Reproductive Activity and Biochemical Composition of *Penaeus setiferus* and *Penaeus aztecus* in the Gulf of Mexico

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ABSTRACT

Penaeus setiferus and P. aztecus compose the bulk of the Texas catch of shrimp, the most valuable fishery product in the state. This study compares the maturation and reproduction of P. setiferus and P. aztecus near an offshore brine diffuser (at a depth of 21 m) to that at two control locations. The parameters used in this evaluation included frequency of capture of ripe mated females, number of eggs and percent hatch from onboard spawns, stage of ovarian development of females, and relative size and biochemical composition (total carbohydrate, total lipid and total protein) of the gonad and hepatopancreas of males and females.

Seven 10-day collecting cruises were conducted between October 1979 and September 1980, and approximately 3,000 shrimp were dissected and analyzed. Descriptions and photographs of characteristic size and color of each stage of ovarian maturation are presented for each species. Fecundity and hatching rate averaged 257,000 and 55 percent, respectively, for P. setiferus, and 167,800 and 77.6 percent, respectively, for P. aztecus. Reproductive activity of P. setiferus was observed from May through September with a peak in June while P. aztecus was reproductively active from July through October with a peak in September. Size at first maturity was estimated as 160-170 mm total length for females of P. setiferus and P. aztecus, 150-160 mm for P. setiferus males, and 110-120 mm for P. aztecus males. Gonad size in males reached an asymptote of maximum size at 170-180 mm total length in P. setiferus and 150-160 mm in P. aztecus.

Spawning grounds of P. setiferus and P. aztecus were readily distinguished by the abundance of mature P. setiferus and scarcity of mature P. aztecus at the two nearshore blocks (10-23 m depth) and by the abundance of mature P. aztecus and near absence of P. setiferus at the deep block (35-44 m depth). Thus, the reproduction of P. aztecus took place outside the brine diffuser area. Reproductive activities of P. setiferus were concentrated in the shallowest stations (10-15 m) of both nearshore blocks, indicating little threat of direct exposure to brine.

Comparisons of dry weight and biochemical composition of gonads and hepatopancreas of P. setiferus males and females between the two nearshore blocks (test and control) indicated no significant differences with two exceptions: (1) the carbohydrate content of the hepatopancreas of both males and females at the test block was significantly higher than at the control block; and (2) the weight of the hepatopancreas of Stage 4 females at the test block was significantly greater than at the control block. These were considered nutritional differences, which may indirectly affect reproduction, but direct differences in gonad weight or composition were not observed between the test and control blocks. Biochemical analyses of hepatopancreas and ovary of P. setiferus and P. aztecus at each maturation stage yielded information on the process of organic storage and mobilization in each species.

INTRODUCTION

As a result of the Energy Policy Conservation Act of 1975, strategic oil reserves are being stored in underground caverns created by dissolving salt from the interiors of coastal salt domes. Most of the brine produced by this process is discharged offshore in the Gulf of Mexico. One of the first salt dome sites scheduled for this process was Bryan Mound near Freeport, Texas. The brine produced at this site is discharged into the Gulf at a depth of about 21 m. Daily discharges of brine may be as much as 684,000 barrels and will continue for 66 months. The brine has a salinity of about 250 ppt and a salt composition different from that of seawater. Because nearshore Gulf waters are spawning grounds for many commercially valuable species, there is concern that the introduction of this brine could be detrimental to the fisheries.

Shrimp is the most valuable commercial fishery product of the Gulf. In 1980, shrimp accounted for approximately 75 percent of the dockside value of commercial fishery landings in Texas ($153.9 million). Shrimp landings in Freeport accounted for $28 million (NMFS, 1981). The white shrimp Penaeus setiferus and the brown shrimp P. aztecus make up the

bulk of the shrimp catch off Texas. These species are reported to spawn offshore at depths ranging from 9 to 31 m and from 27 to 100 m, respectively (Lindner and Anderson, 1956; Renfro and Brusher, 1982). Thus, brine discharge from the Bryan Mound site could infringe on the spawning grounds of both species, depending upon direction of currents and the persistence of the brine plume. Assessment of the effect of brine discharge on shrimp populations requires information on the relative abundance of ripe adults near the discharge point and the effect of the brine on their reproductive performance.

Giese (1959a) described a number of methods for evaluating the breeding condition and reproductive cycle of marine invertebrates. These methods include observation of spawning, collection of larvae, examination of maturity of gametes in the gonads, measurement of relative size of gonads, determination of biochemical composition of gonads and storage organs, and laboratory studies of factors that induce maturation and spawning.

Several of these methods have been used to characterize the reproductive patterns of P. setiferus and P. aztecus. Although spawning of these species in their natural habitats has not been observed, both species have been observed to spawn in captivity with viable eggs shortly after being captured in a fully ripe and mated condition (Cook and Murphy, 1966; Cook, 1969). Very little information is available concerning frequency of capture of ripe mated females, or on measurements of fecundity of spawns, percent hatching of eggs, and percent metamorphosis of nauplii to protozoeae. These are valuable indicators of reproductive performance. However, these indicators do not provide information concerning the less understood and more lengthy process of maturation that precedes spawning. The same argument applies to collection of eggs and larvae from plankton samples (Giese, 1959a). Gonadal maturation can be monitored throughout the year, without relying on the presence of spawning animals or of eggs and larvae, by evaluating the stage of maturation of individuals large enough to reproduce. This method has been used to monitor annual reproductive cycles of the following penaeids: Hymenopenaeus robustus (Anderson and Lindner, 1971); Metapenaeus affinis (Pillay and Nair, 1971); M. monoceros (Nalini, 1976); M. stebbingi, P. semisulcatus and Trachypenaeus granulosus (Badawi, 1975); P. aztecus (Renfro and Brusher, 1982); and P. setiferus (Lindner and Anderson, 1956; Renfro and Brusher, 1982). Ovarian maturation stages of P. setiferus and P. aztecus can be recognized externally by ovary color and size (King, 1948; Brown and Patlan, 1974) and histologically by oocyte size, yolk deposition, presence of follicle cells and presence of cortical rods (King, 1948; Duronslet et al., 1975). Measurements of relative sizes of gonads (gonad weight relative to body weight) have been used to describe the annual reproductive cycle of several decapod crustaceans (Rahman, 1967; Heath and Barnes, 1970; Armitage et al., 1972) including a penaeid (Pillay and Nair, 1971). Gonad size also has been used to evaluate effects of laboratory treatments on maturation of captive P. setiferus, P. vannamei and P. stylirostris (Lawrence et al., 1980; Chamberlain and Lawrence, 1981a, 1981b).

The biochemical composition of the gonad changes with maturation and thus can indicate the reproductive state of an organism. In some marine invertebrates, storage of organic reserves precedes gametogeniesis and is vital for reproducion. Giese (1959b) noted that the increase in size of gonads of the ochre star, Pisaster ochraceus, was out of proportion to the small amount of food ingested. He also noted that gonads did not develop in animals that had been starved. Biochemical analyses of invertebrates have shown that carbohydrate, protein and lipid levels increase in the gonad and decrease in the storage organ during maturation (Giese et al., 1958, 1964; Tucker and Giese, 1962; Nimitz and Giese, 1964; Lawrence et al., 1965a, 1965b, 1966; Allen and Giese, 1966; Towle and Giese, 1966, 1967; Vasu and Giese, 1966; Nimitz, 1979; Moss and Lawrence, 1972). This process of nutrient storage and transport has been documented in several crustaceans, including crawfish (Collatz, 1969; Rice and Armitage, 1974), brachyurans (Adiyodi and Adiyodi, 1971; Diwan and Nagabhushanam, 1974), an anomuran (Nagabhushanam and Kulkarni, 1979) and penaeid shrimp (Kulkarni and Nagab-

hushanam, 1979; Lawrence et al., 1979a). Biochemical information of this type increases understanding of the sequence of events in maturation and spawning and suggests probable nutritional requirements for reproduction.

The primary goal of this study was to evaluate the impact of the Bryan Mound discharge on the reproductive performance of P. setiferus and P. aztecus. The secondary goal was to characterize the variations in reproductive activity and biochemical composition of each species with respect to season, location, body size and maturation stage.

During each sampling period the following were determined: (1) frequency of capture of ripe mated females and viability of their offspring; (2) stage of ovarian maturation of all females of reproductive size; and (3) weight and biochemical composition of gonads and hepatopancreas of selected males and females.

MATERIALS AND METHODS

Sampling Design

Seven cruises, each of approximately 10 days' duration, were conducted off the Texas coast between 24 October 1979 and 11 September 1980 (Table 1). During each cruise, shrimp were collected from three sampling blocks (Figure 1) measuring 24 by 24 km. Each block was partitioned into nine sampling stations measuring 8 by 8 km. Block A was located about 48 km off Freeport in P. aztecus fishing grounds; Blocks B and C were located inshore near Freeport and Port O'Connor, respectively, in P. setiferus fishing grounds. The brine diffuser is located about 23 km off Freeport within Station 8 of Block B. This sampling design uses blocks A and C as deepwater and nearshore control areas, respectively, for comparison with Block B, the test area.

Shrimp were collected with a 12.2-m otter trawl. Three 10-minute tows were made at each station during each cruise. In addition, a series of three 15-minute tows were made at the most productive station of each row (group of three stations within a block parallel to shore) to ensure that enough shrimp were collected from each depth. Tows were conducted during the day in the inshore blocks and after sunset in the offshore block, due to the diurnal and nocturnal activity patterns of P. setiferus and P. aztecus, respectively.

Table 1. Dates of offshore collecting cruises

Cruise	Dates
1	24-30 October, 6-7 November 1979
2	25 February-1 March, 7-10 March 1980
3	5-14 May 1980
4	3-11 June 1980
5	9-16 July 1980
6	12-20 August 1980
7	3-11 September 1980

On-Board Evaluations

As soon as the trawl catch was brought on board the shrimp were placed in buckets of seawater. Species, sex, total length (TL, the distance, to the nearest mm, from the tip of the telson to the tip of the rostrum), and maturation stage were recorded for each. If more than 100 shrimp were captured in a tow, a subsample of 100 was processed.

Ovarian maturation of P. setiferus was classified into five stages based on ovary color and size as described by King (1948) and modified for application to numerous other species (Tuma, 1967; Rao, 1968; Anderson and Lindner, 1971; Farmer, 1974; Aquacop, 1975; Badawi, 1975; Santiago, 1977; and Nalini, 1976). To classify maturation stages of P. aztecus, the ovary color descriptions of P. aztecus (Brown and Patlan, 1974) were incorporated into King's (1948) five-stage classification system.

An attempt was made to classify the maturation of male gonads into discrete stages based on size and external appearance of the terminal ampoules. The five-stage classification of the external structures of the male genitalia of Metapenaeus stebbingi (Tirmizi and Javed, 1976) was not adopted in this study because this system could not discern variations in gonadal maturation of males

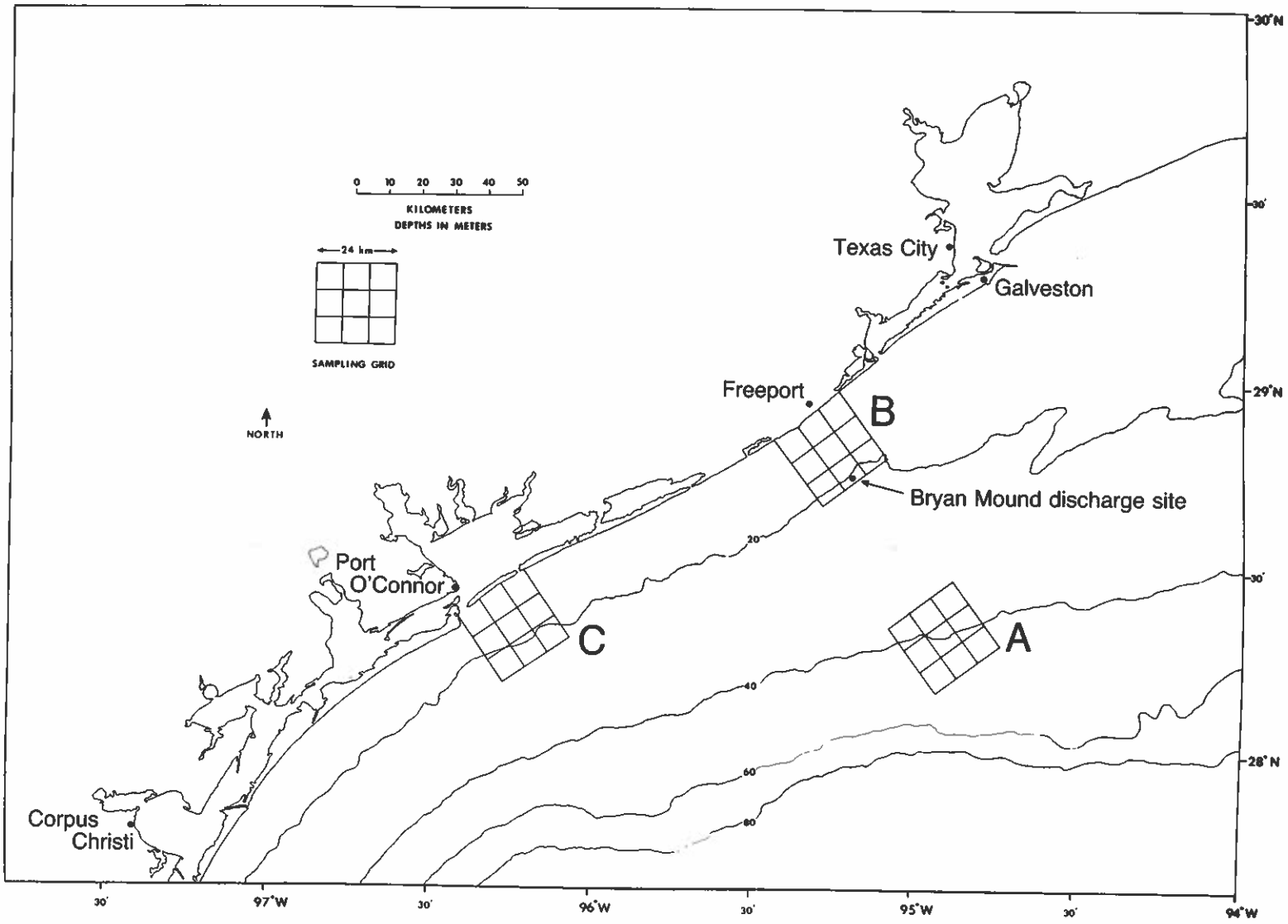


Figure 1. Location of sampling stations near Bryan Mound brine discharge site (Block B) and corresponding stations at the deep (Block A) and near-shore (Block C) control blocks in the Gulf of Mexico.

with completely developed secondary sexual characteristics. No other published information was found concerning discrete external maturation stages of male penaeids.

