These results, however, are somewhat tentative, because no attempt was made to show the potential fertility of all individuals involved. Furthermore, triplicate runs for each type of cross were not completed, and the progress in development of successive batches of eggs from each female was not recorded. The latter consideration is very crucial in light of Thakur's (1960) results. He found that an interbreeding attempt between two Caridina species yielded no larvae in three batches of eggs. However, each successive batch reached a greater state of maturity until larvae were finally hatched from the fourth batch. The relative state of maturity for successive batches of eggs was not recorded in crosses involving P. intermedius. Therefore, it is impossible to comment conclusively on the feasibility of crossbreeding P. intermedius with P. pugio and P. vulgaris because breeding attempts may have been terminated before developmental progress between successive batches of eggs ceased. Future hybridization attempts should be conducted under more controlled conditions, similar to the conditions of the crosses between P. pugio and P. vulgaris in the present study.

Attempts to determine mechanisms of habitat partitioning between P. intermedius and P. pugio or P. vulgaris would also be desirable since P. intermedius is morphologically intermediate to P. pugio and P. vulgaris and no niche differences have been discovered to explain the coexistence of P. intermedius with these two similar species.

Observations of juvenile morphology indicate that under the conditions of this experiment P. pugio juveniles take on some adult morphological characteristics at an age of 40 days. At the same age, P. vulgaris specimens have taken on adult characteristics only in rare cases. More importantly, some P. vulgaris juveniles appeared morphologically similar to P. pugio. This precludes the reliable use of the morphological characters concerned for species diagnosis among juveniles.

Only one characteristic consistently differentiated the juveniles examined. This involved the relative lengths of the segments of the second pereiopod. Among P. pugio the lengths of the carpus and merus were approximately equal. Among P. vulgaris, the carpus was somewhat shorter than the merus. In characters where spination distinguishes these species P. vulgaris is equipped with more spines or teeth than P. pugio. Consequently, it may take more time to develop this extra armature. In any case, the data give evidence for a close relationship between these two species.

Analysis designed to examine morphological characteristics of the juveniles of all three species over an extended period of time may reveal the age at which juveniles take on adult characteristics and it may determine the relative similarities of juveniles during development in characteristics which are normally species specific. Investigations to determine the effect of different environmental parameters in this maturation period may also be useful.

For the most part, larvae of the two species are morphologically alike but they display discreet differences during several instars (Broad & Hubschman, 1962). The current research indicates that larval developmental characters and postlarval length were not significantly different. Some differences were encountered between juveniles of the species but in most cases these deviations were not consistent. The only real difference was in the length of the juveniles. Here, P. vulgaris juveniles were significantly smaller than P. pugio juveniles.

It should be noted that the juveniles examined had not become sexually dimorphic because they had not differentiated completely in the structure of the first two pairs of pleopods. Males are distinguished by the growth on the first pair of pleopods of an inner ramus which is larger and has more setae than that of females. In addition, males have on the second pair of pleopods an accessory appendage which is not present in females (Meehan, 1936).

In both species females greatly outnumbered males among lab reared juveniles. According to chi-square analysis, there was less than a 0.5% chance that the observed deviation from a 1:1 sex ratio was due to chance. All 41 of the P. pugio males reared had only partially developed accessory appendages.

Among the 25 P. vulgaris males reared 24 were partially developed and one was fully developed. Partially developed males had buds or very small accessory appendages rather than fully developed ones. In addition, partially developed males of both species had an inner ramus on the first pair of pleopods which was small and generally resembled that of females. Therefore, the observed sex ratio is not a true indication of the actual sex ratio of this sample.

Interpopulational Hybridization

Hybridization of geographically separated populations of Palaemonetes pugio is feasible under the experimental conditions of this study. Developmental characteristics between the larvae yielded from each mating type were significantly different (5% level) in only two instances. In both cases larvae from interpopulational crosses of Rudee Inlet females and Gulf Breeze males demonstrated significantly lower survival rates than the progeny from either of the intrapopulational control crosses. However, survival was not significantly different from the reciprocal interpopulational crosses, at the 5% level.

This particular mating type also had the smallest mean hatch size of any of the crosses studied. However, the hatches of both interpopulational mating types and the Gulf Breeze intrapopulational type were significantly smaller than intrapopulational crosses of Rudee Inlet shrimp. If the Rudee Inlet females of the intrapopulational crosses were significantly larger than the females of the other cross, this result would be expected. However, the length of these females was not measured. These data are therefore somewhat tentative.

Another aspect delineating the limited fertility of this cross was the occurrence of a relatively large number of dead larvae at the time of hatching. Interpopulational crosses of Rudee Inlet females and Gulf Breeze males produced still-born larvae (those dead at birth) at a rate of 10 to 104 specimens per hatch, while other mating types yielded less than five per hatch.

The relative inferiority of the progeny of this mating type was expressed as mortality at many stages of development rather than as abnormal growth rates or sizes of postlarvae. Hatch sizes were smaller due, possibly, to zygote inviability. Larvae which were still-born may have been deficient in their ability to tolerate stressful situations, such as those encountered during the hatching process. Mortality during development may be the result of an inability to compete with siblings for food, etc. Taken together, these phenomena indicate reproductive incompatability which may result from the divergence of the gene pools of these populations. Since this research was not set up to analyze hatch size and still-born rates, further study specifically designed to contrast these parameters among the different mating types is desirable.

Chi-square analysis showed that sexual dimorphism in the structure of the first two pairs of pleopods does not occur at 40 days of age under the present conditions. This analysis revealed a probability of less than 5% that the observed deviation from the expected 1:1 sex ratio was due to chance. In some crosses the probability was less than 0.5%.

The current investigation was not designed specifically to analyze variation due to parentage. Nevertheless, significant differences in larval development time, postlarval length, and juvenile length were discovered among broods within mating types. In addition, highly significant differences occurred due to the rearing dish in which a group of larvae were raised even though they came from the same female. It is suggested that an alternative method of rearing larvae be used in future studies to avoid this large source of variation. Multiple compartment plastic trays containing one larva per compartment are a possible alternative.

Nested analyses of variance were implemented to test for these possible differences. If these sources of variation had not been taken into account (by rearing broods and portions of broods separately) the observed variation might have falsely been attributed to mating type or to species in the case of the P. pugio vs. P. vulgaris research previously discussed. However, significant differences among culture dishes may obscure real variation between mating types. To account for this, the 10% significance level was used in evaluating differences among mating types where differences among culture dishes were significant. At this level, postlarvae from Gulf Breeze intrapopulational crosses were significantly smaller than postlarvae from Rudee Inlet intrapopulational crosses. If it is legitimate to use the 10% significance level, the difference in postlarval length between these populations may be a further expression of the divergence of their gene pools. Gene pool divergence may be caused by infrequent occurrences of migratory adults or larvae between contiguous populations. Over a large geographic range effected by dissimilar environmental stresses such genetic divergence may be an indication of cline formation. As a result of a clinal situation, geographic variation of a character may lead to geographic speciation. In fact, speciation by distance over a continuously occupied range has been proposed for many marine organisms (Kinne, 1975).

Several noteworthy phenomena occurred during the course of this research. In some instances, when females were isolated prior to mating attempts, ovaries ripened then yellowed and disappeared overnight instead of ripening and being deposited on the pleopods. The same event took place during interspecific mating attempts of P. pugio females and P. vulgaris males.

Eggs were not in evidence on the female or within the hatching area.

They may have been laid and immediately consumed by the breeding pair or dropped out of the breeding chamber and into the undergravel filter. In any event, resorption of the ovaries in a time span of 24 hours is unlikely.

