

Abstract.—Two species of *Loligo* are abundant in northern Gulf waters: the long-finned squid, *Loligo pealei*, and the arrow squid, *Loligo plei*. Variability within species and similarities between the species often hamper accurate identification. The two species more closely resemble each other in areas of sympatry, and there is overlap in almost all of the diagnostic characters. Small specimens of *Loligo* are not easily identified, and there are few studies detailing their morphometry. Because of the taxonomic uncertainties associated with the identification of juveniles and subadults of *L. pealei* and *L. plei*, the species were differentiated by isoelectric focusing, and morphometric characters and indices of potential use in species separation were evaluated. Emphasis was placed on those taxonomic characters suitable for use in the field. Best discrimination between the two species was achieved with combinations of measurements of characters and calculated indices associated with cartilaginous structures (funnel cartilage length, gladius width [GW], nuchal cartilage length, and rachis width [RW]) and the shape of the gladius. An arbitrary cutoff in GW/RW ratio of 2.7 (ratio of the greatest width of the gladius to the width of the free rachis at the junction of the vane) correctly classified 100% of the *L. plei* and 91% of the *L. pealei*. The overall shape of the gladius (broader and more rounded in *L. pealei*), presence or absence of marginal ribs in the vane of the gladius, and the nature of the junction of the vane and free rachis (junction gradual and not distinct in *L. pealei*) were also useful in distinguishing the two species.

Morphometry of juvenile and subadult *Loligo pealei* and *L. plei* from the northern Gulf of Mexico

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Although squid are conspicuous components of the marine ecosystem and form a substantial portion of the invertebrate biomass in the northern Gulf of Mexico, many aspects of their life history and structural characteristics are poorly known. Three species of loliginid squid are abundant in northern Gulf waters (Rathjen et al., 1979): the brief squid, *Lolliguncula brevis* (Blainville 1823), the long-finned squid, *Loligo pealei* Lesueur 1821 (also known as the longfin inshore squid [Roper et al., 1984]), and the arrow squid, *Loligo plei* Blainville 1823. The lack of knowledge about the species of *Loligo* in the Gulf can, in part, be attributed to the difficulties in identifying juveniles. Recent interest in locating new or underutilized squid species, or both, and the recognition of the importance of documenting changes in marine biodiversity have

increased emphasis on the need to improve taxonomic resolution in this important invertebrate group.

Although the adults of *L. pealei* and *L. plei* have been described by numerous authors (e.g. Haefner, 1964; LaRoe, 1967; Cohen, 1976; Roper et al., 1984), variability within species and similarities between the species often hamper precise identification. Species variability has been documented in the long-finned squid, *L. pealei*. Cohen (1976) noted that populations of long-finned squid varied widely both within and between geographic areas. In areas of sympatry with *L. plei*, the two species more closely resemble each other, and with the exception of gladius shape and the hectocotylus in adult males, there

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is overlap in all other diagnostic characters (Cohen, 1976). This problem is particularly acute in the northern Gulf of Mexico, where the two species are common. Hixon (1980) noted that all external characters and morphometric indices overlapped in immature specimens taken in waters off Texas. Difficulties in separating the species were best summarized by LaRoe (1967) who stated, "there are differences between the species, but there is a continuum between them in their range of variation for most characters; there appear to be no distinct boundaries to separate the two species."

The purpose of this study is to examine variability in morphological characteristics between juveniles and subadults of *L. pealei* and *L. plei* in the northern Gulf of Mexico and to determine diagnostic criteria for their identification. Emphasis is placed on taxonomic characters suitable for use in the field. Because of the taxonomic uncertainties associated with identification of immature and small specimens of the two *Loligo* species, an electrophoretic technique (isoelectric focusing) was used to establish species-specific protein patterns. Biochemical differentiation of species provided more accurate assessment of morphological variability between the two species. This contrasts with previous studies in which mor-

phological variability was assessed by using specimens identified by morphological criteria.

Materials and methods

Field procedures

Squid samples were collected by personnel of the National Marine Fisheries Service, Mississippi Laboratories, Pascagoula, Mississippi, aboard the National Oceanic and Atmospheric Administration Ship RV *Oregon II*. Samples used here were obtained during the Southeast Area Monitoring and Assessment Program (SEAMAP) resource survey Cruise 93-03 (205), conducted from 6 June to 21 July 1993. Otter-trawl samples ($n=230$) were taken in an area from 88° to 97°30'W (Mobile, Alabama to Brownsville, Texas) in depths from 9 to 110 meters (Fig. 1). Station selection and field processing procedures followed established SEAMAP protocol.¹ All specimens of *Loligo*

¹ Nichols, S., and G. Pellegrin. 1989. Trends in catch per unit effort for 157 taxa caught in the Gulf of Mexico Fall Groundfish Survey, 1972-88. Summary Rep., Mississippi Laboratories, NMFS, NOAA, 158 p.