Fully mature, mated female P. setiferus were immediately placed for spawning into individual 95-liter plastic garbage cans filled with unfiltered, aerated seawater. The disodium salt of EDTA (a chelator) and erythromycin and minocycline (antibiotics) were added immediately to the water in concentrations of 10 g/liter, 0.18 mg/liter and 0.09 mg/liter, respectively. The following morning, females were removed and dissected for biochemical analysis. The number of eggs per spawn was estimated by thoroughly mixing the water and removing five random samples with a 10-ml Hensen-Stemple pipette. Each sample was placed in a 40-ml petri dish and monitored on the second and third day after spawning to determine percent hatch and percent metamorphosis to protozoea. The eggs remaining after the samples were taken were sieved from the water and frozen for biochemical analysis. It was assumed initially that mature P. aztecus would not spawn on board without temperature manipulation, for which the vessel was not equipped. Consequently, no attempt was made to spawn them. On Cruise 5, however, spawning occurred without temperature manipulation. Thereafter, fully mature P. aztecus were routinely spawned using the same procedure as described for P. setiferus.

From the 771 tows, approximately 3,000 shrimp were selected for immediate dissection and freezing. Criteria for selection included size, molt stage and health. Generally, only individuals were selected that were large enough to reproduce (minimum total length: males, 125 mm; females, 150 mm), in the intermolt stage, and alive and healthy. When catches were poor, healthy shrimp of smaller size or other molt stages were used in order to evaluate the effect of size and molt stage on maturation.

The gonad and hepatopancreas (a major nutrient-storage organ of crustaceans) were dissected from each shrimp and placed in separate aluminum pans that had been numbered and weighed. These were immediately placed in a freezer. On Cruise 1, some male gonadal tissues were not completely removed from all shrimp collected. These data were excluded from analyses. The maturation stage of males and females was evaluated after the gonads were dissected and their size and color were more apparent. The color of ovaries was compared to a numbered grid of colors (Color Visualizer Chart, Grumbacher, Inc., New York, NY) and the most similar color recorded. Pigmentation at the surface of the ovary was ignored. In addition, selected males and females at various stages of gonadal development were photographed (35 mm, color, 160 ASA Tungsten film) during the dissection. Additional photographs of representative P. setiferus collected near Port Aransas and Port Mansfield are included in this paper.

Biochemical Analysis

Frozen gonad, hepatopancreas and egg samples were transported to the laboratory and partially dehydrated in a frostless freezer. The samples were then completely dried under vacuum in a dessicator at room temperature and weighed. Analyses of carbohydrate, protein and lipid required dry tissue weights of 3-7 mg, 3-7 mg, and 75-200 mg, respectively. Some samples, notably gonads of immature males and females, were too small for lipid determination. Rather than to omit this analysis for small samples, several samples of the same species, sex, maturation stage and station were occasionally pooled. The results of each pooled lipid analysis were assigned to one sample corresponding to a shrimp of intermediate length in the pooled group; or if only two samples were pooled the results were assigned randomly. Total lipid was determined by extracting and weighing the lipid by the method of Freeman et al. (1957). Total carbohydrate was estimated by boiling the dried tissue with five percent trichloroacetic acid, centrifuging, and testing the supernatant by the method of Dubois et al. (1956). Protein content was determined by the method of Lowry et al. (1951). All chemicals were reagent grade.

Statistical Analysis

Organ weights and the amount and percentage composition of biochemical constituents

in organs are reported in dry weight (g). In comparisons among animals of different sizes in which size was not the variable being considered, all organ weights and biochemical contents were linearly adjusted to the appropriate weights for a hypothetical animal of standard weight. Standard weights were arbitrarily established as 40 g whole body wet weight (about 172 mm) for females, and 30 g (about 159 mm) for males (Giese, 1967). Adjustments were done by multiplying a given organ dry weight by the ratio of actual whole body wet weight to a calculated whole body wet weight. Calculated whole body wet weights were determined from total length of each shrimp using length/weight regression equations (Fontaine and Neal, 1971). The advantage of this method for adjusting data for animal size over the gonad index method (organ dry weight ÷ calculated body wet weight x 100; Farmanfarmaian et al., 1958) is the avoidance of mixed units (organ dry weights ÷ calculated whole body wet weights). Simple linear conversion of organ dry weight to wet weight would have introduced the tenuous assumption of constant water content (Armitage et al., 1972). The assumption of a linear relationship between organ weight and body weight is similar to both the standard size and the organ index method.

An arcsine transformation was applied to normalize all percentage data before parametric statistical analysis. Statistical Analysis Systems software was used for all statistical analyses (Helwig and Council, 1979). Statistical differences were considered significant at the .01 level.

Length at first maturity was estimated by determining the minimum length at which a significant increase in gonad dry weight occurred. This inflection point was found by arbitrarily grouping the data for each species by 10-mm increments of total length and testing for significant differences in gonad dry weight among these groups using analysis of variance and Duncan's multiple range comparison.

Student's t-tests, following tests for equal variance, were used to isolate differences in P. setiferus populations between Blocks B and C. Tests were performed on standardized organ dry weights and biochemical content of the pooled male population and of females by maturation stage (only Stages 2-4 had sufficient observations for analysis). This analysis included only individuals whose total length was equal to or greater than the minimum length at first maturity.

Data used for comparison among ovarian maturation stages were restricted to (1) females whose total length was equal to or greater than minimum length at first maturity; (2) only P. aztecus at Block A and only P. setiferus at Blocks B and C; and (3) periods of reproductive activity, May through September for P. setiferus, and July through October for P. aztecus.

In comparisons of ovary dry weight to literature values expressed as wet weight a conversion factor of 3.85 was applied to the dry weight and a factor of 0.26 was applied to the wet weight data. These factors were determined from unpublished data.

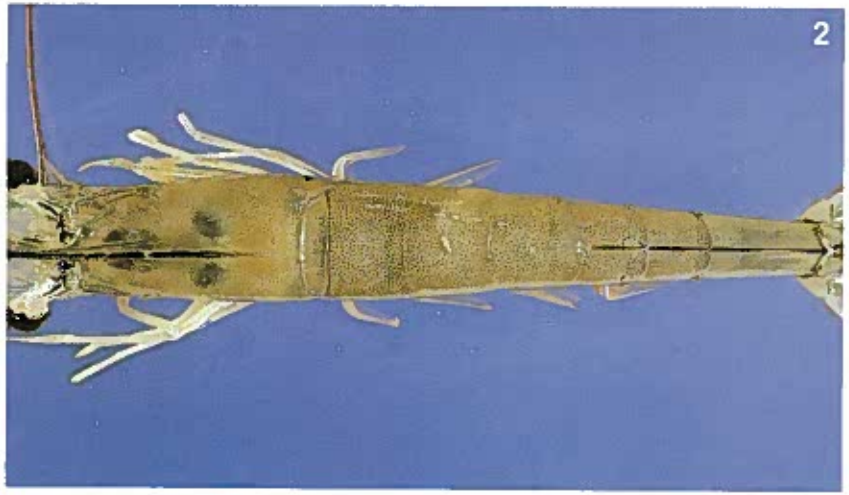
RESULTS

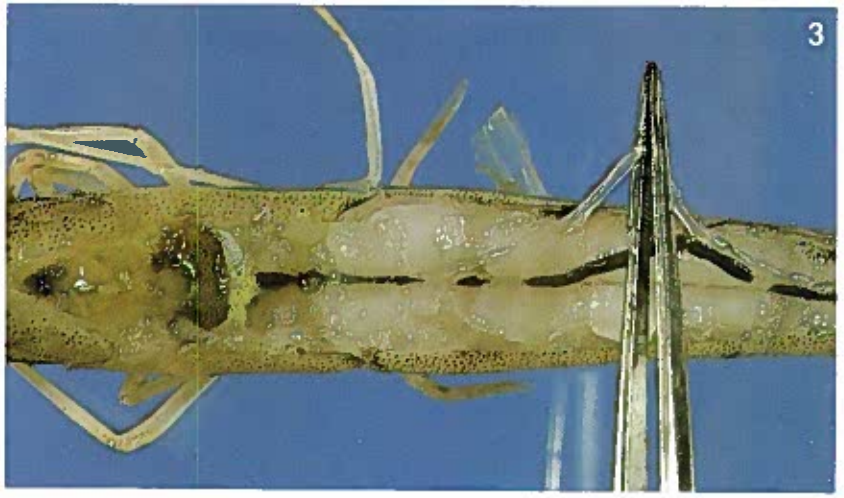
Maturation Stages

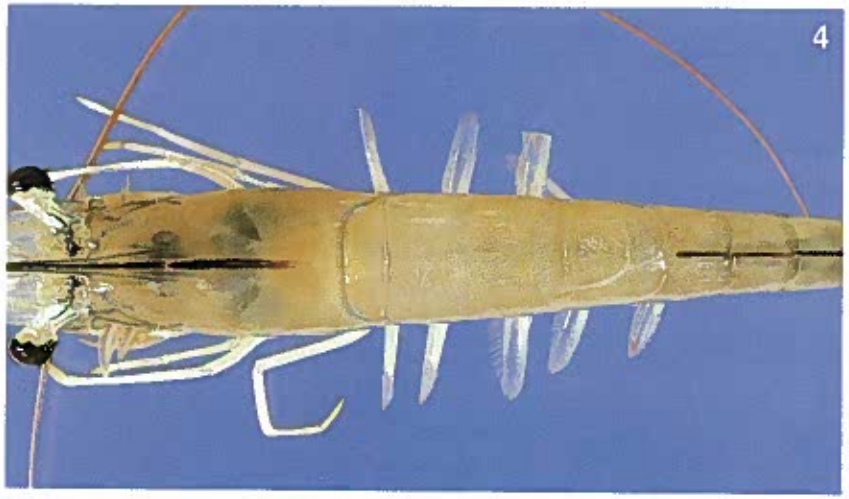
During maturation, consistent changes in relative size and coloration of the ovaries of P. setiferus and P. aztecus allowed visual discrimination of maturation stages in the field. Ovary size and color were similar for both species for Stages 1, 2 and 5, but different for Stages 3 and 4 (Tables 2 and 3). The external and dissected appearance of each maturation stage is described below.

Stage 1, Undeveloped. Ovary was not visible externally (Figure 2). Upon dissection, it appeared thin, transparent and without pigmentation and weighed 0.1-0.2 g (dry weight based on standard female of 40 g total weight) (Figure 3).

Stage 2, Beginning Development: Anterior and posterior lobes of the ovary were faintly visible externally (Figures 4 and 6). Upon dissection the ovary appeared opaque or milky with no distinctive coloration. The dorsal surface of the ovary had scattered grey-green pigmentation in P. setiferus (Figure 5) and red pigmentation in P. aztecus (Figure 7). The standardized dry weight of the ovary was approximately 0.2-0.3 g.







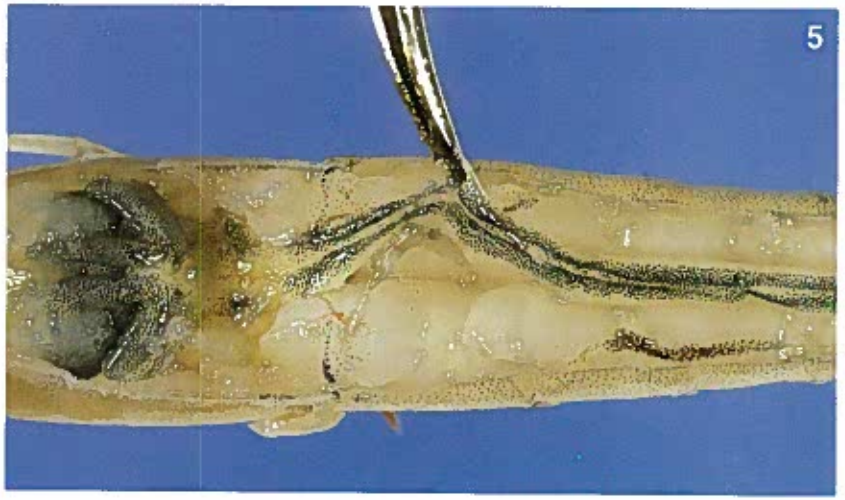
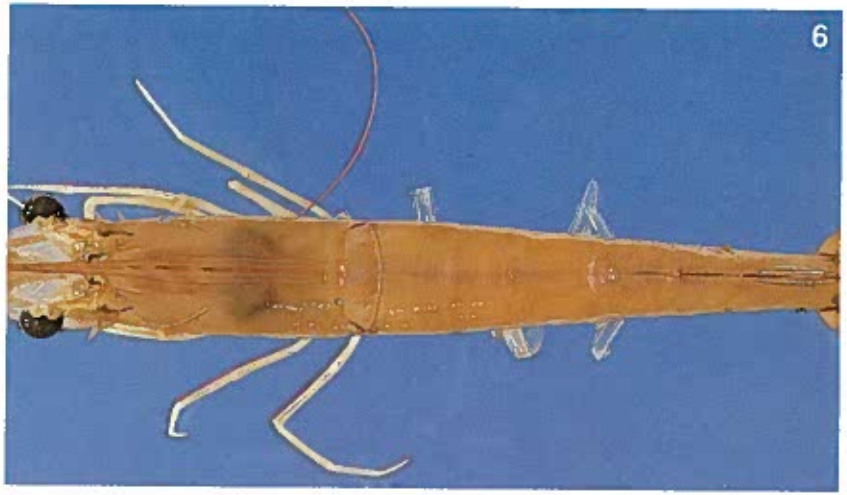
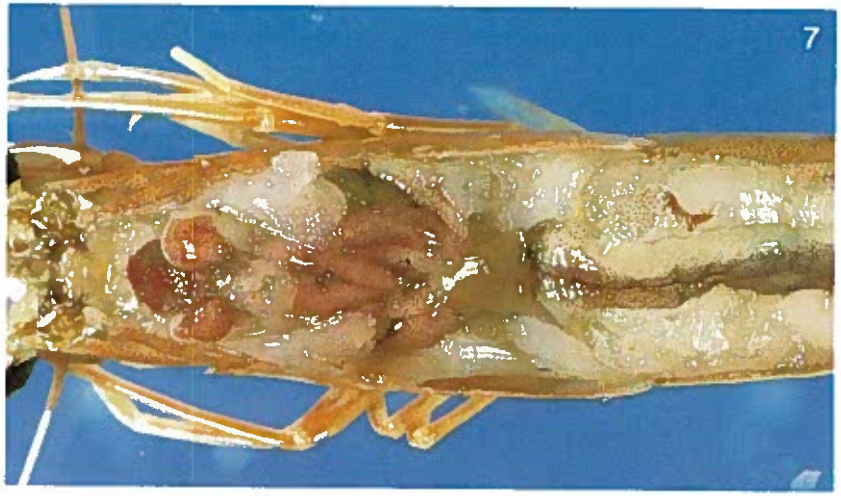
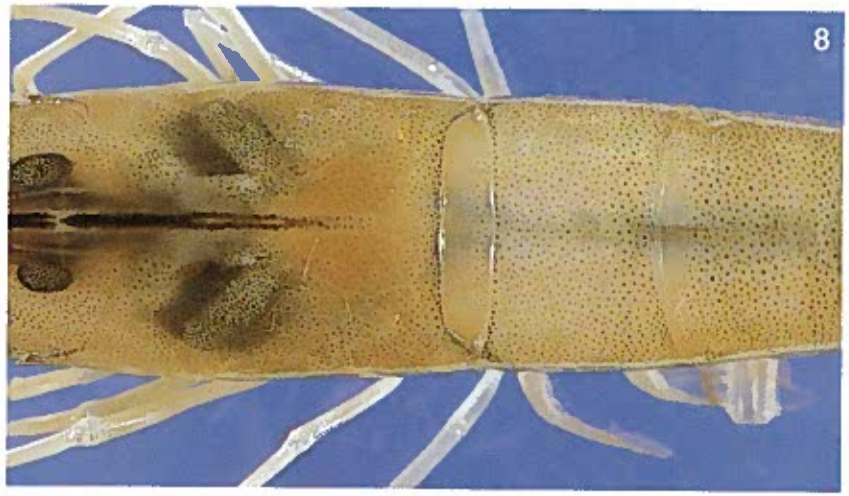