A second phenomenon worthy of mention was observed early in this study. If gravid females were handled within three to four days following egg deposition, they normally dropped all or part of their egg mass. Females handled after this critical three to four-day period did not exhibit this behavior.

A final occurrence deserving comment involved the color change of Palaemonetes pugio and P. vulgaris progeny at metamorphosis. Most larvae are colorless, as are the adults, however, some exhibit yellow, greenish-yellow, brown, or reddish brown pigmentation. There were never two color types on any one larva. Following metamorphosis these pigments were not expressed, so the postlarvae appeared colorless in general appearance. There were rare cases when postlarvae had a uniform reddish-brown tint but no yellow, greenish-yellow, or brown postlarvae were observed.

CONCLUSION

1. Under the laboratory conditions of this study 25°C, 25 °/oo, and a 14.5/9.5 light-dark period, interspecific hybridization of *Palaemonetes pugio* and *P. vulgaris* was not feasible.

2. Preliminary interspecific hybridization of *P. intermedius* with *P. pugio* and *P. vulgaris* under the current laboratory conditions was not possible. Research under more controlled conditions would be desirable.

3. Differences between *P. pugio* and *P. vulgaris* progeny in survival rates, larval development time, and postlarval length were not significantly different at the 5% level.

4. At 40 days of age the length of *P. pugio* juveniles was significantly larger than that of *P. vulgaris* juveniles.

5. Juveniles of *P. pugio* and *P. vulgaris* at 40 days of age have not developed complete sexual dimorphism.

6. *P. vulgaris* juveniles at 40 days of age were not completely developed as per the adult diagnostic features described by Holthuis (1952), while *P. pugio* had developed the adult characteristics investigated.

7. Species identity cannot be conclusively assigned to 40-day-old juveniles of *P. pugio* and *P. vulgaris*.

8. Gulf Breeze and Rudee Inlet populations of *P. pugio* were interbred and produced larvae.

9. Progeny from reciprocal interpopulational and intrapopulational crosses were similar in duration of larval development, postlarval length, and juvenile length.

10. Survival rates were significantly different (5%) only between interpopulational crosses involving Rudee Inlet females and Gulf Breeze males relative to the control crosses.

11. Intrapopulational hatch sizes of _P._ _pugio_ from Rudee Inlet were significantly larger than the hatch sizes of the other three mating types.

12. _P._ _vulgaris_ and _P._ _pugio_ exhibit a small but highly significant regression coefficient, attributing increases in postlarval length to longer development times.

13. Differences in postlarval length, larval development time, survival and juvenile length explained by culture dish population density were insignificant at the 5% level for _P._ _pugio_ and _P._ _vulgaris_ young.

14. Differences in duration of larval development, postlarval length, and juvenile length, due to parentage were significant in most cases analyzed.

15. Differences among culture dishes within females were significant for duration of larval development, postlarval length, and juvenile length.

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Appendix A

Developmental history of the progeny from interpopulational crosses involving Rudee Inlet females and Gulf Breeze males.

The data include the initial population (IP) of each dish, percent survival during development (% S), arcsine transformations of percent survival (arc), larval development time in days (days), and the postlarval length in millimeters of shrimp which metamorphosed on those days (length).

Cross 1

Dish #1
IP = 18
% S = 44.44
arc = 41.78

Days	Length
18	6.75
19	7.15
24	7.30
25	7.35
29	7.25
	7.35
31	7.75
	7.85

Dish #2
IP = 18
% S = 61.11
arc = 51.41

Days	Length
20	7.10
21	7.25
	7.35
23	7.15
	7.20
	7.20
25	7.50
29	7.95
	7.35
30	7.15
31	7.75

Cross 2

Dish #1
IP = 24
% S = 66.67
arc = 54.76

Days	Length
12	6.10
	6.20
14	6.70
	6.40
	6.75
15	7.20
	7.10
	7.00
16	7.05
	7.20
18	6.90
	7.30
	7.70
	7.65
21	7.75
23	7.80

Dish #2
IP = 24
% S = 70.83
arc = 57.29

Days	Length
12	6.05
	6.35
13	6.35
14	6.60
	6.60
	6.70
	6.55
	6.60
	6.45
	6.45
16	7.05
17	7.20
	7.25
	7.35
18	6.85
20	7.60
23	7.60

Cross 3

Dish #1
IP = 14
% S = 71.43
arc = 57.67

Days	Length
17	6.50
18	6.35
20	7.00
	6.95
	6.90
	6.75
	7.10
21	7.15
29	7.45
	7.60

Dish #2
IP = 9
% S = 77.78
arc = 61.89

Days	Length
14	6.50
	6.55
	6.65
15	6.85
	7.00
18	6.85
26	7.80

Appendix B

Developmental history of the progeny from Gulf Breeze female and Rudee Inlet male crosses.

The data include the initial population (IP) of each dish, percent survival during development (% S), arcsine transformations of percent survival (arc), larval development time in days (days), and the postlarval length in millimeters of shrimp which metamorphosed on those days (length).

Cross 1

Dish #1
IP = 20
% S = 75.00
arc = 60.00

Days	Length
15	6.50
17	7.30
	7.40
19	7.50
22	7.70
	7.15
	7.25
25	7.70
26	8.10
	8.10
27	7.90
29	7.95
31	7.90
38	8.05
54	8.95

Dish #2
IP = 29
% S = 86.21
arc = 68.19

Days	Length
15	6.65
17	7.05
	6.95
22	7.60
	7.05
	7.45
24	7.30
	7.35
	7.40
	7.35
	7.45
25	7.35
26	7.25
	7.35
27	7.15
31	7.35
32	7.35
34	7.85
	7.65
38	7.85
	7.80
41	8.60
42	8.30
47	8.25
51	8.40

Cross 2

Dish #1
IP = 15
% S = 86.67
arc = 68.61

Days	Length
18	6.50
	6.60
	6.80
	6.65
19	7.65
20	7.35
21	7.65
	7.55
23	7.20
	7.75
26	6.85
	7.50
	7.35

Dish #2
IP = 27
% S = 77.78
arc = 61.89

Days	Length
16	6.45
17	6.95
	7.00
	6.60
	6.80
18	7.00
	7.15
	7.35
	7.25
	7.25
	7.20
	7.15
19	7.10
	7.35
21	7.40
	7.55
	7.40
22	7.40
23	7.50
28	7.55
31	8.75

Cross 3

Dish #1
IP = 25
% S = 88.00
arc = 69.73

Days	Length
14	6.55
	6.55
15	6.55
	6.60
	6.65
	6.60
	6.60
	6.65
	6.70
16	6.75
17	6.85
	6.90
	6.90
	7.35
	6.75
	7.10
	7.10
18	7.45
20	6.70
	7.40
21	7.85
30	8.20

Dish #2
IP = 11
% S = 100.00
arc = 90.00

Days	Length
18	7.05
19	6.40
	6.95
	6.90
	6.80
	6.75
	6.75
	6.70
20	7.15
21	7.30
23	7.40

Appendix C

Developmental history of the progeny from Gulf Breeze intrapopulational crosses

The data include the initial population (IP) of each dish, percent survival during development (% S), arcsine transformations of percent survival (arc), larval development time in days (days), and the postlarval length in millimeters of shrimp which metamorphosed on those days (length).