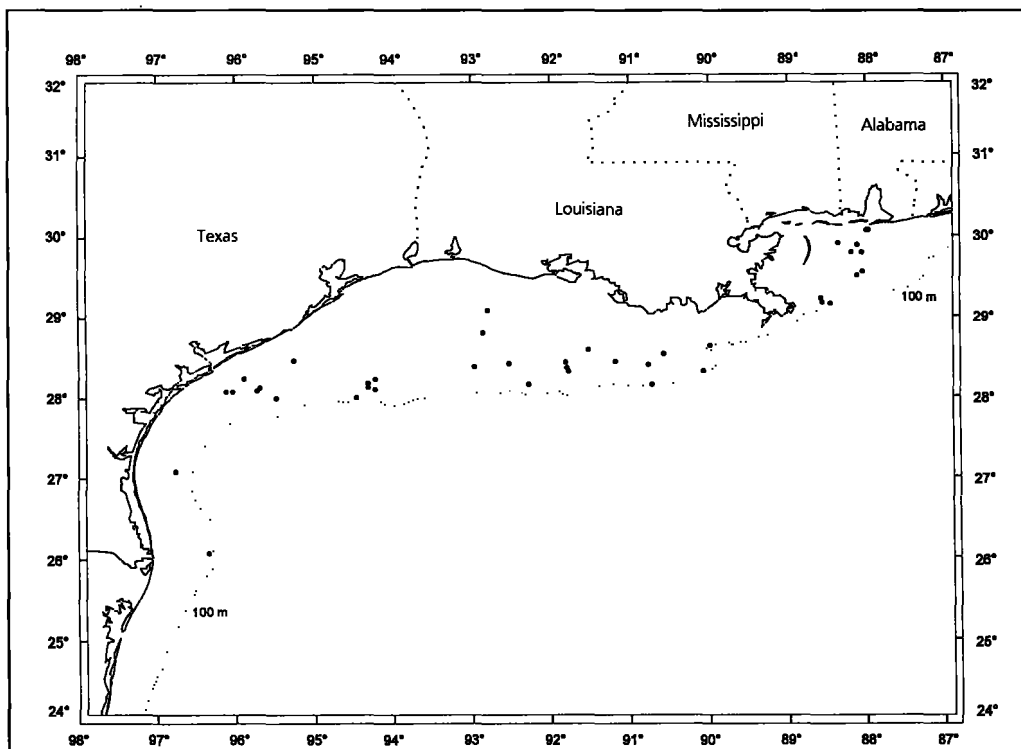


Figure 1

Location of trawl stations in the northern Gulf of Mexico where *Loligo pealei* and *Loligo plei* were collected.

were sorted from the trawl samples, counted, and weighed. They were frozen (-20°C) on board within two hours of capture to deter tissue degradation. Large, more readily identifiable squids were frozen separately for later use in biochemical determinations of species-specific protein patterns, whereas the smaller squids from each station were frozen together. All samples were sealed in polyethylene bags in which the air had been squeezed out to prevent freezer-burn during storage. The frozen squids were placed in chilled containers for transportation from the ship to the Gulf Coast Research Laboratory (GCRL), Ocean Springs, Mississippi, following completion of the cruise. Squids were stored at -20°C in the laboratory.

Laboratory procedures

Initial research efforts centered on establishing nonvariant protein patterns that could be used to separate the two *Loligo* species. Ten large specimens of each *Loligo* species (greater than 200-mm mantle length) were selected from the frozen samples and tentatively identified at GCRL with the morphological criteria provided by Bane and Wagner,² Voss (1956), LaRoe (1967), Cohen (1976), and Roper et al. (1984). These criteria included shape of the hectocotylus in males, overall body shape, the size and shape of the fins, and the dentition of the tentacular suckers. A sample of mantle tissue was removed from each squid, refrozen, and stored at -70°C . Frozen squids were shipped on dry ice to the National Museum of Natural History, Washington, D.C., for confirmation of field identifications. Following verification of identification of specimens based on morphological criteria, mantle tissue from each individual was analyzed electrophoretically and diagnostic protein bands were determined.

To evaluate morphometric parameters of potential value in field separation of *L. plei* and *L. pealei*, specimens of juvenile and subadult *Loligo* spp. ($n=304$) ranging in size from 29.0 to 238.0 mm mantle length (ML) were selected from the squid samples frozen in the field. Squids were selected from each of three geographic areas over the complete range of depths sampled (less than 18 m, 18–36 m, 37–55 m, 56–73 m, 74–110 m) within each area. Designation of geographic areas followed Nichols and Pellegrin¹: Texas (Brownsville to Galveston), West Delta (Galveston, TX, to the Mississippi River), and East Delta (Mississippi River to Mobile, AL). Juvenile and subadult squids were selected from 41 stations without regard

to probable species or sex. Specimens were thawed, individually numbered, and weighed to the nearest 0.1 g. Visual inspection for the presence of nidamental glands or the spermatophoric sac was used to separate females from males.