Figure 2. Penaeus setiferus female, external view. Stage 1. 170 mm TL, caught 1 May 1980, inshore waters near Port Mansfield. 26°35'N, 97°15'W. **Figure 4.** Penaeus setiferus female, external view. Stage 2. 189 mm TL, caught 1 May 1980, inshore waters near Port Mansfield. 26°35'N, 97°15'W.

Figure 3. Penaeus setiferus female, dissected view with heart removed. Stage 1. 140 mm TL, caught 26 Feb. 1980, inshore waters near Freeport. 28°55'N, 95°15'W. **Figure 5.** Penaeus setiferus female, dissected view with heart removed. Stage 2. 161 mm TL, caught 1 May 1980, inshore waters near Port Mansfield. 26°35'N, 97°15'W.







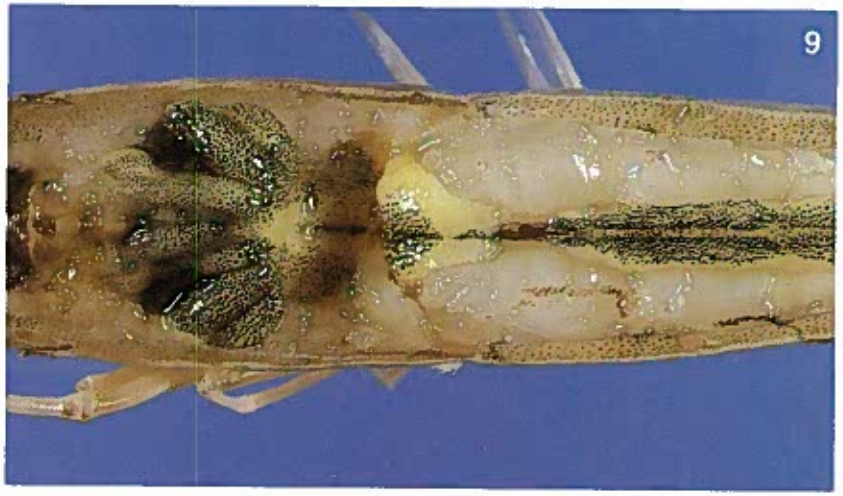
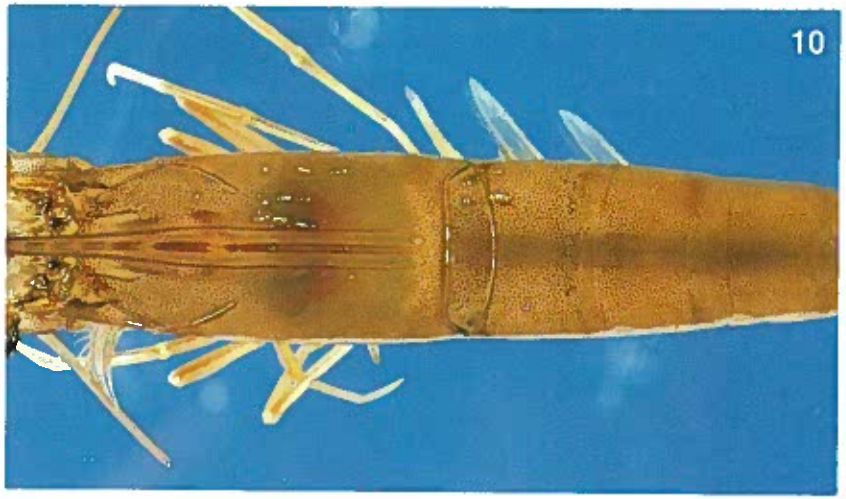
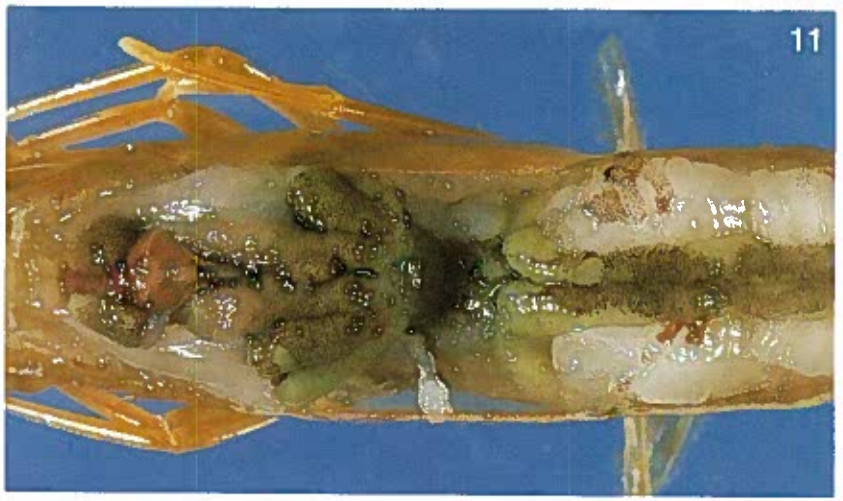
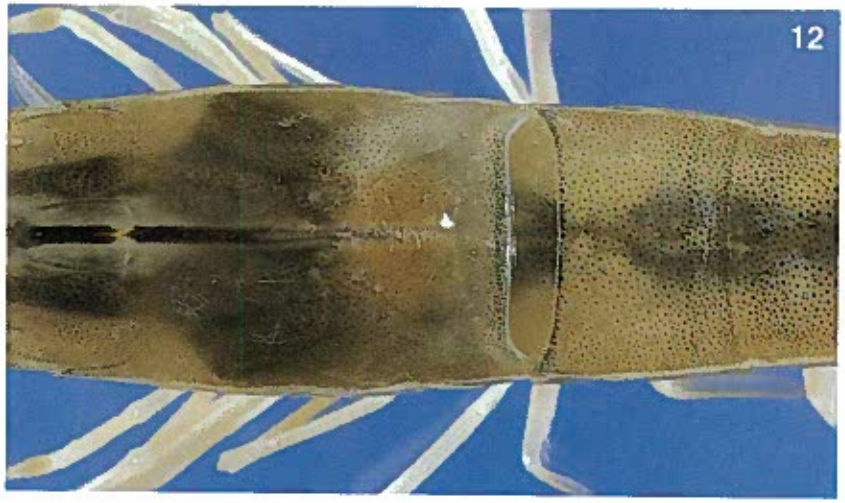


Figure 6. Penaeus aztecus female, external view. Stage 2. 148 mm TL, caught 25 Oct. 1979, offshore waters near Freeport. 28°20'N, 94°50'W. **Figure 8.** Penaeus setiferus female, external view. Stage 3. 165 mm TL, caught 1 May 1980, inshore waters near Port Mansfield. 26°35'N, 97°15'W.

Figure 7. Penaeus aztecus female, dissected view. Stage 2. 165 mm TL, caught 25 Oct. 1979, offshore waters near Freeport. 28°20'N, 94°50'W. **Figure 9.** Penaeus setiferus female, dissected view with heart removed. Stage 3. 165 mm TL, caught 1 May 1980, inshore waters near Port Mansfield. 26°35'N, 97°15'W.







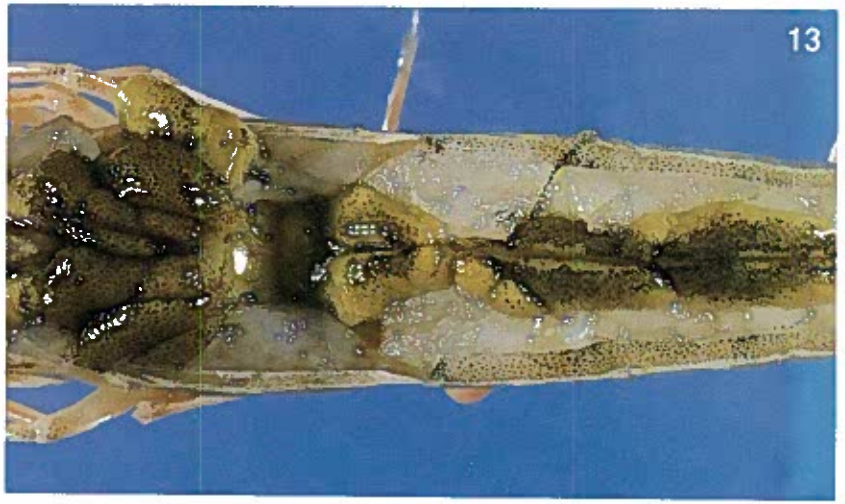
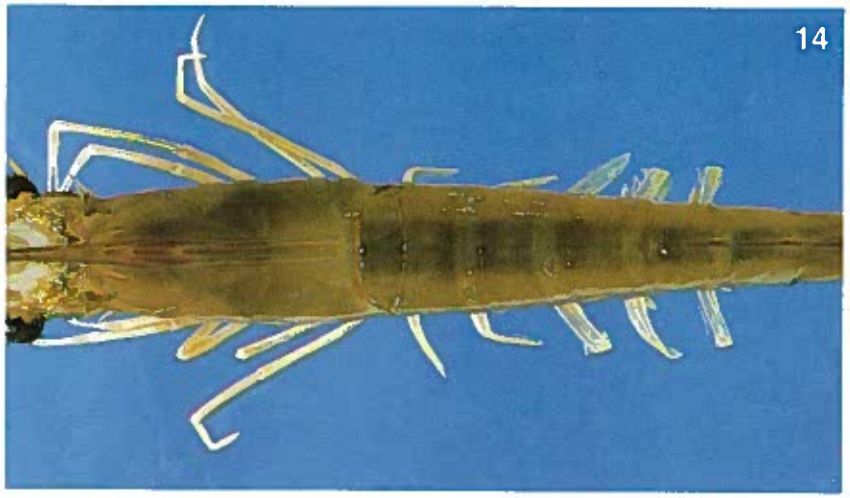


Figure 10. Penaeus aztecus female, external view. Stage 3. 154 mm TL, caught 28 February 1980, offshore waters near Freeport. 28°20'N, 94°50'W. **Figure 12.** Penaeus setiferus female, external view. Stage 4. 164 mm TL, caught 1 May 1980, inshore waters near Port Mansfield. 25°35'N, 97°15'W.

Figure 11. Penaeus aztecus female, dissected view with heart removed. Stage 3. 170 mm TL, caught 29 February 1980, offshore waters near Freeport. 28°20'N, 94°50'W. **Figure 13.** Penaeus setiferus female, dissected view with heart removed. Stage 4. 164 mm TL, caught 1 May 1980, inshore waters near Port Mansfield. 26°35'N, 97°15'W.