Cross 1

Dish #1
IP = 19
% S = 94.74
arc = 76.69

Days	Length
13	6.20
	6.20
15	6.40
	6.40
	6.00
	6.30
	6.10
	6.60
	6.70
16	6.20
17	7.00
18	6.90
	7.00
	7.00
19	7.00
	7.00
20	6.70
21	7.30

Dish #2
IP = 19
% S = 89.47
arc = 71.09

Days	Length
13	6.40
14	6.30
	6.40
15	6.50
	6.20
	6.20
	6.00
	6.50
	6.30
16	6.50
	6.80
	6.50
18	7.00
	6.90
	6.50
	6.90
20	7.10

Cross 2

Dish #1
IP = 37
% S = 86.49
arc = 68.44

Days	Length
13	6.20
	6.40
14	6.10
	6.20
	6.20
15	6.40
	6.70
	6.60
	6.60
	6.60
	6.65
	6.35
	6.80
	6.70
	6.50
	6.50
	6.70
	6.70
	6.75
	6.30
	6.35
	6.50
	6.50
	6.45
	6.50
17	6.80
	6.75
	7.20
18	6.80
19	6.95
21	6.85
23	7.00

Dish #2
IP = 37
% S = 94.59
arc = 76.56

Days	Length
13	6.00
	6.30
14	6.40
	6.30
	6.40
15	6.25
	6.70
	6.70
	7.10
	6.40
	6.40
	6.50
	6.95
	6.85
	6.45
	6.50
	6.70
	6.70
	6.75
	6.70
	6.60
16	6.70
	6.70
17	6.80
	6.70
	6.75
	6.75
18	6.80
	6.85
19	7.00
	6.95
	7.05
20	7.65
	7.35
22	7.60

Cross 3

Dish #1
IP = 20
%S = 70.00
arc = 56.79

Days	Length
16	6.50
17	6.30
18	6.60
	6.30
19	6.80
	6.75
	7.85
21	6.95
	6.75
22	7.05
	7.00
23	6.95
32	7.10
	7.50

Dish #2
IP = 23
% S = 95.65
arc = 77.89

Days	Length
17	6.10
18	6.55
20	6.60
	6.65
	6.70
21	6.95
	6.95
	6.75
	6.80
	6.95
23	6.55
	6.80
	6.80
	6.75
	6.90
24	6.80
	6.95
	7.05
25	7.15
26	6.90
27	7.10
34	7.25

Dish #3
IP = 23
% S = 91.30
arc = 72.85

Days	Length
14	6.00
17	6.50
	6.45
	6.35
18	6.35
	6.30
	6.40
21	6.90
	6.95
	7.05
	7.05
	6.90
	7.00
22	6.90
24	7.05
	7.35
	7.20
25	7.00
27	6.95
28	7.65
	7.20

Appendix D

Developmental history of the progeny
from intraspecific crosses of P. pugio.
Data were also used in populational study
(Rudee Inlet intrapopulational crosses).

The data include the initial population (IP) of each dish, percent survival during development (% S), arcsine transformations of percent survival (arc), larval development time in days (days), and the postlarval length in millimeters of shrimp which metamorphosed on those days (length).

Cross 1

Dish #1
IP = 36
% S = 91.67
arc = 73.26

Days	Length
13	7.05
	7.60
	7.55
	6.90
	7.10
	7.10
14	7.45
	7.40
	7.25
	7.10
15	7.40
	7.25
	7.30
	7.15
	7.30
	7.25
	7.20
	7.25
	7.25
	7.35
	7.40
16	7.40
	7.35
	7.30
	7.35
	7.35
	7.40
17	7.70
	7.80
20	8.10
21	8.10
	7.50
	7.60

Dish #2
IP = 36
% S = 91.67
arc = 73.26

Days	Length
13	7.10
	7.05
14	7.10
	7.15
	7.15
	6.95
	7.00
15	6.70
	7.15
	6.90
	7.30
	7.30
	7.35
	7.65
	7.50
16	7.20
	7.55
	7.35
	6.85
	7.70
	7.45
	7.45
	7.50
	7.10
17	7.75
	7.30
18	7.50
	7.50
19	7.50
	7.85
	7.35
21	7.60
	8.10

Dish #3
IP = 53
% S = 83.02
arc = 65.65

Days	Length
13	6.95
	6.90
14	7.35
	7.30
15	7.25
	7.25
	7.30
	7.35
	7.25
	7.50
	7.40
	7.35
	7.10
	6.90
16	6.60
	7.45
	7.45
	7.45
	7.35
	7.40
	6.95
	6.95
	7.00
	7.50
	7.35
	7.40
17	7.20
	7.10
	7.65
18	7.95
	8.25
	7.50
	7.75
	7.75
19	7.50
	7.45
20	7.10
21	8.10
22	7.90
	7.80
	7.65
23	8.15
	7.90
	7.75

Cross 2

Dish #1
IP = 21
% S = 75.00
arc = 60.00

Days	Length
15	6.45
	6.50
17	6.95
	6.70
18	7.10
20	7.15
	7.15
	7.10
	7.00
	6.90
	7.10
	6.75
	6.85
24	7.00
25	7.05

Dish #2
IP = 21
% S = 76.19
arc = 60.80

Days	Length
13	6.30
15	6.70
	6.65
17	6.90
	6.95
18	7.20
	7.40
	7.35
20	7.25
	6.65
	7.00
	6.75
22	6.80
23	6.80
	7.35
24	7.40

Cross 3

Dish #1
IP = 36
% S = 85.71
arc = 67.78

Days	Length
20	6.85
	6.95
	6.85
	7.05
	6.95
	6.85
	6.65
	7.15
	7.15
21	7.10
	7.10
22	7.35
	7.40
	7.55
23	7.60
	7.00
	6.80
	7.65
	7.30
	7.20
24	7.05
	7.60
25	7.20
	7.20
	7.45
26	7.25
28	7.95
	7.75
	7.80

Dish #2
IP = 36
% S = 88.89
arc = 70.54

Days	Length
17	6.40
18	6.45
20	7.05
	6.80
	6.80
	6.75
	6.85
	7.00
	6.80
	6.90
	6.90
	6.85
	6.95
	6.85
	6.75
21	6.95
22	7.40
	7.25
	7.40
23	7.45
	7.35
	7.25
	7.15
	7.30
24	7.30
25	7.50
	7.20
	7.45
28	7.45
	7.35
31	7.95
35	7.40

Dish #3
IP = 78
% S = 76.92
arc = 61.27

Days	Length
17	6.75
18	6.85
	6.80
	6.80
	6.70
	6.30
	6.45
	6.70
	6.60
	6.85
	6.70
	6.75
	6.80
	6.90
	6.95
20	6.65
	6.55
	6.85
	7.15
	6.75
	6.80
	6.90
	6.90
	6.80
21	6.90
	7.10
	7.25
	7.10
	7.10
	7.00
22	7.25
23	7.25
	6.95
	7.05
	7.15
	7.15
24	7.15
	7.05
	7.40
	7.10
	7.30
	7.35
	7.30
	7.20
	7.20
	7.30

Dish #3
Cont'd

Days	Length
24	7.15
	7.20
	7.05
	7.10
25	7.35
	7.25
	7.45
	7.20
	7.25
26	7.35
	7.40
28	7.70
30	7.60
31	7.55

Cross 4

Dish #1
IP = 21
% S = 61.90
arc = 51.88

Days	Length
21	7.25
	6.95
22	6.90
24	6.95
	7.35
	7.05
	7.00
25	7.15
26	7.10
	7.15
28	7.75
	7.60
31	7.60

Dish #2
IP = 21
% S = 66.67
arc = 54.76

Days	Length
20	6.70
	7.10
21	6.70
22	6.85
23	7.05
25	6.80
	7.05
26	6.95
	7.20
	7.00
28	7.05
29	7.30
	7.10
	7.35

Cross 4

Dish #3
IP = 36
% S = 94.29
arc = 76.19

Days	Length
14	6.65
	6.85
	6.80
	6.75
	6.90
	6.65
	6.80
	6.85
	6.70
	6.80
	6.65
	6.60
15	6.20
	6.90
	6.50
	6.70
	6.85
	6.80
	6.60
16	6.65
	6.65
	6.75
	6.65
	6.45
	6.70
	6.60
17	7.15
18	7.00
	7.10
	6.95
	7.05
	6.70
20	7.30