The diagnostic measurements and indices used by Voss (1956), Haefner (1964), Cohen (1976), and Hixon (1980) were reviewed for their potential usefulness in taxonomic resolution of the *Loligo* species. Eleven morphometric measurements and selected indices (ratios of measured characters) of potential value in separating species and sex within a species were determined (Table 1; Fig. 2).

Morphometric characters were measured with digital calipers to the nearest 0.1 mm. Because some individuals were damaged, the number of measured characters per individual varied. Squids >100.0 mm ML were not well represented in the sample aliquot. Large males of both *L. pealei* and *L. plei* were included in the aliquot, but there were few females of similar size. For that reason, size limitations were placed on the data used in regression analyses, with

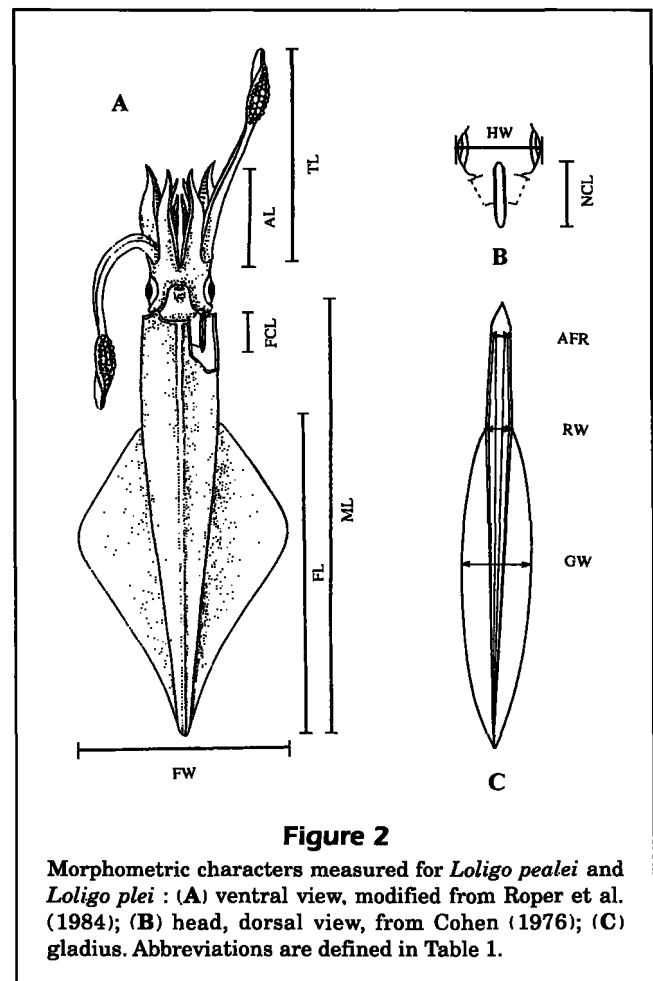


Figure 2

Morphometric characters measured for *Loligo pealei* and *Loligo plei*: (A) ventral view, modified from Roper et al. (1984); (B) head, dorsal view, from Cohen (1976); (C) gladius. Abbreviations are defined in Table 1.

² Bane, G. W., and A. Wagner. 1985. Key to important squids of the northern Gulf of Mexico. Coastal Fisheries Institute, Center for Wetland Resources, Louisiana State Univ., Baton Rouge, LA, LSU-CFI-85-21, 10 p.

the limits dependent upon the size-frequency distribution of the specimens. Size-class ranges for tests of sexual dimorphism were from 50.0 to 150.0 mm ML for *L. pealei* and from 40.0 to 110.0 mm ML for *L. plei*. When characters and indices were compared between the two species, squids from 20.0 to 150.0 mm ML were used in the analyses. Both individual characters and indices were regressed against mantle length to determine their potential value in differentiating species, or sex within a species, for squids of a given size.

After external measurements were recorded for each squid, the mantle was opened and the gladius removed. Drawings of the gladii were made by means of photocopies of the actual gladii as patterns. A piece of mantle tissue was taken from each squid, frozen immediately, and stored at -70°C . Specimens were refrozen and stored at -20°C .