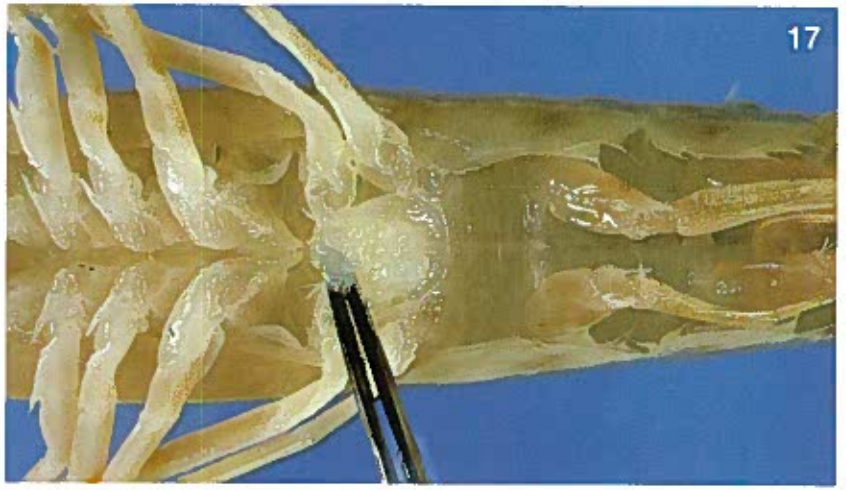
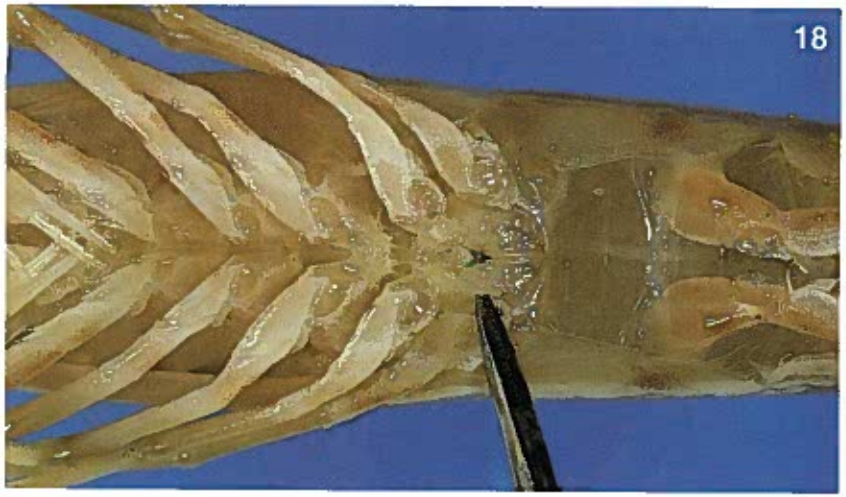
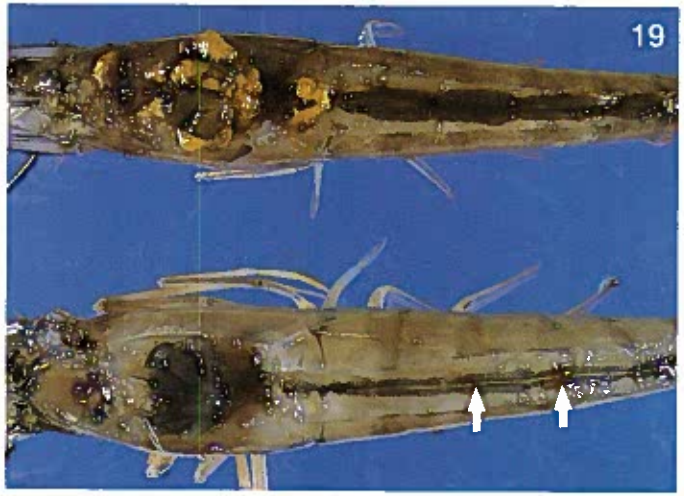
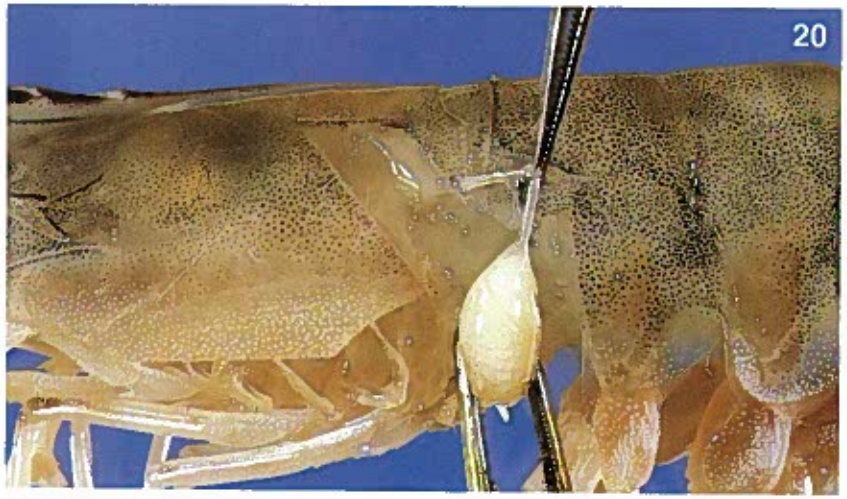


Figure 14. Penaeus aztecus female, external view. Stage 4. 176 mm TL, caught 10 June 1980, offshore waters near Freeport. 28°20'N, 94°50'W. **Figure 16.** Penaeus setiferus mated Stage 4 female, ventral view. Sperm mass visible at tip of probe between third and fourth pereiopods. Remnant of spermatophore visible just above sperm mass and to left of probe. Caught July 1981, inshore waters near Port Aransas. 28°30'N, 96°50'W.

Figure 15. Penaeus aztecus female, dissected view with heart removed. Stage 4. 176 mm TL, caught 10 June 1980, offshore waters near Freeport. 28°20'N, 94°50'W. **Figure 17.** Penaeus aztecus female, 183 mm TL, with soft exoskeleton. Portion of spermatophore (held in forceps) still visible externally, indicating recent mating. Caught 9 June 1980 offshore waters near Freeport. 28°20'N, 94°50'W.







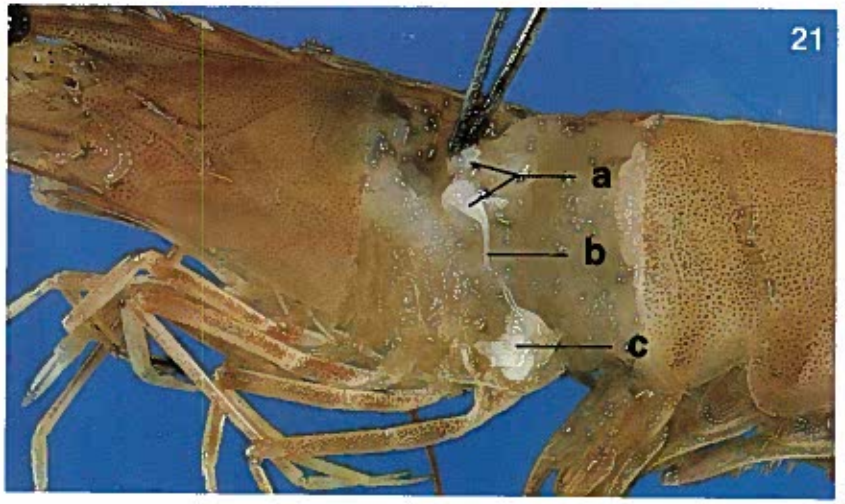


Figure 18. Penaeus aztecus female, 190 mm TL, with spermatophore completely enclosed within thelycum and not visible externally. Caught 9 June 1980 in offshore waters near Freeport. 28°20'N, 94°50'W. **Figure 20.** Penaeus setiferus male, 148 mm TL, with one vas deferens (upper forceps) and one terminal ampoule (lower forceps) partially removed. Caught 9 June 1981, inshore waters near Port Mansfield. 26°35'N, 97°15'W.

Figure 19. Penaeus setiferus females, dissected views with hearts removed. Stage 4 (upper) and Stage 5 (lower). Note orange patches of undischarged ova (see arrows) in posterior lobes of Stage 5 ovary. Caught 10 June 1981, inshore waters near Port Aransas. 28°30'N, 96°50'W. **Figure 21.** Penaeus aztecus male with one testis (a), vas deferens (b) and terminal ampoule (c) exposed. Caught 1 May 1980, offshore waters near Freeport. 28°20'N, 94°50'W.

Stage 3, Nearly Ripe: The ovaries of both P. setiferus and P. aztecus were easily recognized externally by enlarged anterior and posterior lobes (Figures 8 and 10). P. setiferus ovaries were usually light yellow with green chromatophores and a mean standardized dry weight of 0.9 g (Figure 9). P. aztecus ovaries were usually light yellow-green with red chromatophores and a mean standardized dry weight of 0.6 g (Figure 11).

Stage 4, Ripe: Externally, the anterior and posterior lobes of the ovaries of both P. aztecus and P. setiferus appeared broad and dark (Figures 12 and 14). The color of the middle lobe was easily distinguished through the thin membrane between the abdomen and cephalothorax. P. setiferus ovaries were usually light yellow-orange with a mean standardized dry weight of 1.6 g (Figures 13 and 19). P. aztecus ovaries were dark yellow-green with a mean standardized dry weight of 1.1 g (Figure 15). The mated condition of P. setiferus was easily distinguished when a complete spermatophore was attached to the thelycum, but closer observation was required if the geminate body of the spermatophore had been dislodged (Perez-Farfante, 1975) leaving only the deposited sperm mass attached near the third pair of pereiopods (Figure 16). The presence of a sperm mass in the thelycum of P. aztecus was easily detected immediately after mating in recently molted females when the spermatophore was only partially inserted within the thelycum (Figure 17). However, the thelycum of mated P. aztecus typically showed no external spermatophore, slightly darkened medial edges on the lateral plates of the

Table 2. Frequency of occurrence of various ovarian colors with each maturation stage of Penaeus setiferus. Percentage of ovaries within a maturation stage with a given color is indicated in parentheses. Color judgements were made after ovaries were exposed by dissection.

Color	Ovarian Maturation Stage					Total
	1	2	3	4	5	
Clear	66 (98.5)*	1 (2.1)				67
Milky white		37 (77.1)*	18 (18.6)		5 (71.4)*	60
Light yellow		1 (2.1)	62 (63.9)*	5 (6.3)		68
Medium yellow			5 (5.2)	5 (6.3)		10
Dark yellow				4 (5.0)		4
Light yellow-orange		1 (2.1)	7 (7.2)	52 (65.0)*	1 (14.3)	61
Medium yellow-orange				8 (10.0)	1 (14.3)	9
Dark yellow-orange			1 (1.0)	5 (6.3)		6
Light yellow-green	1 (1.5)	8 (16.7)	4 (4.1)			13
Medium yellow-green				1 (1.3)		1
Total	67	48	97	80	7	299

*Most common ovary color of the indicated maturation stage.

thelycum where the spermatophore was inserted, and an opaque convexity of the thelycum (Figure 18).

Stage 5, Spent: Evaluations of Stage 5 ovaries were based on females that had spawned aboard the vessel the previous night. External appearance and standardized dry weight of the Stage 5 ovaries were similar to those of Stage 2 ovaries. We were unable to distinguish between Stage 2 and Stage 5 ovaries without dissection. After dissection of the Stage 5 ovary small areas of Stage 4 coloration were usually apparent in the posterior lobe (Figure 19), and the ovary appeared more flaccid and watery than at Stage 2. In incomplete spawns, the anterior and middle lobes were evacuated, but the posterior lobe still contained large quantities of Stage 4 ova.

Classification of male gonads into maturation stages based on external and dissected appearance (Figures 20-21) was largely arbitrary because of the continuous nature of the gonadal developent from a small translucent structure to a larger off-white structure. Dissected terminal ampoules, vas deferens and

Table 3. Frequency of occurrence of various ovarian colors with each maturation stage of Penaeus aztecus. Percentage of ovaries within a maturation stage with a given color is indicated in parentheses. Color judgements were made after ovaries were exposed by dissection.

Color	Ovarian Maturation Stage					Total
	1	2	3	4	5	
Clear	37 (97.4)*					37
Milky white	1 (2.6)	64 (80.0)*	2 (0.82)		3 (60.0)*	70
Light yellow		6 (7.5)	18 (7.4)			24
Medium yellow			1 (0.4)	2 (1.4)		3
Dark yellow			1 (0.4)	1 (0.7)		2
Light yellow-orange		3 (3.8)	3 (1.2)			6
Medium yellow-orange				1 (0.7)	1 (20.0)	2
Light yellow-green		7 (8.8)	179 (73.4)*	33 (23.2)		219
Medium yellow-green			3 (1.2)	26 (18.3)		29
Dark yellow-green			2 (0.8)	53 (37.3)*	1 (20.0)	56
Light green			25 (10.3)	11 (7.8)		36
Dark green					13 (9.2)	13
Light blue-green				10 (4.1)	2 (1.4)	12
Total	38	80	244	142	5	509

*Most common ovary color of the indicated maturation stage.

Table 4. Estimated number, hatching rate and biochemical composition of eggs of ripe, mated *Penaeus aztecus* and *P. setiferus* that spawned aboard the research vessel shortly after collection.

Date	Hour	Block, Station	TL	No. eggs ($\times 10^3$)	Hatch rate (%)	% metamorphosis	% Composition of Eggs: Carbohydrate	Protein	Lipid
					Penaeus aztecus				
17 Aug	2030	A7	179	158	53	44	3.15	5.3	25.8
18 Aug	2030	A6	165	190	72	ND	3.08	9.0	46.2
18 Aug	2030	A6	ND	2	0	ND	ND	6.2	7.5
18 Aug	2030	A6	ND	40	85	ND	2.95	7.8	15.8
18 Aug	2130	A6	ND	51	97	ND	2.24	3.5	15.4
19 Aug	2030	A1	170	36	80	ND	3.05	5.1	21.5
19 Aug	2030	A1	166	22	50	ND	3.38	9.7	6.6
19 Aug	2030	A1	153	60	59	ND	2.53	6.8	5.4
19 Aug	2100	A1	160	27	70	ND	2.71	6.7	8.1
19 Aug	2130	A1	179	19	57	ND	2.76	4.6	10.7
8 Sep	2030	A7	195	22	21	ND	3.24	22.9	11.2
8 Sep	2000	A7	170	116	99	ND	3.53	17.6	22.0
10 Sep	2000	A6	169	169	94	ND	2.27	11.1	13.9
10 Sep	2000	A6	171	206	70	ND	2.36	14.9	16.0
10 Sep	2000	A6	157	30	90	ND	1.98	14.3	8.5
10 Sep	0100	A7	151	6	0	ND	ND	ND	8.9
7 Sep	1200	C8	148	46	48	44	3.55	16.1	13.2
					Penaeus setiferus				
5 May	1835	B3	150	63[a]	0[b]	0[b]	--	--	--
6 May	1845	B3	170	155	0[b]	0[b]	--	--	--
6 May	1915	B3	163	114[a]	0[b]	0[b]	--	--	--
6 May	1845	B3	169	179	0[b]	0[b]	--	--	--
6 Jun		C2	ND[c]	0	0	0	--	--	--
6 Jun		C2	175	358	72	72	ND	ND	ND
9 Jul	1400	B3	189	217[ad]	11	11	ND	ND	ND
11 Jul	1825	B3	ND[c]	241	13	14	ND	ND	ND
13 Jul	1830	C1	187	320	64	66	ND	ND	ND
13 Jul	1830	C1	178	169	72	70	ND	ND	ND
13 Jul	1830	C1	192	215	64	65	ND	ND	ND
13 Jul	1830	C1	181	236	90	80	ND	MD	ND
17 Aug	1530	C9	194	440	52	42	2.16	6.0	15.4
7 Sep	1730	C4	165	13[a]	25	25	1.05	16.8	9.5

[a] Partial spawn.