Dish #4
IP = 36
% S = 91.67
arc = 73.26

Days	Length
13	6.25
14	6.95
	6.75
	7.00
	6.70
	6.80
	6.70
	6.75
	6.65
	6.70
	6.70
	6.70
15	6.90
	6.70
	6.85
	7.05
	6.95
	7.05
16	7.05
	7.10
	7.25
	6.90
	6.75
17	6.95
	7.05
	7.00
18	6.80
	6.80
	7.00
	7.15
20	7.35
	7.40
	7.40

Dish #5
IP = 30
% S = 90.00
arc = 71.57

Days	Length
14	6.60
	6.70
	6.80
	6.95
15	6.70
	6.55
	6.60
	7.25
	6.75
	6.95
16	6.70
	6.85
	6.85
	6.85
	6.80
	6.85
	6.70
17	6.40
	7.20
	6.55
18	7.05
	7.30
	6.95
	7.25
	7.20
19	7.25
20	7.35

Appendix E

Developmental history of the progeny
from intraspecific crosses of *P. vulgaris*.

The data include the initial population (IP) of each dish, percent survival during development (%S), arcsine transformations of percent survival (arc), larval development time in days (days), and the postlarval length in millimeters of shrimp which metamorphosed on those days (length).

Cross 1

Dish #1
IP = 26
% S = 76.92
arc = 61.27

Days	Length
14	6.10
	6.50
15	6.70
	6.60
	7.00
	7.20
	7.10
	6.70
	7.00
	6.90
	6.75
	6.90
	6.95
	7.10
16	6.75
	7.30
	7.10
	7.10
	7.15
18	7.55

Dish #2
IP = 36
% S = 75.00
arc = 60.00

Days	Length
15	6.95
16	6.95
	6.75
	6.75
	6.85
	6.85
	6.90
	6.75
	6.70
17	6.95
	6.75
	6.80
	6.85
18	6.00
	6.80
	6.75
19	7.00
	7.40
	7.55
20	7.25
	7.55
	7.45
	7.45
21	7.15
22	7.55
23	7.55
30	7.60

Dish #3
IP = 36
% S = 63.89
arc = 53.07

Days	Length
15	6.60
16	6.70
	6.65
	6.40
	6.75
	6.40
	6.55
	6.65
18	6.50
	7.10
	7.00
	7.00
	6.80
19	6.55
20	6.60
	6.90
	6.95
22	7.60
	7.50
	6.40
	7.15
	6.95
26	7.65

Dish #4
IP = 39
% S = 71.79
arc = 57.92

Days	Length
15	6.55
	6.60
	6.45
16	6.50
	6.55
	6.45
	6.80
	6.60
	6.50
	6.70
	6.80
	6.70
	6.65
	6.60
17	6.85
	6.95
18	6.40
	6.85
	6.45
	6.10
	6.50
	6.90
	6.95
	7.05
20	6.70
	7.05
	6.65
21	7.30

Cross 2

Dish #1
IP = 36
% S = 86.11
arc = 68.11

Days	Length
15	6.80
	6.85
	6.75
	6.80
	6.55
	6.55
	6.70
	6.60
	6.65
	6.55
	6.90
16	6.60
	7.00
	6.70
	6.70
17	6.65
	7.00
	6.95
	7.00
	6.90
	6.70
	7.10
	7.05
18	6.85
	7.40
	6.70
	7.25
	7.15
	7.50
20	6.75
	6.95

Dish #2
IP = 36
% S = 52.78
arc = 46.61

Days	Length
16	6.45
	6.40
	6.40
	6.50
	6.65
	6.50
	6.50
	6.90
	5.90
	7.00
17	7.00
	6.75
	6.75
18	6.75
	6.85
	6.95
20	6.90
22	6.95
	6.85

Dish #3
IP = 46
% S = 65.22
arc = 53.85

Days	Length
16	6.80
	6.45
	6.45
17	6.40
	6.40
	6.55
	6.55
	6.55
	6.50
	6.55
	6.70
18	7.00
	6.90
	6.95
	6.75
	6.90
	6.90
	7.05
	7.10
	7.05
	7.00
19	6.85
20	6.90
	7.00
	7.00
	6.95
21	6.75
22	6.70
24	7.00

Cross 3

Dish #1
IP = 20
% S = 85.00
arc = 67.21

Days	Length
15	6.90
	6.95
	6.95
	6.50
	6.60
16	6.75
17	7.00
	6.80
	6.80
	7.40
18	7.10
	7.10
19	6.80
	6.85
	6.75
26	7.10
	6.95

Dish #2
IP = 20
% S = 73.68
arc = 59.15

Days	Length
15	6.60
	7.20
17	7.30
	7.30
	7.25
	7.30
	7.10
	7.15
	7.15
18	7.15
	7.35
	7.00
19	6.95
	7.00

Cross 3

Dish #3
IP = 36
% S = 83.33
arc = 65.88

Days	Length
17	6.45
	6.35
18	6.10
	6.50
	6.40
	6.15
	6.55
19	6.70
20	6.50
	6.55
	6.00
	6.60
22	6.50
	6.90
	7.05
	6.85
	6.85
	6.75
	6.90
	6.70
	6.65
23	6.35
	6.30
	6.35
	6.40
	6.55
	6.55
24	6.35
	6.30
28	7.10

Dish #4
IP = 36
% S = 80.00
arc = 63.43

Days	Length
16	6.00
	6.00
	6.10
17	6.15
	6.15
	6.25
18	6.60
20	6.80
	7.00
21	6.65
	6.75
	6.70
	6.65
	6.40
	7.10
	6.90
	6.75
	6.75
	6.80
	6.70
	6.70
22	6.20
	7.25
23	6.80
	6.55
	6.70
24	6.85
28	7.35

Dish #5
IP = 18
% S = 94.44
arc = 76.31

Days	Length
17	6.45
	6.15
18	6.40
	6.55
	6.20
	6.35
	6.70
	6.45
	6.55
20	6.70
	6.70
	6.65
	6.80
21	6.55
	6.50
22	7.00
	6.85

Appendix F

Juvenile length at 40 days of age of progeny from interpopulational crosses involving Rudee Inlet females and Gulf Breeze males.

Data are tabulated by cross and culture dish. The lengths of juveniles in millimeters (measured from the tip of the rostrum to the tip of the telson, omitting setae) are given.

Cross 1

Dish 1	Dish 2
9.45	11.40
11.45	10.20
13.85	10.35
12.45	10.55
10.60	12.15
10.15	10.50
9.45	11.75
11.10	10.55
	9.50
	11.60
	12.25

Cross 2

Dish 1	Dish 2
11.85	15.25
15.15	15.30
11.50	12.00
15.40	14.05
14.65	14.10
16.60	13.65
13.00	14.15
13.35	12.95
12.70	12.75
14.05	15.45
12.60	15.70
13.70	13.95
13.85	14.05
15.45	15.25
14.35	13.35
	13.80
	12.35

Cross 3

Dish 1	Dish 2
13.35	15.10
11.90	13.35
12.15	11.80
12.15	13.55
9.65	12.60
13.20	13.60
11.55	14.05
10.75	
11.70	
11.90	

Appendix G

Juvenile length at 40 days of age
of progeny from interpopulational crosses involving
Gulf Breeze females and Rudee Inlet males.

Data are tabulated by cross and culture dish. The lengths of juveniles in millimeters (measured from the tip of the rostrum to the tip of the telson, omitting setae) are given.

Cross 1

Dish 1	Dish 2
13.35	11.35
10.50	10.50
11.95	12.60
10.10	9.15
8.45	10.95
9.10	11.35
10.90	8.60
11.30	10.45
11.35	11.30
11.15	12.10
13.30	9.35
9.85	11.40
11.40	9.10
11.20	11.65
9.90	11.45
	11.05
	12.50
	8.30
	9.05
	11.40
	10.60
	9.65
	12.15
	9.95

Cross 2

Dish 1	Dish 2
9.75	13.75
10.80	13.65
11.30	15.35
12.05	12.65
10.10	13.50
12.50	12.25
10.90	14.35
12.55	12.35
11.55	14.75
11.60	12.45
11.50	12.50
11.65	11.05
11.15	13.05
	12.40
	12.70
	12.70
	11.30
	10.95
	11.90
	12.85
	12.70

Cross 3

Dish 1	Dish 2
12.25	13.10
13.10	12.75
12.15	13.25
12.90	11.45
12.45	11.35
14.05	11.65
11.80	11.70
12.30	12.25
11.80	12.20
12.35	13.10
11.10	
11.40	
11.60	
11.55	
12.40	
11.85	
10.95	
10.70	
10.60	
9.30	

Appendix H

Juvenile length at 40 days of age of progeny from Gulf Breeze intrapopulational crosses.

Data are tabulated by cross and culture dish. The lengths of juveniles in millimeters (measured from the tip of the rostrum to the tip of the telson, omitting setae) are given.

Cross 1

Dish 1	Dish 2
13.45	11.60
10.70	11.95
12.70	11.75
11.00	12.60
11.45	12.30
12.80	11.10
11.50	12.65
12.55	11.80
10.55	11.65
10.60	12.55
10.80	11.65
13.75	11.05
11.75	

Cross 2

Dish 1	Dish 2
9.15	10.90
11.35	12.25
10.35	10.85
10.05	10.95
11.30	11.40
10.60	11.00
10.45	10.50
11.55	11.70
10.85	10.40
12.60	10.45
11.20	11.10
10.50	10.35
11.35	10.30
11.90	10.80
10.10	10.50
9.85	10.55
10.55	10.40
11.90	11.20
12.50	10.60
11.75	11.05
10.50	10.65
11.35	10.70
10.95	11.10
10.90	11.00
10.40	11.25
11.00	10.70
10.75	10.70
11.10	11.45
11.75	12.25
11.40	10.45
10.70	10.20
	11.75

Cross 3

Dish 1	Dish 2
13.90	10.45
11.95	10.30
10.70	11.65
11.50	11.60
11.45	12.05
10.50	11.85
11.50	11.20
11.10	11.10
12.25	13.55
12.25	12.70
12.50	10.25
11.75	11.20
12.35	12.35
12.30	11.30
12.60	11.85
12.55	12.80
9.10	10.30
12.20	11.00
12.90	10.95
12.70	12.25
11.15	11.75
11.05	

Appendix I

Juvenile length at 40 days of age
of progeny from intraspecific crosses of *P. pugio*.
These data were also used in the populational study
(Rudee Inlet intrapopulational crosses).

Data are tabulated by cross and culture dish. The lengths of juveniles in millimeters (measured from the tip of the rostrum to the tip of the telson, omitting setae) are given.

Cross 1

Dish 1	Dish 2	Dish 3
12.60	12.55	11.60
13.55	12.10	12.10
13.50	11.05	11.10
13.40	14.25	12.35
12.30	11.35	10.95
15.10	12.00	12.10
11.40	11.20	10.75
13.45	12.15	11.15
12.10	12.45	12.80
14.25	13.80	12.65
13.30	14.05	12.90
12.45	12.00	11.85
11.00	11.55	11.90
14.75	12.45	13.65
12.70	12.90	11.55
12.05	13.75	10.90
12.50	13.85	13.25
12.00	10.75	12.70
11.75	13.90	12.65
12.65	14.20	12.40
11.60	14.15	13.25
10.65	12.70	12.55
12.15	13.05	10.05
12.35	12.05	12.75
12.15	13.95	13.45
11.35	11.15	11.90
12.35	13.65	10.20
12.40	13.40	11.40
13.75	12.05	12.20
11.50	11.40	9.95
13.10	12.85	11.70
12.35	10.95	12.30
10.25	10.45	13.10
		11.45
		12.60
		12.75
		9.90
		10.55
		10.35
		11.20
		10.25
		10.35

Cross 3

Dish 1	Dish 2	Dish 3	Dish 3 Cont's
12.75	11.55	10.30	
13.75	12.20	10.45	11.55
12.40	12.50	12.25	12.00
11.90	11.20	9.45	12.95
12.50	10.80	10.40	12.30
12.45	12.15	10.85	
11.90	12.20	11.50	
12.65	12.15	11.15	
11.00	11.55	10.75	
10.65	12.50	13.05	
12.20	12.35	11.25	
14.55	11.30	10.80	
11.55	8.15	11.85	
11.65	13.40	11.40	
11.85	13.00	11.90	
12.20	13.15	11.45	
10.90	10.85	11.60	
12.90	13.20	11.50	
13.00	12.75	13.70	
10.65	12.90	11.05	
11.95	12.00	10.95	
11.05	12.55	11.95	
10.95	10.95	12.70	
12.30	10.15	11.95	
13.40	11.20	10.15	
12.85	12.15	9.40	
11.10	12.75	10.60	
	13.35	12.20	
	9.00	10.65	
	12.30	10.50	
	11.95	11.90	
		10.70	
		12.30	
		11.15	
		13.50	
		13.30	
		10.90	
		12.25	
		11.65	
		12.40	
		10.90	
		12.00	
		10.80	
		13.25	
		11.25	
		10.95	
		11.80	
		11.70	
		11.75	
		10.65	
		10.55	
		10.45	
		10.85	

Cross 4

Dish 1	Dish 2	Dish 3
12.70	12.75	11.45
12.80	14.30	14.05
13.25	13.45	13.90
12.90	13.85	13.70
13.35	13.60	13.55
13.50	13.50	13.75
14.35	14.05	13.00
14.00	13.65	13.25
14.45	11.80	13.05
14.45	13.65	12.95
14.00	13.85	12.30
11.95	14.00	13.15
11.90	14.85	12.95
14.20	14.30	13.65
12.95	12.75	13.80
13.65	13.20	13.85
13.35	15.05	13.30
13.65	12.70	13.95
11.85	13.40	14.65
12.10	12.10	14.55
13.50	14.90	13.40
14.50	13.35	13.90
11.15	11.50	13.65
12.40	13.15	14.15
11.55	14.05	14.05
15.25	12.55	12.05
14.40	12.15	
12.60	14.35	
11.55	12.60	
12.45	12.50	
13.20	12.55	
12.65		

Appendix J

Juvenile length at 40 days of age of progeny from intraspecific crosses of *P. vulgaris*.

Data are tabulated by cross and culture dish. The lengths of juveniles in millimeters (measured from the tip of the rostrum to the tip of the telson, omitting setae) are given.