[b] Inadequate aeration of water; eggs did not hatch and were not analyzed.

[c] Died in spawning tank.

[d] Spermatophore found dislodged in spawning tank, 9 July, 1600 hours.

ND indicates that data were not determined.

testes, composing the reproductive system of a male P. setiferus, are shown in Figure 22. The appearance of a dissected reproductive system of a male P. aztecus is similar to that of a male P. setiferus. No published description of visual maturation stages for males was found except for Persyn's (1977) observation that mature P. setiferus males can be distinguished by slightly yellow, hardened spermatophores. Consequently, gonad dry weight was used as the primary indicator of male maturation.

Reproductive Performance

Mated P. setiferus were collected only during the months of May through September (Table 4). Most of the mated females were collected one to two hours before sundown, although two were collected in midafternoon. All six mated females from Block B were collected at Station 3, while the eight mated females from Block C were collected from four different stations. Frequency of capture of mated female P. setiferus averaged approximately 26 percent of Stage 4 female P. setiferus in collections within two hours of sundown. During July, the most productive month for mated P. setiferus, 40 percent of the Stage 4 females at Block C (n = 20) were mated. All mated P. setiferus (n = 14), with the exception of one that died, spawned on the night of capture after they were placed in aerated seawater. The number of eggs released ranged from 13,000 to 440,000 per spawn. Spawns releasing less than 150,000 were considered "incomplete" (Table 4). The mean number of eggs per spawn from the nine complete spawns was 257,600. Fecundity (F) increased logarithmically and significantly with increasing total length according to the linear regression equation

$$\log F = -3.1911 + 3.7944 \log TL.$$

For complete spawns, rates of eggs hatching to nauplii and of naupliar metamorphosis to protozoea averaged 55 percent (range, 13-90 percent) and 53 percent (range, 14-80 percent), respectively. Although the temperature in one spawning tank increased to 33°C the afternoon following spawning, 72 percent of the eggs hatched and metamorphosed normally.

More than 90 percent of the P. aztecus females large enough to spawn appeared to be impregnated with a sperm mass regardless of maturation stage. Consequently, no attempt was made to record the presence of sperm in each female. However, presence of sperm in thelyca of P. aztecus females did not necessarily indicate that spawning was imminent. Of the ripe females placed in spawning tanks, only about 55 percent spawned, and only 16 percent spawned more than 100,000 eggs per spawn. Spawns of less than 100,000 eggs probably represented abortions of premature ova induced by the stress of capture and placement in surface water (27-30°C) that was warmer than bottom water (21-24°C) from which they were collected. For normal spawns (n = 5), the mean number of eggs per spawn, percent hatch and percent metamorphosis to protozoea were 167,800, 78 percent and 44 percent, respectively. All females that spawned normally were collected between 2000 and 2030 hours CDT. All ripe females placed in spawning tanks were from Block A with the exception of one from Block C, Station 8, which yielded 53,000 eggs with a 48 percent hatching rate.

No significant correlations were found between arcsine-transformed percent carbohydrate, lipid, and protein of eggs and percent hatch rate. However, this may have been related to the small sample size.

Organ Weight and Biochemical Composition

Size-Dependent Variations

Much of the variation in dry weight and biochemical composition of gonad and hepatopancreas was related to shrimp size. The dry weight of the hepatopancreas increased linearly with increase in total length (Figures 22 and 23). Large differences in hepatopancreas dry weight occurred between sexes. For females of both species the mean dry weight of the hepatopancreas increased from 0.1 g at 100 mm to 0.8 g at 200 mm. In males of both species the increase in hepatopancreas dry weight was less rapid -- from 0.1 g at 100 mm to 0.3 g at 170 mm.

The dry weight of the gonad did not increase linearly with total length. In females, a non-continuous linear relationship

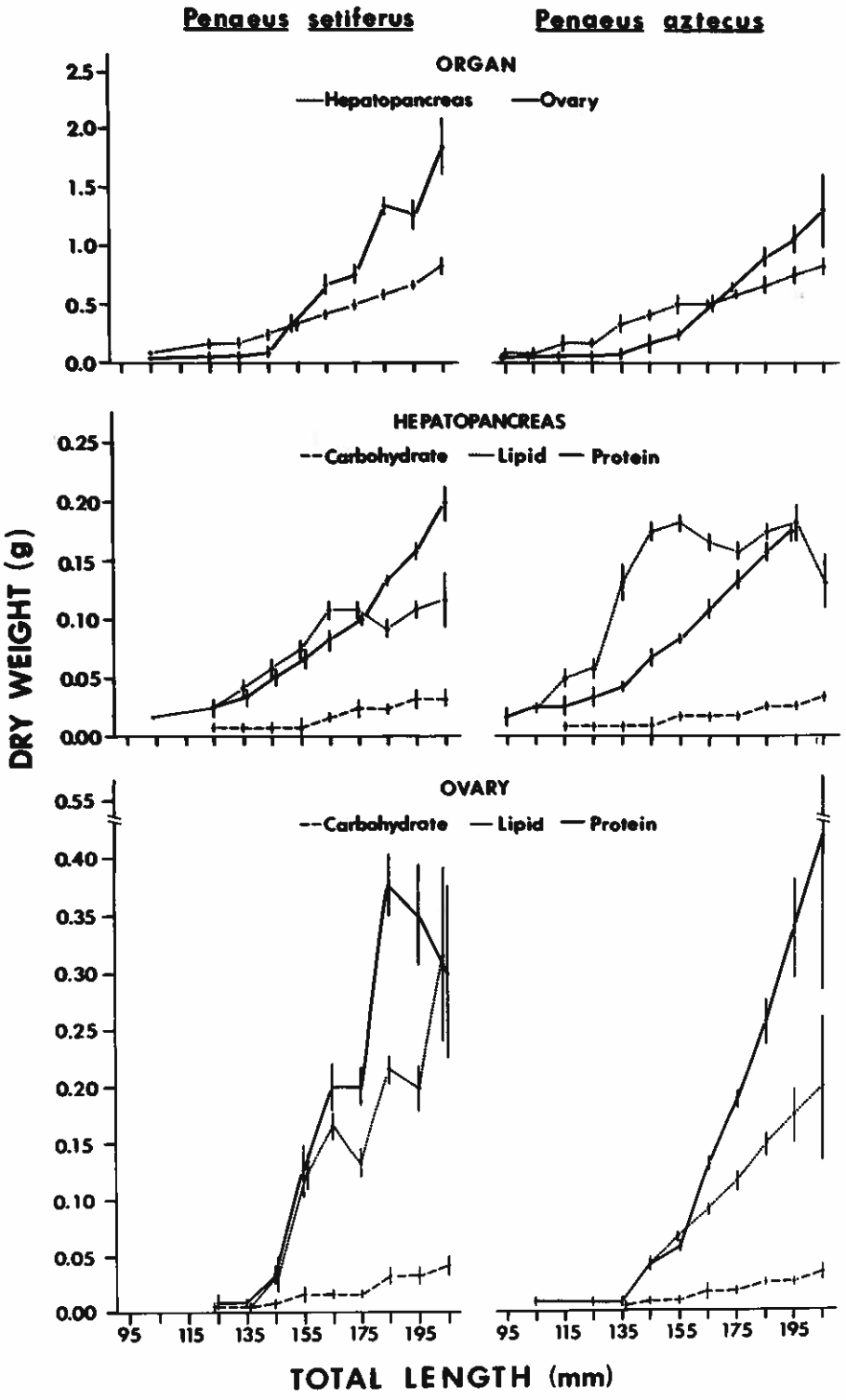


Figure 22. Mean organ weight and carbohydrate, lipid and protein content of the hepatopancreas and ovary of female Penaeus setiferus and P. aztecus for each 10-mm increment of TL. Vertical bars represent standard errors.

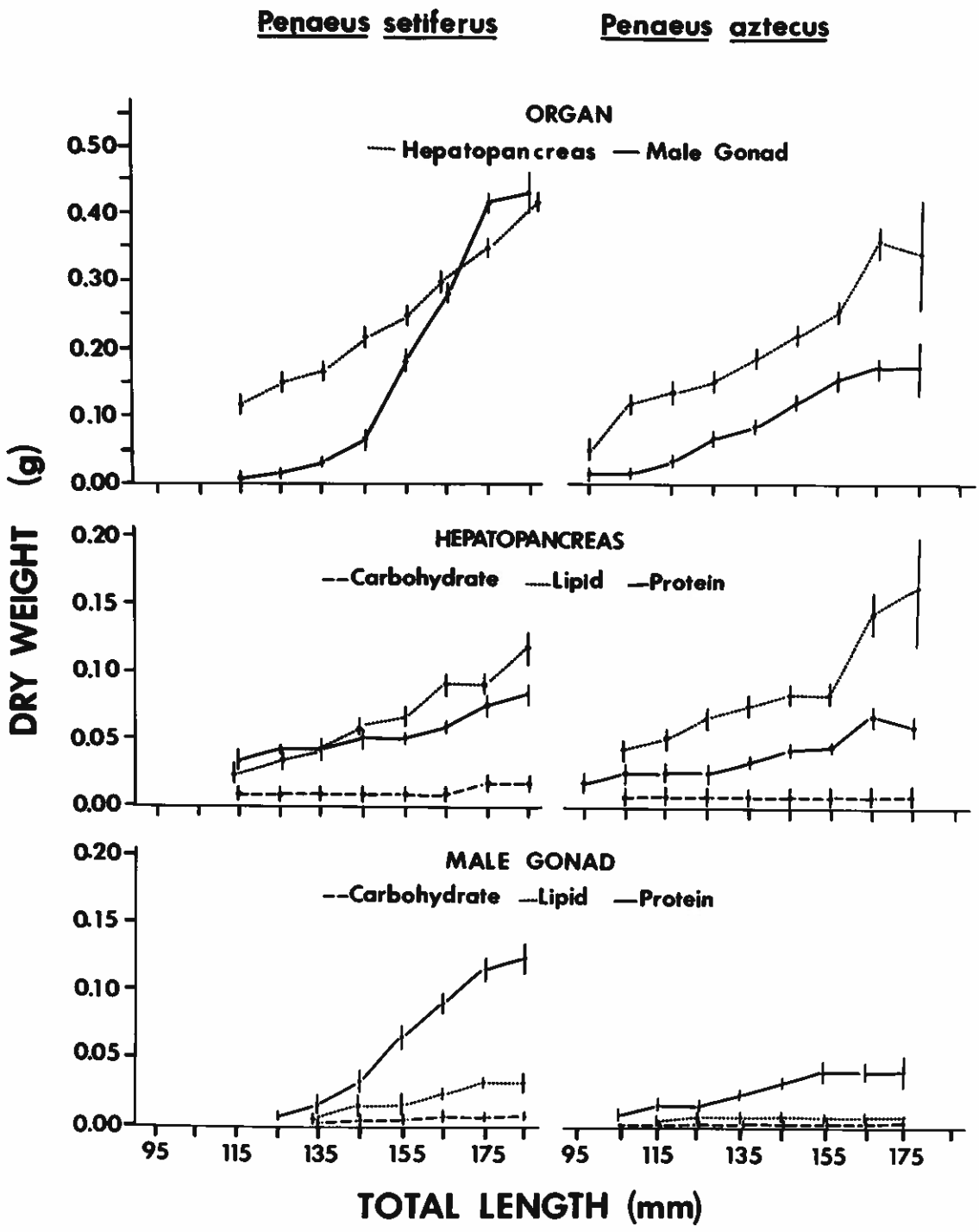


Figure 23. Mean organ weight and carbohydrate, lipid and protein content of the hepatopancreas and gonad of male Penaeus setiferus and P. aztecus for each 10-mm increment of TL. Vertical bars represent standard errors.

was apparent in which mean gonad weight remained small in small shrimp and then inflected and increased linearly for larger shrimp (Figure 22). The inflection point was considered to represent the onset of maturation. In males, the pattern of increase in gonad weight appeared sigmoidal with two inflections. The first inflection was considered to be the onset of maturation and the second to represent the asymptote of gonad growth. Significant differences in mean gonad dry weight occurred among 10-mm increments of total length for both species and sexes. The increment in total length at which the first significant increase in gonad dry weight occurred was considered the length at first maturity (Table 5). The increment in total length at which the last significant increase occurred in male gonad dry weight was considered as the minimum length at asymptotic gonad size (Table 5). These estimates demonstrate marked sexual dimorphism in length at the onset of maturation and length at first maturity in P. aztecus and less marked sexual dimorphism in P. setiferus. Gonad dry weight of P. setiferus males at the minimum length at asymptotic gonad size (0.40 g) was more than twice that of P. aztecus males at the corresponding length (0.14 g) (Figure 23).

Lipid levels in the hepatopancreas generally exceeded protein levels, and both lipid and protein levels greatly exceeded levels of carbohydrate. Carbohydrate, lipid and protein levels in the hepatopancreas increased with increase in total length in both species and sexes (Figures 22 and 23). However, the increase in hepatopancreas lipid content reached a plateau in females of both species as they approached the length at first maturity (Figure 22). Large P. aztecus males and females stored more lipid in the hepatopancreas (0.15 and 0.17 g, respectively) than large P. setiferus males and females (0.10 and 0.10 g, respectively) of similar size. Protein content in the hepatopancreas was significantly greater in females of both species than in males of similar length (Figures 22 and 23).

In both male and female gonads, carbohydrate, lipid and protein levels remained low in small shrimp and increased significantly as they approached the length at first maturity. Protein appeared to be the major constituent of the male gonad, while protein and lipid dominated the female gonad (Figures 22 and 23). Ovaries contained more of all three biochemical constituents than male gonads because ovaries weigh so much more.

Table 5. Estimated minimum total length (mm) at onset of gonadal maturation (OM), at first maturity (FM) and at ultimate (asymptotic) gonadal maturation (GM) of male and female P. setiferus and P. aztecus. Values were estimated graphically.

	OM	FM	GM
P. setiferus			
males	140-150	150-160	170-180
females	150-160	160-170	*
P. aztecus			
males	110-120	110-120	150-160
females	140-150	160-170	*

* Ovary size did not reach an asymptote but increased linearly with total length.