Cross 1

Dish 1	Dish 2	Dish 3
10.80	11.45	11.15
12.10	10.05	12.10
11.15	11.25	9.85
10.90	9.65	10.75
10.85	11.60	10.50
11.30	9.40	11.60
10.80	9.45	9.15
10.90	9.40	9.55
12.00	10.65	10.95
11.30	10.85	10.45
9.80	9.25	10.80
10.45	10.55	10.05
11.65	11.80	10.30
11.10	10.60	10.90
10.80	10.10	10.95
10.35	10.20	11.35
10.00	10.80	11.30
9.95	10.25	11.10
9.90	10.00	10.30
11.75	10.45	10.55
9.45	10.30	10.45
10.55		11.75
9.85		11.20
10.80		9.90
8.70		10.75
		10.35
		10.30
		7.95

Cross 2

Dish 1	Dish 2	Dish 3
10.05	10.05	8.30
12.20	10.85	9.85
10.45	10.70	11.25
10.85	10.60	11.20
10.55	10.45	9.55
10.40	10.10	11.25
9.30	11.05	9.45
11.05	10.55	10.55
10.65	10.30	9.40
9.45	11.30	9.10
9.90	9.20	9.85
11.00	10.40	11.25
11.30	9.95	10.75
10.30	10.25	10.85
10.55	9.20	11.00
11.05	9.25	9.40
10.10	8.15	11.35
10.20	9.75	9.80
12.00	10.30	11.55
11.85		9.75
10.30		9.90
10.50		9.85
11.15		9.50
11.40		9.40
8.75		9.70
10.45		9.95
9.60		9.80
10.45		9.80
10.50		10.35
11.45		
10.55		

Cross 3

Dish 1	Dish 2	Dish 3
9.80	10.30	10.00
8.90	11.40	11.80
9.25	9.75	11.35
9.45	10.35	9.95
10.00	10.65	10.85
9.25	8.80	10.80
11.35	9.50	9.90
10.45	10.90	11.65
10.10	11.45	10.40
9.90	9.75	11.50
9.60	10.10	10.10
10.10	11.25	9.75
9.75	9.90	10.50
9.50	10.10	9.35
9.85	10.50	10.85
7.60	10.60	10.70
10.65	7.85	
8.75	8.30	
8.55	10.00	
8.15	9.55	
10.20	8.55	
10.60	10.15	
10.45	10.60	
9.00	11.35	
8.90		
10.50		
10.70		

Appendix K

The postlarval length and juvenile length
of shrimp reared from a single
intraspecific cross of _P. pugio_.

Length (in millimeters) was measured from the tip of the rostrum to the tip of the telson, omitting setae.

pl = postlarval length
jl = juvenile length

pl	jl
6.45	12.35
7.00	11.65
7.00	13.40
7.05	12.20
7.05	12.25
6.90	12.00
6.90	12.45
7.00	12.95
7.00	11.80
6.85	13.35
6.95	12.70
6.95	11.25
6.80	12.45
7.15	14.25
7.00	11.85
7.00	11.80
7.10	12.35
7.10	12.60
7.00	11.70
7.00	11.65
6.90	12.70
7.10	12.30
7.10	10.90
7.00	11.65
7.00	11.15
7.25	12.30
7.15	13.60
7.05	12.75
7.10	12.80
7.20	13.60
7.15	12.50
7.15	11.95
7.25	11.80
7.20	12.30
7.10	10.75
7.10	12.60
7.15	11.75
7.15	12.05
7.20	12.95
7.35	11.60
7.35	12.80
7.25	14.35
7.30	12.30
7.60	12.90
7.45	10.55
7.40	13.05
7.75	12.55
7.85	13.95
7.60	11.75
7.40	12.05
7.55	11.60
7.55	11.10
7.60	10.80
7.70	10.85
7.70	11.95
7.65	12.50
6.55	8.30

Appendix L

The mean and the standard error of the mean for the number of dorsal and ventral spines on the rostrum of juveniles 40 days of age.

Data are presented for the progeny of interpopulational and intrapopulational crosses of P. pugio from Rudee Inlet and Gulf Breeze and for the progeny of intraspecific crosses of P. vulgaris. Rudee Inlet intrapopulational crosses of P. pugio and intraspecific crosses of P. pugio are one in the same.

RI = Rudee Inlet
GB = Gulf Breeze
ds = dorsal spines
vs = ventral spines

		Mean	Standard error
RI ♀ X GB ♂	ds	8.22	0.81
	vs	2.92	0.29
GB ♀ X RI ♂	ds	8.09	0.66
	vs	2.92	0.24
GB ♀ X GB ♂	ds	7.61	0.53
	vs	2.94	0.21
RI ♀ X RI ♂ (*P. pugio* intraspecific crosses)	ds	8.24	0.39
	vs	2.98	0.14
P. vulgaris intraspecific crosses	ds	8.22	0.47
	vs	3.17	0.18

Appendix M

Morphological observations of juvenile shrimp 40 days of age from interpopulational crosses involving Rudee Inlet females and Gulf Breeze males.

Data are tabulated according to cross and culture dish. Characters recorded include a description of the dorsal tip of the rostrum, the number of dorsal rostral spines on the carapace, the relative lengths of the segments of the second pereiopod as they occur in both sexes, the number of teeth on the inner margins of the dactylus and fixed finger of the second pereiopod, the number of spines on the dorsal and ventral margins of the rostrum, and the degree of development of sexually dimorphic characters in males.

O = observation
F = frequency of occurrence
c = length of carpus
p = length of palm
ch = length of whole chela
m = length of merus

Cross 1

	Dish 1		Dish 2	
	O	F	O	F
Dorsal Rostral Tip	Naked	8	Naked	11
Dorsal Rostral Spines on Carapace	1	8	1	11
Carpus Second Pereiopod	♀ c > p, c = m	6	♀ c > p, c = m	8
	♂ c < ch, c = m	2	♂ c < ch, c = m	3
Teeth Dactylus / Fixed Finger	0/0	8	0/0	11
Rostral Spines Dorsal / Ventral	9/4	2	9/3	4
	9/3	1	8/4	1
	8/4	1	8/3	5
	8/3	4	7/3	1
Development of Sexually Dimorphic Characters of Males	Partial	2	Partial	2
			Full	1

Cross 2

	Dish 1		Dish 2	
	O	F	O	F
Dorsal Rostral Tip	Naked	15	Naked	17
Dorsal Rostral Spines on Carapace	1	15	1	17
Carpus Second Pereiopod	♀ c > p c = m	10	♀ c > p c = m	7
	♂ c < ch c = m	5	♂ c < ch c = m	10
Teeth Dactylus / Fixed Finger	$\frac{0}{0}$	15	$\frac{0}{0}$	17
Rostral Spines Dorsal / Ventral	10/3 9/3 8/3 8/2 7/2	1 4 6 3 1	10/3 9/3 9/2 8/3 8/2 7/3	1 3 1 6 5 1
Development of Sexually Dimorphic Characters of Males	Partial Full	1 4	Partial Full	3 4

Cross 3

	Dish 1		Dish 2	
	O	F	O	F
Dorsal Rostral Tip	Naked	10	Naked	7
Dorsal Rostral Spines on Carapace	1	10	1	7
Carpus Second Pereiopod	♀ $c > p$ $c = m$	7	♀ $c > p$ $c = m$	5
	♂ $c < ch$ $c = m$	3	♂ $c < ch$ $c = m$	2
Teeth $\frac{\text{Dactylus}}{\text{Fixed Finger}}$	$\frac{0}{0}$	10	$\frac{0}{0}$	7
Rostral Spines $\frac{\text{Dorsal}}{\text{Ventral}}$	9/3	3	9/3	1
	8/3	3	8/3	4
	8/2	3	7/3	2
	7/3	1		
Development of Sexually Dimorphic Characters of Males	Partial	3	Partial	2

Appendix N

Morphological observations of juvenile shrimp 40 days of age from interpopulational crosses involving Gulf Breeze females and Rudee Inlet males.

Data are tabulated according to cross and culture dish. Characters recorded include a description of the dorsal tip of the rostrum, the number of dorsal rostral spines on the carapace, the relative lengths of the segments of the second pereiopod as they occur in both sexes, the number of teeth on the inner margins of the dactylus and fixed finger of the second pereiopod, the number of spines on the dorsal and ventral margins of the rostrum, and the degree of development of sexually dimorphic characters in males.

O = observation
F = frequency of occurrence
c = length of carpus
p = length of palm
ch = length of whole chela
m = length of merus

Cross 1

	Dish 1		Dish 2	
	O	F	O	F
Dorsal Rostral Tip	Naked	15	Naked	24
Dorsal Rostral Spines on Carapace	1	15	1	24
Carpus Second Pereiopod	♀ c > p, c = m	12	♀ c > p, c = m	23
	♂ c < ch, c = m	3	♂ c < ch, c = m	1
Teeth $\frac{\text{Dactylus}}{\text{Fixed Finger}}$	$\frac{0}{0}$	15	$\frac{0}{0}$	24
Rostral Spines $\frac{\text{Dorsal}}{\text{Ventral}}$	9/3	4	10/3	1
	8/3	9	9/3	7
	8/2	1	8/3	10
	7/3	1	8/2	3
			7/3	2
			7/2	1
Development of Sexually Dimorphic Characters of Males	Partial	3	Partial	1

Cross 2

	Dish 1		Dish 2	
	O	F	O	F
Dorsal Rostral Tip	Naked	13	Naked	21
Dorsal Rostral Spines on Carapace	1	13	1	21
Carpus Second Pereiopod	♀ c > p c = m	11	♀ c > p c = m	7
	♂ c < ch c = m	2	♂ c < ch c = m	14
Teeth Dactylus / Fixed Finger	0/0	13	0/0	21
Rostral Spines Dorsal / Ventral	9/3	4	10/3	1
	8/3	7	9/3	6
	8/2	1	8/4	1
	7/3	1	8/3	10
			8/2	1
			7/3	1
			7/2	1
Development of Sexually Dimorphic Characters of Males	Partial	2	Partial	13
			Full	1

Cross 3

	Dish 1		Dish 2	
	O	F	O	F
Dorsal Rostral Tip	Naked	20	Naked	10
Dorsal Rostral Spines on Carapace	1	20	1	10
Carpus Second Pereiopod	♀ $c > p$, $c = m$	11	♀ $c > p$, $c = m$	7
	♂ $c < ch$, $c = m$	9	♂ $c < ch$, $c = m$	3
Teeth Dactylus / Fixed Finger	0/0	20	0/0	10
Rostral Spines Dorsal / Ventral	9/3	1	8/3	9
	8/3	13	7/3	1
	7/3	4		
	7/2	2		
Development of Sexually Dimorphic Characters of Males	Partial	9	Partial	3

Appendix O

Morphological observations of juvenile shrimp
40 days of age from
Gulf Breeze intrapopulational crosses.

Data are tabulated according to cross and culture dish. Characters recorded include a description of the dorsal tip of the rostrum, the number of dorsal rostral spines on the carapace, the relative lengths of the segments of the second pereiopod as they occur in both sexes, the number of teeth on the inner margins of the dactylus and fixed finger of the second pereiopod, the number of spines on the dorsal and ventral margins of the rostrum, and the degree of development of sexually dimorphic characters in males.

O = observation
F = frequency of occurrence
c = length of carpus
p = length of palm
ch = length of whole chela
m = length of merus

Cross 1

	Dish 1		Dish 2	
	O	F	O	F
Dorsal Rostral Tip	Naked	13	Naked	12
Dorsal Rostral Spines on Carapace	1	13	1	12
Carpus Second Pereiopod	♀ c > p c = m	11	♀ c > p c = m	9
	♂ c < ch c = m	2	♂ c < ch c = m	3
Teeth Dactylus Fixed Finger	$\frac{0}{0}$	13	$\frac{0}{0}$	12
Rostral Spines Dorsal Ventral	9/3 8/4 8/3 7/3 5/3	1 1 6 4 1	9/3 8/3 8/2 7/3 7/2	1 6 1 2 2
Development of Sexually Dimorphic Characters of Males	Partial	2	Partial	3

Cross 2

	Dish 1		Dish 2	
	O	F	O	F
Dorsal Rostral Tip	Naked	31	Naked	33
Dorsal Rostral Spines on Carapace	1	31	1	33
Carpus Second Pereiopod	♀ $c > p$, $c = m$	19	♀ $c > p$, $c = m$	24
	♂ $c < ch$, $c = m$	12	♂ $c < ch$, $c = m$	9
Teeth Dactylus / Fixed Finger	0/0	31	0/0	33
Rostral Spines Dorsal / Ventral	8/3	18	9/3	1
	8/2	1	9/2	1
	7/3	11	8/4	1
	7/2	1	8/3	20
			7/3	10
Development of Sexually Dimorphic Characters of Males	Partial	12		9

Cross 3

	Dish 1		Dish 2	
	O	F	O	F
Dorsal Rostral Tip	Naked	22	Naked	21
Dorsal Rostral Spines on Carapace	1	22	1	21
Carpus Second Pereiopod	♀ c > p, c = m	18	♀ c > p, c = m	17
	♂ c < ch, c = m	4	♂ c < ch, c = m	4
Teeth Dactylus / Fixed Finger	0/0	22	0/0	21
Rostral Spines Dorsal / Ventral	9/3	2	9/3	1
	8/3	7	8/4	1
	7/3	12	8/3	8
	7/2	1	7/3	9
			7/2	2
Development of Sexually Dimorphic Characters of Males	Partial	4	Partial	4

Appendix P

Morphological observations of juvenile shrimp
40 days of age from intraspecific crosses of P. pugio.
These data were used also in the populational study
(Rudee Inlet intrapopulational crosses).

Data are tabulated according to cross and culture dish. Characters recorded include a description of the dorsal tip of the rostrum, the number of dorsal rostral spines on the carapace, the relative lengths of the segments of the second pereiopod as they occur in both sexes, the number of teeth on the inner margins of the dactylus and fixed finger of the second pereiopod, the number of spines on the dorsal and ventral margins of the rostrum, and the degree of development of sexually dimorphic characters in males. Damaged specimens were examined but only identifiable characteristics were recorded.

O = observation
F = frequency of occurrence
c = length of carpus
p = length of palm
ch = length of whole chela
m = length of merus

Cross 1

	Dish 1		Dish 2		Dish 3	
	O	F	O	F	O	F
Dorsal Rostral Tip	Naked	32	Naked	33	Naked	43
Dorsal Rostral Spines on Carapace	1	32	1	33	1	43
Carpus Second Pereiopod	♀ c > p c = m	29	♀ c > p c = m	27	♀ c > p c = m	38
	♂ c < ch c = m	4	♂ c < ch c = m	6	♂ c < ch c = m	5
Teeth <u>Dactylus</u> Fixed Finger	$\frac{0}{0}$	33	$\frac{0}{0}$	33	$\frac{0}{0}$	43
Rostral Spines <u>Dorsal</u> Ventral	9/3 8/3 8/2 7/3	6 21 1 4	10/4 9/3 8/3 7/3	1 5 21 6	10/3 9/3 8/3 8/2 7/3 7/2	2 7 20 1 11 2
Development of Sexually Dimorphic Characters of Males	Partial	4	Partial	6	Partial	5

Cross 3

	Dish 1		Dish 2		Dish 3	
	O	F	O	F	O	F
Dorsal Rostral Tip	Naked	28	Naked	31	Naked	59
Dorsal Rostral Spines on Carapace	1	28	1	31	1 1.5	58 1
Carpus Second Pereiopod	♀ c > p c = m ♂ c < ch c = m	24 3	♀ c > p c = m ♂ c < ch c = m	26 5	o c > p c = m o c < ch c = m	58 1
Teeth <u>Dactylus</u> Fixed Finger	$\frac{0}{0}$	27	$\frac{0}{0}$	31	$\frac{0}{0}$	59
Rostral Spines <u>Dorsal</u> Ventral	10/3 9/3 9/2 8/3	1 16 1 10	10/3 9/3 8/3	4 19 8	10/4 10/3 9/4 9/3 8/4 8/3 7/3	1 3 2 33 1 18 1
Development of Sexually Dimorphic Characters of Males	Partial	3	Partial	5	Partial	1