Spatial Variations

Comparisons of organ weight and biochemical content between stations are limited to P. setiferus from Blocks B and C, because only one adult P. setiferus was obtained from Block A, and too few mature P. aztecus were obtained from Blocks B and C to allow statistical comparisons. Student's t-tests indicate that hepatopancreas carbohydrate levels in shrimp from Block B were consistently greater than those from Block C in males and females of Stages 2, 3 and 4 (Table 6). Stage 4 females from Block B also had significantly greater hepatopancreas dry weights than those from Block C. Gonad weight and biochemical content did not differ significantly between blocks. We consider the differences in hepatopancreas carbohydrate content to reflect the nutritional condition of the shrimp more than their reproductive state. Therefore, data for P. setiferus from Blocks B and C were pooled in subsequent analyses of seasonal variations and variations with ovarian maturation.

Seasonal Variations

Standardized gonad dry weights of P. setiferus males and females varied greatly among months. Concurrent gonadal development of males and females occurred during May through September. Penaeus setiferus with well-developed gonads were most numerous from May through July (Figure 24). Standardized mean gonad dry weight was greatest during May, 1.25 and 0.33 g for males and females, respectively. Multiple regression analysis of sine- and cosine-transformed day-of-year functions vs. standardized gonad dry weight yielded significant relationships for both males (r^2 = 0.61) and females (r^2 = 0.38) (Table 7). These models indicate synchronized development of males and females with maximum gonad size occurring in early June and minimum gonad size in mid-December.

Penaeus aztecus males and females showed a less pronounced seasonal cycle of standardized gonad dry weight than P. setiferus (Figure 25). Depressed ovary dry weights were apparent in February to March when only one Stage 4 female was caught, but relatively high levels were found in all other collections. Ovary weights were greatest from July through October. Gonad dry weights of P. aztecus males showed even less seasonal variation than P. aztecus females, with a slight depression in February to March and a peak in September. Multiple regression analysis of sine and cosine functions vs. standardized gonad weight of P. aztecus indicated no significant relationship with males and a significant relationship with females (r^2 = 0.03). The regression model for females indicated maximum gonad size during early May (Table 7).

Variations with Ovarian Maturation

Major changes in organ weight and biochemical composition were related to stage of ovarian maturation. Hepatopancreas dry weight remained relatively constant for the first four maturation stages in both P. setiferus and P. aztecus (Figure 26). The increase between Stages 2 and 3 and the subsequent decrease between Stages 3 and 4 were not significant in either species, but the decrease between Stages 4 and 5 was significant in both species. Hepatopancreas standardized dry weights of P. aztecus were significantly larger than those of P. setiferus in Stages 2-5.

Table 6. Mean dry weight (mg) and mean biochemical content (mg) of gonads and hepatopancreas of Penaeus setiferus between locations.

			Hepatopancreas					Gonad				
	Stage	Block	N	Wt.	Carb.	Prot.	Lip.	N	Wt.	Carb.	Prot.	Lip.
Males	---	B	116	262	11*	51	73	104	294	5	93	24
	---	C	109	256	9*	53	68	101	273	5	81	23
Females	2	B	43	478	22*	97	97	33	389	10	92	39
		C	33	425	16*	88	87	31	344	7	84	38
	3	B	76	499	24*	109	87	74	901	20	251	144
		C	54	499	21*	116	89	52	898	18	271	140
	4	B	55	460*	22*	94	81	51	1,604	40	418	285
		C	64	420*	18*	101	70	64	1,587	36	469	281

* Means differ significantly between locations.

Table 7. Summary of multiple regression analysis of sine and cosine functions of day-of year of collection (χ) on the dependent variable, standardized gonad dry weight of male and female Penaeus setiferus and female P. aztecus. (No significant relationship was found with male P. aztecus.)

Source	DF	SS	MS	F	Prob>F	Source	Coefficient	Partial SS	F	Prob>F
					Female Penaeus setiferus					
Model	2	84.3971	42.1986	167.56	0.0001	sine($2\pi\chi/365$)	0.107708	2.8466	11.30	0.0008
Error	539	135.7395	0.2518			cosine($2\pi\chi/365$)	-0.634381	83.0481	329.77	0.0001
Corrected total	541	220.1367				intercept	0.417891			
R^2 = 0.383										
					Male Penaeus setiferus					
Model	2	4.2736	2.1368	333.04	0.0001	sine($2\pi\chi/365$)	0.070146	1.1271	175.67	0.0001
Error	422	2.7076	0.0064			cosine($2\pi\chi/365$)	-0.145361	3.0565	476.39	0.0001
Corrected total	424	6.9811				intercept	0.141097			
R^2 = 0.612										
					Female Penaeus aztecus					
Model	2	4.8474	2.4237	16.26	0.0001	sine($2\pi\chi/365$)	-0.084353	2.6012	17.45	0.0001
Error	1104	164.5346	0.1490			cosine($2\pi\chi/365$)	0.066944	1.1746	7.88	0.0051
Corrected total	1106	169.3820				intercept	0.453807			
R^2 = 0.029										

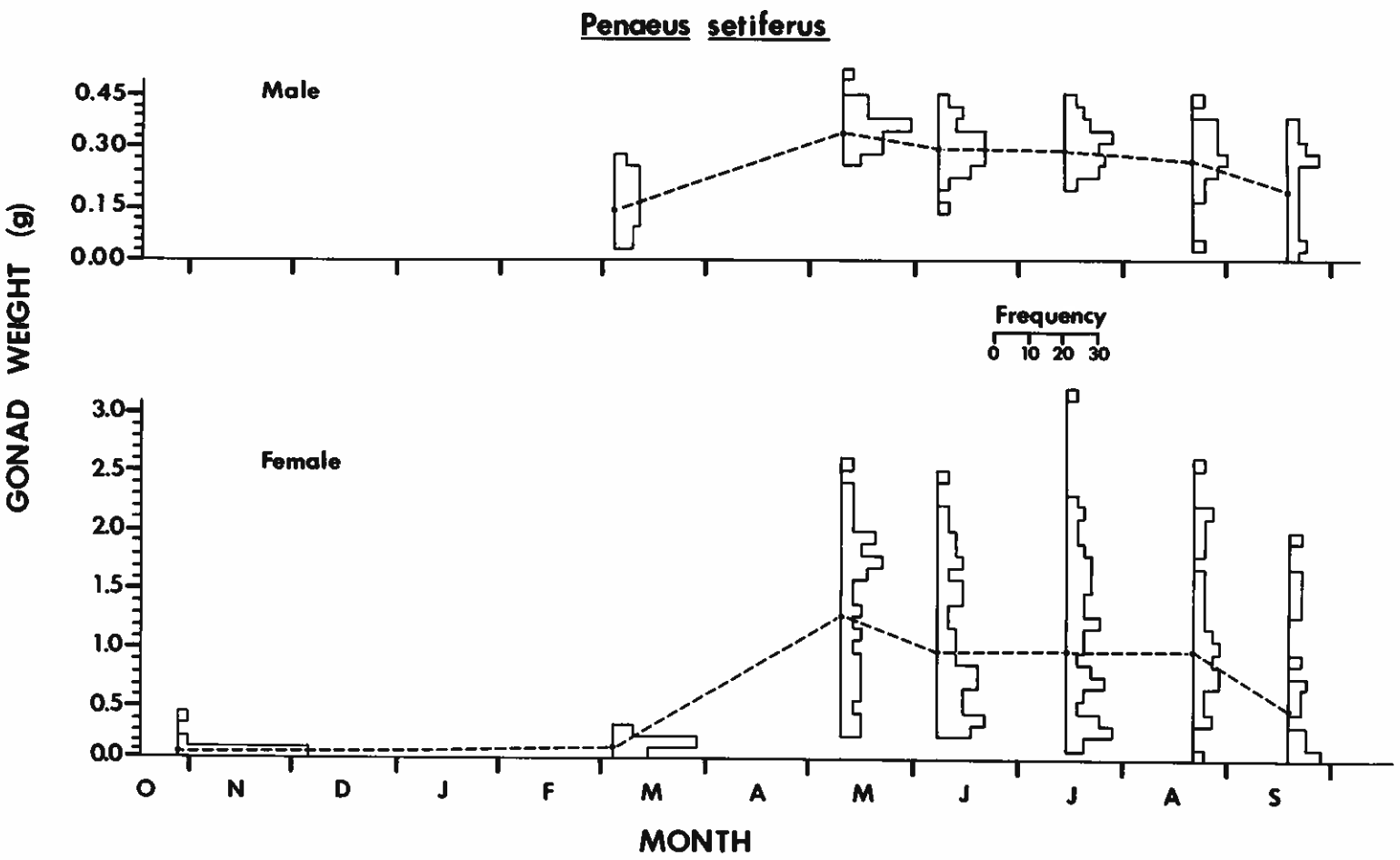


Figure 24. Gonad dry weight frequency and mean gonad dry weight (dashed line) for Penaeus setiferus males and females during each collecting cruise.

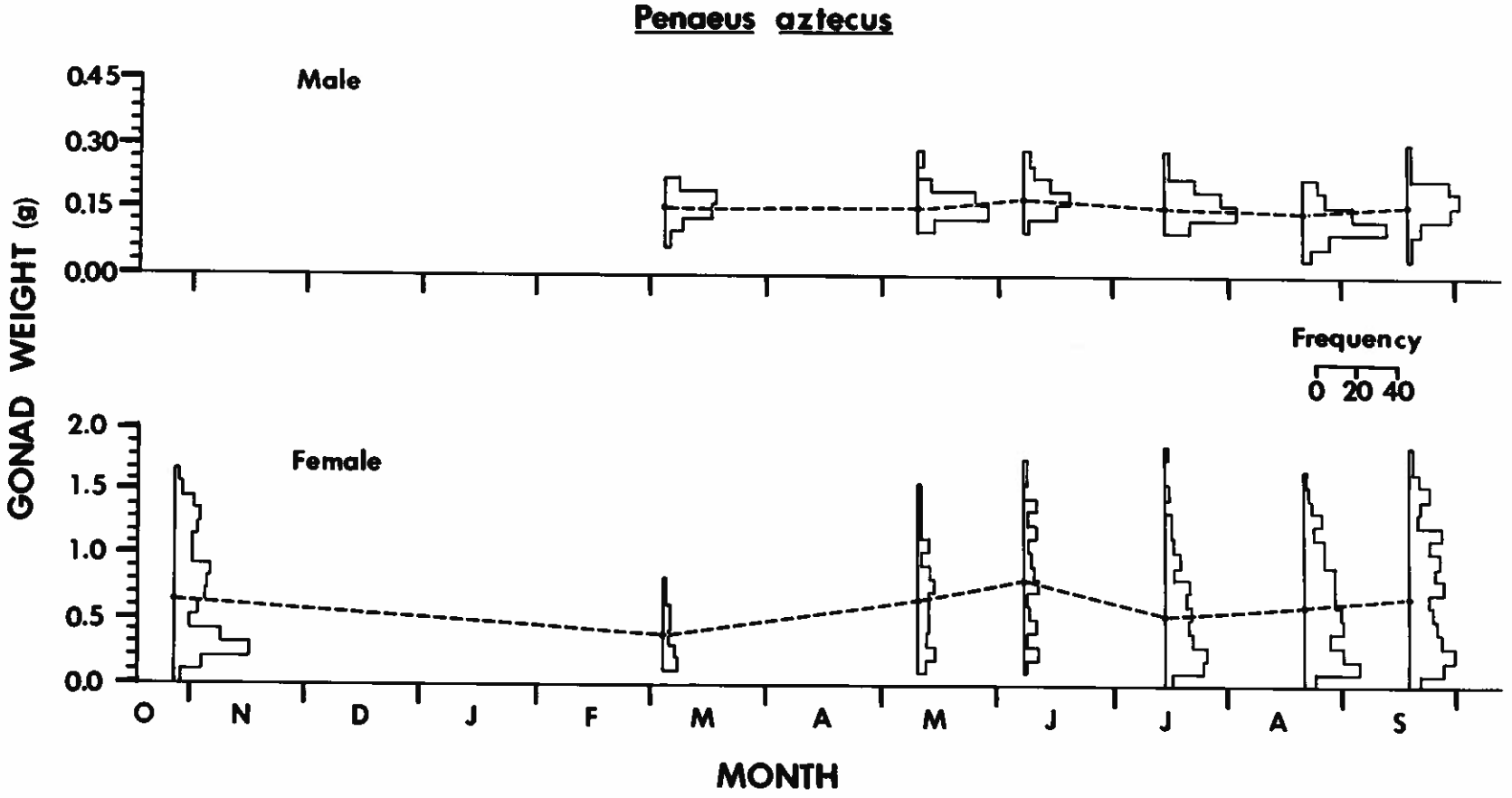


Figure 25. Gonad dry weight frequency and mean gonad dry weight (dashed line) for Penaeus aztecus males and females during each collecting cruise.

In P. setiferus, lipid was the major component of the hepatopancreas at Stage 1 but decreased progressively in both dry weight content and percent composition between Stages 3 and 5 to a level significantly less than that of protein (Figure 26, Table 8). Hepatopancreas carbohydrate and protein increased significantly in both dry weight content and percent composition between Stages 2 and 3. Between Stages 3 and 4 and between Stages 4 and 5, the content of carbohydrate and protein in the hepatopancreas decreased, while the percent composition remained the same.

In the hepatopancreas of P. aztecus, lipid content and percentage composition did not differ significantly among maturation stages. Lipid was the largest component of the hepatopancreas at Stages 1 and 2, but lipid levels did not differ significantly from protein levels in Stages 3-5. Hepatopancreas carbohydrate and protein levels increased significantly in both content and percent composition between Stages 2 and 3 but did not change significantly thereafter. A notable pattern in the hepatopancreas of both species was the consistent decrease in the dry weight but not in the percentage composition of each biochemical component from Stage 3 to Stage 4 and from Stage 4 to Stage 5. These changes resulted from decreasing hepatopancreas dry weight, whereas the changes from Stage 2 to Stage 3 reflected changes in relative composition of the hepatopancreas.

In both species the dry weight of the ovary increased significantly between Stages 2 and 4 and then decreased significantly between Stages 4 and 5. The dry weight of the ovary at Stages 3 and 4 was greater in P. setiferus than in P. aztecus. As a result, carbohydrate, protein and lipid levels in the ovary were greater at Stages 3 and 4 in P. setiferus than in P. aztecus. The content of carbohydrate, lipid and protein in the ovary of both species followed the same pattern of significant changes among maturation stages as did dry weight.

Table 8. Mean percentage composition (dry weight) of carbohydrate, lipid and protein in the hepatopancreas and ovary of Penaeus setiferus and P. aztecus. Means with the same superscript did not differ significantly among maturation stages. Statistical analyses were performed on arcsine-transformed data, but means presented below are not transformed.

Maturation Stage	Hepatopancreas			Ovary		
	Carbohydrate	Lipid	Protein	Carbohydrate	Lipid	Protein
			Penaeus setiferus*			
1	3.29^{a}	35.05^{a}	16.65^{a}	3.21^{a}	9.50^{ab}	27.48^{ab}
2	4.08^{a}	20.49^{b}	20.52^{a}	2.21^{b}	9.20^{a}	21.71^{b}
3	4.55^{b}	16.51^{c}	22.59^{a}	2.15^{b}	14.87^{b}	28.08^{a}
4	4.57^{b}	16.26^{c}	22.28^{a}	2.37^{c}	17.60^{c}	27.60^{ab}
5	4.34^{ab}	15.01^{c}	23.70^{a}	2.20^{b}	8.77^{a}	24.91^{ab}
			Penaeus aztecus*			
1	3.40^{ab}	28.15^{a}	18.26^{a}	2.16^{a}	28.03^{a}	8.48^{a}
2	3.20^{b}	27.53^{a}	21.05^{a}	2.22^{a}	23.00^{a}	9.84^{a}
3	3.44^{a}	24.95^{a}	23.04^{b}	2.25^{a}	25.23^{b}	14.86^{a}
4	3.50^{a}	23.90^{a}	23.04^{b}	2.81^{b}	27.30^{c}	19.06^{a}
5	3.35^{ab}	24.23^{a}	25.62^{b}	2.07^{a}	22.52^{a}	10.29^{a}

* These data are limited to the reproductive location (Blocks B and C for P. setiferus and Block A for P. aztecus), season (May-September for P. setiferus and July-October for P. aztecus) and size (>160 mm TL) for each species.

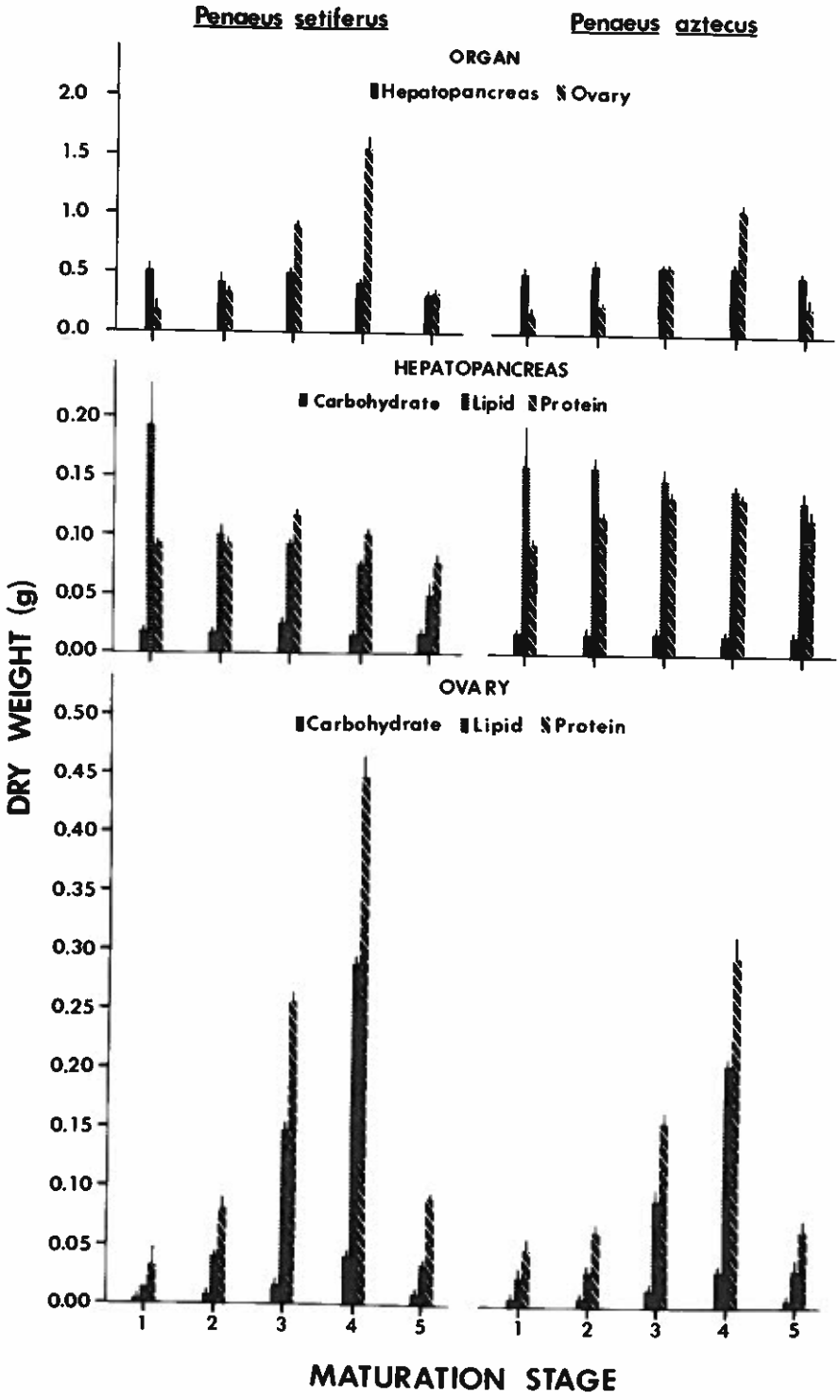


Figure 26. Organ weight and carbohydrate, lipid and protein content of ovary and hepatopancreas of Penaeus setiferus and P. aztecus females by ovarian maturation stage. Vertical bars represent standard error.

The percentage composition of protein in the ovary did not change significantly among maturation stages in P. aztecus and increased only from Stage 2 to Stage 3 in P. setiferus (Table 8). The percentage composition of carbohydrate in the ovary increased significantly from Stage 3 to Stage 4 and decreased significantly from Stage 4 to Stage 5 in both species. The percentage composition of lipid in the ovary increased significantly from Stage 2 to Stage 3 to Stage 4 and then decreased significantly from Stage 4 to Stage 5 in both species.

DISCUSSION

Maturation Stages

The gross external and dissected appearance of the ovary in Stages 1, 2, 3 and 5 of P. setiferus were similar to those of P. aztecus with the exception of differences in pigmentation on the dorsal surface of the ovary. The most marked interspecific difference was in the color of the ovary at Stage 4. The ovary color of P. aztecus at Stage 4 ranged from blue-green to yellow-green, while that of P. setiferus ranged from dark yellow to yellow-orange. These descriptions match those of Cook and Murphy (1969), Brown and Patlan (1974) and Aquacop (1975) for P. aztecus and agree closely with those of King (1948), Heegaard (1953) and Cook and Murphy (1969) for P. setiferus. However, Cook and Murphy (1969) described an olive or green tint in ripe ovaries of P. setiferus that neither we nor Burkenroad and Gunter observed (see Heegard, 1953; editorial note, p. 79). The reported green coloration may refer to (1) the pigmentation on the dorsal surface of the ovary and not to the ovary per se; (2) to the color of the ovaries in the process of resorption; or (3) to ovaries of dead shrimp. (We have observed that shrimp ovaries occasionally turn green after death.) It is also possible that differences in ovary coloration may result from dietary differences of the females colected at different locations in different years. The darkest ovary colors (Tables 2 and 3) in both P. setiferus and P. aztecus were associated with the largest standardized ovary weights and with the most advanced maturation stage.

Reproductive Performance

Little information has been published concerning mating and spawning of P. setiferus and P. aztecus. Heegaard (1953) was unsuccessful in obtaining viable eggs from mated P. setiferus. Johnson and Fielding (1956) reported spawning of P. setiferus in ponds, but he did not estimate percent of females spawning, fecundity or hatching rate. Brown et al. (1979) and Lawrence et al. (1980) induced maturation and spawning of P. setiferus in captivity, but Brown et al. (1979) did not obtain viable eggs, and neither monitored individual fecundity and hatching rate. Aquacop (1975) achieved spawning of unilaterally eyestalk-ablated P. aztecus in captivity and reported poor fecundity and hatching rate. Biologists at the Galveston Laboratory of the National Marine Fisheries Service have published numerous papers on rearing of postlarvae from eggs of wild P. setiferus and P. aztecus (Cook and Murphy, 1966, 1969, 1971; Cook, 1969; Brown, 1971; Mock and Murphy, 1972; Mock and Neal, 1974). However, only Mock (1972) reported fecundity and hatching rates -- and his data were limited to only three P. aztecus females.

Biologists at Marifarms, Inc., a commercial shrimp farm near Panama City, Florida, have extensively studied the availability of mated P. setiferus as a source of fertile eggs for their culture operation (Howell and Akamine, pers. comm.). Their data indicated peak abundance of mated females near Apalachicola Bay, Florida, from April through September, from three hours before sundown until one hour after sundown. Parker (1977) collaborated with Marifarms, Inc., and found their time frame effective for collecting mated P. setiferus off Port Aransas, Texas. The present study confirms these results. Mated P. setiferus were collected from May through September with a diurnal peak one to two hours before sundown.

Persyn (1977) noted that 11 to 50 percent of the ripe P. setiferus collected off the coast of northwest Florida near Apalachicola Bay were mated. Howell and Akamine (pers. comm.) indicated that 25 percent of the 2,795

ripe P. setiferus collected during 1980 were mated. In the present study, approximately 26 percent of the Stage 4 P. setiferus collected within two hours of sunset had mated. These estimates of natural mating frequency may be conservative because some females probably lost their spermatophores during capture. Also, the sperm mass of some mated females in advanced prespawning stages (when the geminate body of the spermatophore becomes disengaged from the female (Perez-Farfante, 1975)) is difficult to detect and, at times, may have escaped notice. For example, Howell and Akamine (pers. comm.) and Brown (pers. comm.) have recorded fertile spawns of P. setiferus females that had no visible spermatophore or remnant when removed from the trawl. The percentage of Stage 4 P. setiferus capable of viable spawns has been estimated by artificially inseminating all Stage 4 females with no spermatophore and no visible sperm mass immediatly after capture (Bray et al., 1982). Most of the inseminated females spawned at least a few viable eggs, 32 percent spawned with four percent viable eggs; 27 percent spawned with 10 percent viable eggs; and 11 percent spawned with 50 percent viable eggs. Thus, at least 10 percent of the apparently unmated, ripe females were capable of yielding hatching rates equivalent to mated females.

Almost all P. aztecus females of reproductive size appeared to be mated regardless of season or maturation stage (Burkenroad, 1939). Females with a soft exoskeleton, indicating a recent molt, typically still displayed a portion of the freshly attached spermatophore externally. Thus, P. aztecus followed the pattern of other closed-thelycum penaeids, such as P. japonicus, of which 99.5 percent of the soft-shelled females (n = 1,424) were impregnated (Hudinaga, 1942), and P. indicus, of which 95.4 percent of the females were impregnated (Emmerson, 1980).

Parker (1977) indicated that 65 percent of the 108 mated P. setiferus that he collected yielded complete, viable spawns that averaged 222,500 eggs with a 55 percent hatching rate. Bray et al. (1982) reported that three of the four mated females he collected yielded spawns that averaged 454,000 eggs with a 50 percent hatching rate. In the present study, 77 percent of the 13 mated P. setiferus yielded complete spawns that averaged 257,000 eggs with a 55 percent hatching rate. Fecundity was logarithmically related to total length of females. Similar logarithmic relationships between fecundity and total length have been reported for P. semisulcatus, Metapenaeus stebbingi, Trachypenaeus granulosus (Badawi, 1975) and Metapenaeus monoceros (Nalini, 1976).

Mock (1972) reported that three wild P. aztecus females, averaging 191 mm total length, spawned an average of 231,000 eggs (range: 71,000-380,000) with an average hatching rate of 12.8 percent (range: 0.5-35.7 percent). Howell and Akamine (pers. comm.) spawned two ripe P. aztecus females averaging 150,000 eggs per spawn with a 67 percent hatching rate. Aquacop (1975) induced small P. aztecus (15-20 g) to mature and spawn in captivity using unilateral eyestalk ablation and reported spawns of only 10,000 to 20,000 eggs, with hatching rates between 0 and 50 percent. These spawns took place when the ovaries were poorly developed, and many of the eggs were abnormally developed. We suspect that a large percentage of the Stage 4 P. aztecus that we placed in spawning tanks were probably induced to spawn prematurely due to handling and stress and the increase in temperature between water in their bottom habitat and the surface water used to fill the spawning tanks. Thus the 12 spawns of 2,000-60,000 eggs with an average hatching rate of 55 percent were considered abortions, while the five spawns of 116,000-206,000 eggs (mean: 167,800) with an average hatching rate of 77.6 percent were considered "normal."

The mean fecundity estimate for P. aztecus (167,000 eggs per spawn) in this study was 35 percent lower than that for P. setiferus (257,000 eggs per spawn). The lower fecundity of P. aztecus relative to P. setiferus might be explained by the 33 percent smaller standardized ovary dry weight in P. aztecus at Stage 4 than P. setiferus. However, the egg diameter in P. aztecus (0.26 mm, Cook and Murphy, 1971) is smaller than that of P. setiferus (0.28 mm, Heegaard, 1953), which may result in more eggs per gram of ovary for P. aztecus than P. setiferus.

Organ Weight and Biochemical Composition

Size at First Maturity

Total lengths for females at onset of maturation and at first maturity were estimated to be 150-160 mm and 160-170 mm, respectively, for P. setiferus and 140-150 mm and 160-170 mm, respectively, for P. aztecus. These estimates represent mean -- not minimum -- lengths. Some individuals would be expected to mature at sizes smaller or larger than these means. For example, eight percent of P. setiferus and 29 percent of P. aztecus that spawned on board were smaller than the length at first maturity. However, none of these females yielded normal spawns, although all were larger than the length at onset of maturation (Table 4). Renfro and Brusher (1964) stated that female P. aztecus begin to develop ovaries at 90 mm and are sexually mature and capable of spawning at 140 mm. Broad (1965) interpreted the length frequency data of Lindner and Anderson (1956) and estimated that P. setiferus females reach maturity at 140 mm. Apparently, Broad (1965) selected 140 mm because it represents the approximate minimum length that Lindner and Anderson (1956) recorded for Stage 3 females. However, according to Figure 27 of Lindner and Anderson (1956), Stage 4 females were seldom found as small as 145 mm and generally measured at least 160 mm. Anderson and Lindner (1958) used the difference in length-weight curves between mature and immature P. setiferus to demonstrate that all shrimp larger than 190 mm total length were mature and that an increasing percentage were mature between 170 and 190 mm.