Cross 4

	Dish 1		Dish 2		Dish 3	
	O	F	O	F	O	F
Dorsal Rostral Tip	Naked	32	Naked	31	Naked	26
Dorsal Rostral Spines on Carapace	1	33	1	31	1	25
					1.5	1
Carpus Second Pereiopod	♀ $c > p$, $c = m$	31	♀ $c > p$, $c = m$	26	♀ $c > p$, $c = m$	16
	♂ $c < ch$, $c = m$	2	♂ $c < ch$, $c = m$	5	♂ $c < ch$, $c = m$	10
Teeth Dactylus / Fixed Finger	$\frac{0}{0}$	33	$\frac{0}{0}$	31	$\frac{0}{0}$	26
Rostral Spines Dorsal / Ventral	10/3	1	9/3	5	9/3	4
	9/3	5	8/3	17	8/3	14
	8/3	22	8/2	1	7/3	8
	8/2	2	7/3	7		
	7/3	2	5/2	1		
Development of Sexually Dimorphic Characters of Males	Partial	2	Partial	5	Partial	10

Appendix Q

Morphological observations of juvenile shrimp
40 days of age from intraspecific crosses of P. vulgaris.

Data are tabulated according to cross and culture dish. Characters recorded include a description of the dorsal tip of the rostrum, the number of dorsal rostral spines on the carapace, the relative lengths of the segments of the second pereiopod as they occur in both sexes, the number of teeth on the inner margins of the dactylus and fixed finger of the second pereiopod, the number of spines on the dorsal and ventral margins of the rostrum, and the degree of development of sexually dimorphic characters in males. Damaged specimens were examined but only identifiable characteristics were recorded.

O = observation
F = frequency of occurrence
c = length of carpus
p = length of palm
ch = length of whole chela
m = length of merus

Cross 1

	Dish 1		Dish 2		Dish 3	
	O	F	O	F	O	F
Dorsal Rostral Tip	Naked	26	Naked	21	Naked	28
Dorsal Rostral Spines on Carapace	2	2	1.5	12	1.5	25
	1.5	12	1	9	1	3
	1	12				
Carpus Second Pereiopod	♀ $c > p$, $c < m$	21	♀ $c > p$, $c < m$	19	♀ $c > p$, $c < m$	21
	♂ $c > p$, $c < m$	4	♂ $c > p$, $c < m$	2	♂ $c > p$, $c < m$	7
Teeth <u>Dactylus</u> Fixed Finger	$\frac{1}{0}$	1	$\frac{2}{1}$	2	$\frac{2}{1}$	7
	$\frac{0}{0}$	24	$\frac{1}{0}$	3	$\frac{1}{0}$	7
			$\frac{0}{0}$	16	$\frac{0}{0}$	14
Rostral Spines <u>Dorsal</u> Ventral	9/4	2	10/3	1	9/4	5
	9/3	4	9/4	1	9/3	7
	8/4	1	9/3	6	8/4	7
	8/3	17	8/4	1	8/3	7
	7/3	2	8/3	10	7/3	2
			7/3	2		
Development of Sexually Dimorphic Characters of Males	Partial	3	Partial	2	Partial	7
	Full	1				

Cross 2

	Dish 1		Dish 2		Dish 3	
	O	F	O	F	O	F
Dorsal Rostral Tip	Naked	31	Naked	19	Naked	29
Dorsal Rostral Spines on Carapace	2 1.5 1	1 27 3	1.5 1	17 2	2 1.5 1	2 24 3
Carpus Second Pereiopod	♀ c > p, c < m ♂ c > p, c < m	26 5	♀ c > p, c < m ♂ c > p, c < m	18 1	♀ c > p, c < m ♂ c > p, c < m	26 3
Teeth <u>Dactylus</u> Fixed Finger	$\frac{2}{1}$ $\frac{1}{1}$ $\frac{1}{0}$ $\frac{0}{0}$	4 4 6 17	$\frac{2}{1}$ $\frac{1}{0}$ $\frac{0}{0}$	3 5 11	$\frac{2}{1}$ $\frac{1}{0}$ $\frac{0}{0}$	3 9 17
Rostral Spines <u>Dorsal</u> Ventral	10/3 9/4 9/3 8/3 7/3	1 2 9 16 3	9/3 8/4 8/3 7/3	2 1 15 1	9/4 9/3 8/3 7/2	6 8 14 1
Development of Sexually Dimorphic Characters of Males	Partial	5	Partial	1	Partial	3

Cross 3

	Dish 1		Dish 2		Dish 3	
	O	F	O	F	O	F
Dorsal Rostral Tip	Naked	27	Naked	24	Naked	17
Dorsal Rostral Spines on Carapace	1.5	17	1.5	16	2	1
	1	10	1	8	1.5	9
					1	7
Carpus Second Pereiopod	♀ c > p, c < m	27	♀ c > p, c < m	22	♀ c > p, c < m	16
	♂ c > p, c < m	0	♂ c > p, c < m	2	♂ c > p, c < m	1
Teeth Dactylus / Fixed Finger	0/0	27	2/1	1	2/1	2
			1/0	6	1/0	5
			0/0	17	0/1	1
					0/0	9
Rostral Spines Dorsal / Ventral	9/4	1	10/3	1	9/4	3
	9/3	9	9/4	4	9/3	2
	8/4	1	9/3	4	8/4	3
	8/3	10	8/4	3	8/3	6
	7/3	6	8/3	7	7/3	1
			7/3	4	6/4	1
			6/3	1	6/3	1
Development of Sexually Dimorphic Characters of Males			Partial	2	Partial	1

Appendix R

Mean, range, and standard deviation (SD) of the lengths of juveniles reared from intraspecific crosses of *P. pugio* and *P. vulgaris*.

Cross	Mean	Range	SD	No. Juveniles
P. pugio	12.40	8.15-15.10	1.34	311
P. vulgaris	10.31	7.60-12.20	0.86	221

Appendix S

Mean, range, and standard deviation (SD) of the arcsine transformations of percent survival for Gulf Breeze intrapopulational crosses and interpopulational crosses involving Rudee Inlet females and Gulf Breeze males. Data are based on percent survival in each culture dish.

Cross	Mean	Range	SD	No. Culture Dishes
GB ♀ X GB ♂	71.47	56.79-77.89	6.77	7
RI ♀ X GB ♂	54.13	41.78-61.89	6.36	6

Appendix T

Mean, range, and standard deviation (SD) of the arcsine transformations of percent survival for Rudee Inlet intrapopulational crosses and interpopulational crosses involving Rudee Inlet females and Gulf Breeze males. Data are based on percent survival in each culture dish.

Cross	Mean	Range	SD	No. Culture Dishes
RI ♀ X RI ♂	66.17	51.88-76.19	7.48	13
RI ♀ X GB ♂	54.13	41.78-61.89	6.36	6

Appendix U

Mean, range, and standard deviation (SD) of hatch sizes (number of larvae per hatch) for intrapopulational and interpopulational crosses of Rudee Inlet and Gulf Breeze *P. pugio*.

Cross	Mean	Range	SD	No. Hatches
RI ♀ X GB ♂	33.5	7-60	19.32	10
GB ♀ X RI ♂	38.7	9-111	29.99	12
GB ♀ X GB ♂	51.0	24-79	20.47	10
RI ♀ X RI ♂	91.5	36-206	49.57	16