Total lengths for males at onset of maturation and at first maturity were estimated to be 150-160 mm and 170-130 mm, respectively, for P. setiferus and 110-120 mm and 150-160 mm, respectively, for P. aztecus. Burkenroad (1934) reported that P. setiferus males first develop ripe sperm at a carapace length of 25 mm (about 119 mm total length) but the spermatophores are not fully developed until the carapace length is 35 mm (about 165 mm total length). Eldred (1958) noted the presence of spermatozoa in the spermatophore of a 90-mm P. duorarum. No information was found on size at first maturity of P. aztecus males.

Ovary weight increased with increasing total length from the length at first maturity to the maximum length encountered. This agrees with spawning data, which indicated a logarithmic relationship between fecundity and total length. Male gonad weight appeared to level off at a maximum size despite further increases in total length. The asymptote occurred at 170-180 mm for P. setiferus and 150-160 mm for P. aztecus. This agrees with Burkenroad's (1934) statement that spermatophores of large mature male P. setiferus were no larger than those of smaller mature males.

Spatial Variations

The spawning grounds of P. setiferus and P. aztecus were readily distinguished by overwhelming species differences in abundance of mature animals between nearshore and deep blocks. Block A was a primary spawning area for P. aztecus, with virtually no P. setiferus, while Blocks B and C, the nearshore blocks, were primarily spawning grounds of P. setiferus. Only a small portion of mature P. aztecus were found in the deepest stations of Block C (Gallaway and Reitsema, 1981). Thus, the location of the brine diffuser in Block B incurred minimum impact on reproduction of P. aztecus.

Comparisons of P. setiferus populations between Blocks B and C indicate that abundance was three times greater at Block B (1,590 shrimp collected) than at Block C (436 shrimp collected), but the percentage of mature females in the catch was slightly greater at Block C (14 percent) than at Block B (9 percent). Also, Block C yielded eight mated females, compared to six from Block B. Thus Block C was approximately as productive a spawning area as Block B despite much less abundance of shrimp. Bryan and Cody (1975) found that the area near Block C (Pass Cavallo) was the most productive area between Yarborough Pass and High Island. No significant differences in gonad weight or biochemical composition were found in P. setiferus between Blocks B and C. However, Stage 4 females from Block B had significantly larger hepatopancreas weights than those from Block C. Also, hepatopancreas carbohydrate levels were greater in both male and female P. seti-

ferus from Block B than in those from Block C. This was considered to reflect primarily a different nutritional -- not reproductive -- state of shrimp between Blocks B and C, because hepatopancreas carbohydrate levels are more sensitive than lipid and protein to starvation and re-feeding (Heath and Barnes, 1970). Also, hepatopancreas carbohydrate levels are consistently low in penaeid shrimp (Lawrence et al., 1979b), crabs (Heath and Barnes, 1970) and crawfish (Armitage et al., 1972) and therefore are of dubious value as a significant metabolic reserve for reproduction.

Mature P. setiferus were most abundant in the shallowest (10-15 m) stations of Blocks B and C during the peak spawning months of May, June and July. In addition, all six mated P. setiferus from Block B were captured from the shallowest (10 m) station in Block B, and four of the eight mated P. setiferus from Block C were captured from the shallowest station (12 m) in Block C. This concurs with the conclusion of Bryan and Cody (1975) that the principal spawning depth for P. setiferus on the Texas coast is about 11 m.

During August and September the abundance of mature P. setiferus decreased sharply, and the few remaining animals moved to deeper (15-23 m) stations of Blocks B and C. This pattern of nearshore spawning during midsummer, followed by movement to deeper water has also been reported by Bryan and Cody (1975) for P. setiferus off the Texas coast.

Most reproductive activity of P. setiferus occurred in the depth range of 10-15 m. Unfortunately, no collections were made during March or April when Lindner and Anderson (1956) reported strong reproductive activity in 15- to 30-m depths off the coast of Louisiana. However, Bryan and Cody (1975) collected at depths of 7, 11, 15 and 22 m during the spring off the Texas coast and reported little reproductive activity at 22 m.

Seasonal Variations

Variations in standardized gonad dry weight indicated pronounced gonadal development of both male and female P. setiferus from May through September. The mean monthly standardized ovary dry weight reached a peak of 1.23 g (gonad index, 11.8) during May. Pillay and Nair (1971) noted that the mean monthly ovary index for Metapenaeus affinis reached a peak of 11.7 g during December. Lawrence et al. (1979b) calculated a mean gonad index of 5.10 for 52 P. setiferus females collected during June and July in the northwestern Gulf of Mexico. The corresponding mean gonad index for 216 P. setiferus females collected during this study was 8.5.

A regression model with sine and cosine functions of day-of-year of collection predicted a synchronous peak in gonad weight of both male and female P. setiferus during early June. Mated female P. setiferus were collected from May through September with peak abundance in May and July.

The abundance of mature P. setiferus based on external maturation stages of females indicated a peak at Block B during June and July and at Block C during May (Gallaway and Reitsema, 1981). Bottom tows with a Gulf V plankton sampler from June through September indicated peak abundance of nauplii and protozoeae during June and July (Gallaway and Reitsema, 1981). Thus, variations in gonad weight, mating frequency, abundance of mature females, and abundance of nauplii and protozoeae indicate a peak reproductive period of May through July for P. setiferus. Lindner and Anderson (1956) and Bryan and Cody (1975) examined the abundance of mature female P. setiferus and concluded that the peak spawning period was in June-July and June, respectively. Baxter and Renfro (1966) found that June was the month of peak migration of postlarval P. setiferus into Galveston Bay.

The seasonality of reproduction of P. aztecus was much less pronounced. Plots of standardized gonad dry weight indicated elevated gonadal development in some females for all months except February and March. The mean monthly standardized ovary dry weight reached a peak of 0.62 g (gonad index, 5.9) during October. A regression model with sine and cosine functions of day-of-year of collection predicted peak gonadal development in November. The abundance of mature females varied strongly from a low in February to a peak in July through October (Gallaway and Reitsema, 1981).

P. aztecus males showed only a slight depression in gonadal development in February

and March and a slight peak in September. Pillay and Nair (1971) also found a depressed cycle of male gonad development in Metapenaeus affinis. Sperm from males of this species, like P. aztecus, is inserted within the thelycum of the female. Thus, spermatophore size may be more limited in these species than in P. setiferus and other open-thelycum species. Plankton collections with a Gulf V bottom sampler from June through September yielded P. aztecus nauplii and protozoeae only during August and September. On-board spawning of P. aztecus was attempted only during August and September and yielded viable larvae in both months. Thus, the composite data indicate that the peak reproductive months for P. aztecus at Block A (35-44 m) were from August through October. This agrees well with Temple and Fischer's (1967) conclusion that larvae of P. aztecus are most abundant in the Gulf from September to November. Renfro and Brusher (1982) stated that spawning of P. aztecus at 46 m occurs throughout the year but that peak activity occurs from October through December and a smaller peak extends from March until May. The seasonal peak in reproductive activity varies from year to year and with location as a function of changes in environmental factors. Consequently, one should be cautious in applying conclusions reached in this study to different locations or future years.

Variations with Ovarian Maturation

During maturation, the standardized ovary dry weight of both P. setiferus and P. aztecus significantly increased between Stages 2 and 4 and decreased between Stages 4 and 5. A similar trend was reported for Parapenaeopsis hardwickii (Nagabhushanam and Kulkarni, 1979). However, this species reached a mean ovary index (wet weight) of only 5.9 at Stage 4. In the present study, the mean ovary indices of P. setiferus and P. aztecus at Stage 4 were 15.1 and 10.1, respectively.

The percentage composition of ovarian carbohydrate, lipid and protein in P. setiferus varied similarly among maturation stages to that of P. aztecus. The percent protein remained relatively constant, while percent lipid increased significantly between Stages 2 and 4 and then decreased between Stages 4 and 5 in both species. Kulkarni and Nagabhushanam (1979) found the same general trends of increase in percentage composition of lipid and glycogen (the major component of total carbohydrate), but they also noted a decrease in percentage composition of protein between Stages 4 and 5, which was not observed in the present study.

P. setiferus and P. aztecus differed in relative composition of protein and lipid in the ovary. In P. setiferus, mean percent ovarian protein in each maturation stage (22-28 percent) exceeded percent ovarian lipid (9-18 percent), while the reverse occurred in P. aztecus (protein, 8-19 percent; lipid, 23-28 perecent). Carbohydrate levels (two to three percent) were similar in both species. Gehring (1974) reported a range of 4.2 to 7.7 percent (wet weight) ovarian lipid composition (equivalent to about 16.1 to 29.6 percent dry weight, assuming a 26 percent dry weight of the ovary) among maturation stages of P. duorarum. This range agrees well with that obtained for P. aztecus in the present study.

However, Gehring reported a significant decrease rather than an increase in percent lipid between Stages 3 and 4. This might be attributed to ovary resorption, because the females used by Gehring were collected offshore and transported live to a laboratory for dissection rather than being dissected immediately after collection. Farmer (1974) observed that ovary resorption in the Norway lobster, Nephrops norwegicus, turned the muscles of the animal a brilliant green. We have observed a yellow-orange color of the abdominal muscles of Stage 4 P. setiferus 5 to 10 hours after collection offshore.

Lawrence et al. (1979b) analyzed the protein, lipid and carbohydrate composition (percent dry weight) of spawned eggs of P. setiferus (42, 20 and 3 percent, respectively) and P. stylirostris (37, 16 and 3 percent, respectively) and found consistently greater protein levels than those obtained for P. setiferus and P. aztecus in this study. Lipid levels for each of the three species that Lawrence et al. (1979b) analyzed are similar to those of P. setiferus but less than those of P. aztecus in the present study. Carbohydrate levels were similar

among all four species encompassed by both studies.

The similarity in biochemical composition of P. setiferus, P. stylirostris and P. vannamei may be a reflection of their taxonomic relationship as members of the open-thelycum subgenus Litopenaeus, while P. aztecus and P. duorarum are members of the closed-thelycum subgenus Farfantepenaeus. The ovarian composition of Parapenaeopsis hardwickii (protein, 3-6 percent; lipid, 3-5 percent; glycogen, 23-32 percent) was lower in protein and lipid and higher in carbohydrate (glycogen) than any of the species discussed above (Nagabhushanam and Kulkarni, 1979). These differences may be due in part to differences in diet. Colvin (1976) reported that the percent dry weight lipid composition of whole P. indicus increased from four to six percent after the animals were fed diets supplemented with various seed oils. Bottino et al. (1980) found that diet greatly influences fatty acid patterns in shrimp. Undoubtedly, some of the differences noted above are also attributable to interspecific differences and analytical techniques. Unfortunately, insufficient data are available to differentiate these effects because the above studies represent the only published literature on protein, lipid and carbohydrate composition of ovaries and eggs of penaeid shrimp. Pandian (1967) reported percent lipid and protein (dry weight) of Crangon crangon eggs to be 59 and 33 percent, respectively. Lipid content of eggs of Palaemon serratus varied from 30 percent at the beginning of embryonic development to 16.7 percent at the end (Martin, 1978). Carbohydrate, lipid and protein composition (dry weight) of eggs were 5, 44 and 47 percent, respectively for the European lobster Homarus gammarus (Pandian, 1970), and 4, 49 and 42 percent, respectively, for the isopod Ligia oceanica (Pandian, 1972). Lipid levels of these eggs were significantly greater than those given above for penaeid ovaries and eggs. However, considering the different analytical techniques used, the protein and carbohydrate levels are comparable to those in the present study and to those of Lawrence et al. (1979b) but differ significantly from those reported by Nagabhushanam and Kulkarni (1979).

The standardized dry weight of the hepatopancreas did not change significantly from Stage 1 to Stage 4 in either P. setiferus or P. aztecus. However, percent lipid decreased during these stages (although not significantly in P. aztecus), while percent carbohydrate and protein increased. From Stage 4 to Stage 5, standardized dry weight of the hepatopancreas decreased in both species. Collatz (1969) reported a decline in hepatopancreas lipid of Orconectes limosus during ovarian development and suggested that the lipid was transported to the ovary. Armitage et al. (1972) hypothesized that lipid transfer from the hepatopancreas to the ovary also occurred in O. nais. Lipid, glycogen and protein levels decrease markedly in the hepatopancreas of Parapenaeopsis hardwickii during ovarian maturation, when they also increase in the ovary (Nagabhushanam and Kulkarni, 1979).

In the present study also, dramatic increases in carbohydrate, lipid and protein content of the ovary between Stages 3 and 4 coincided with decreases in these components in the hepatopancreas. The decline in hepatopancreas lipid during maturation was particularly apparent in females as they reached and exceeded the size at first maturity (Fig. 22). The importance of the hepatopancreas as a reserve for ovarian nutrients is also suggested by the larger hepatopancreas dry weight of females than males of similar size (Figures 22 and 23). However, the magnitude of increase in carbohydrate, lipid and protein in the ovary between Stages 3 and 4 was much greater than the magnitude of decrease in the hepatopancreas. Decreases in hepatopancreas carbohydrate, lipid and protein content between Stages 3 and 4 amounted to only 13.5, 9.4 and 7.9 percent, respectively, of the corresponding increases in ovary content of P. setiferus and 1.0, 11.0 and 0.3 percent, respectively, of the corresponding increases in ovary content of P. aztecus. Thus, both P. setiferus and P. aztecus probably depend on immediate food intake for most of the nutrients required for vitellogenesis. Lipid appeared to be the most significant of the hepatopancreas constituents used by P. aztecus during ovarian maturation, while carbohydrate, protein and

lipid appeared to function as organic reserves in P. setiferus. However, the role of carbohydrate as a significant reserve is questionable because of the typically low levels reported for crustaceans (Armitage et al., 1972; Pandian, 1967, 1970, 1972; Lawrence et al., 1979).

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