Isoelectric focusing (IEF), a technique which separates proteins according to their isoelectric points along a continuous pH gradient (Righetti et al., 1990),

was used to characterize mantle protein patterns. Mantle tissue was minced and then homogenized in BSS buffer (0.05 M Tris at pH 7.5, 0.25 M sucrose, 0.01 M KCl, 0.01 M MgCl_2 , 0.001 M CaCl_2 , 0.001 M phenylmethylsulfonyl fluoride) with an Ultra-TurexTM (Type TP 18/10 S1) homogenizer. The homogenate from each squid was centrifuged separately at 14,000 RPM (23,460 RCF) for 20 minutes at 4°C with a BeckmanTM Model J-21C refrigerated centrifuge. The supernatants were recovered and frozen separately at -70°C . Total protein in each supernate was measured (Bio-RadTM Bradford Protein Assay Kit) in order that the same amount of protein (35 μg) was loaded in each lane of the gel.

Isoelectric focusing separations were made in 0.5-mm polyacrylamide gels with an LKB UltraphorTM apparatus. Proteins were separated with ampholytes of the 4.0–6.5 pH range by using 0.1 M glutamic acid as the analyte and 0.1 M beta-alanine as the catholyte. The gels were prefocused at 2,000 V for 30

Table 1
Morphometric characters and indices in *Loligo pealei* and *Loligo plei*.

Parameter	Definition ¹
Measured characters	
AFR (anterior free rachis)	Width of free rachis at terminus of marginal ribs
AL (arm length)	Length of right arm IV from proximalmost sucker to arm tip (left if right arm missing)
FCL (funnel cartilage length)	Greatest length of entire right funnel cartilage
FL (fin length)	Fin length from point on dorsal mantle midway between anterior fin insertion, to posterior body tip
FW (fin width)	Greatest width of both fins between lateralmost points
GW (gladius width)	Greatest width of gladius vane
HW (head width)	Greatest head width at level of eyes (head strongly ventrodorsally compressed between two fingers)
ML (mantle length)	Mantle length, from dorsal anterior margin to posterior end
NCL (nuchal cartilage length)	Greatest length of entire nuchal cartilage
RW (rachis width)	Width of gladius rachis at junction of vane
TL (tentacle length)	Length of right (left if right tentacle missing) tentacle from junction of arms III and IV to tip
W (weight)	Specimen wet weight to the nearest 0.1 g
Morphometric indices	
AL/ML	Arm length: mantle length index
FCL/ML	Funnel cartilage length: mantle length index
FL/ML	Fin length: mantle length index
FW/FL	Fin width: fin length index
FW/ML	Fin width: mantle length index
GW/ML	Gladius width: mantle length index
GW/RW	Gladius width: rachis width index
HW/ML	Head width: mantle length index
NCL/FCL	Nuchal cartilage length: funnel cartilage length index
NCL/ML	Nuchal cartilage length: mantle length index
RW/ML	Rachis width: mantle length index
TL/AL	Tentacle length: arm length index
TL/ML	Tentacle length: mantle length index
W/ML	Wet weight: mantle length index

¹ Modified from Cohen (1976).

minutes. The samples were then loaded and focused for 3 hours at 2,000 V. The gels were fixed, washed with destaining solution, and stained with Coomassie Blue R-250. They were washed again in destaining solution, dried, and photographed. Protein bands from the juvenile and subadult squids were compared with protein bands from the positively identified adult specimens for species identification.

Statistical analysis of data

Two approaches were used in morphometric analysis of data: comparisons were made between species and by sex within each species. All data were analyzed with Statgraphics Plus, Version 7.1 computer software. Student's *t*-test ($\alpha=0.05$) was used to compare the means of indices to determine if any index could be used to distinguish between the two species or between sexes within a species. Both linear and multiplicative regression techniques were employed to examine the relation of each measured character and each index (dependent variable) to mantle length (independent variable). Correlation coefficients (*r*) and coefficients of determination (r^2) were calculated for each regression to measure strength of the relationship and goodness of fit of the calculated regression line, respectively.

Coefficients of determination were considered high when 85% of the total observed variability was accounted for by the mathematical model. Tests were conducted for differences in the slopes of the regression lines. The null hypothesis was that there was no significant difference ($\alpha>0.05$) between the slopes. A discriminant analysis was employed to identify the relative contribution of measured characters or indices, or both, in discriminating between the two species. For each individual, the characters and indices were entered with their respective species classification codes. Analyses were performed as follows: 1) all characters, 2) all indices, and 3) selected characters or indices, or both (singly or in combination), based on discriminant function coefficients. From these analyses, characters or indices (or both), were ranked by comparing their percentage success in species differentiation.

Results

Biochemical analyses

Isoelectric focusing patterns of mantle tissue proteins are shown in Figure 3. Proteins used to differentiate between the species were labeled *a* through *f* according to their decreasing order of isoelectric points (Table 2). Both species had unique protein bands and

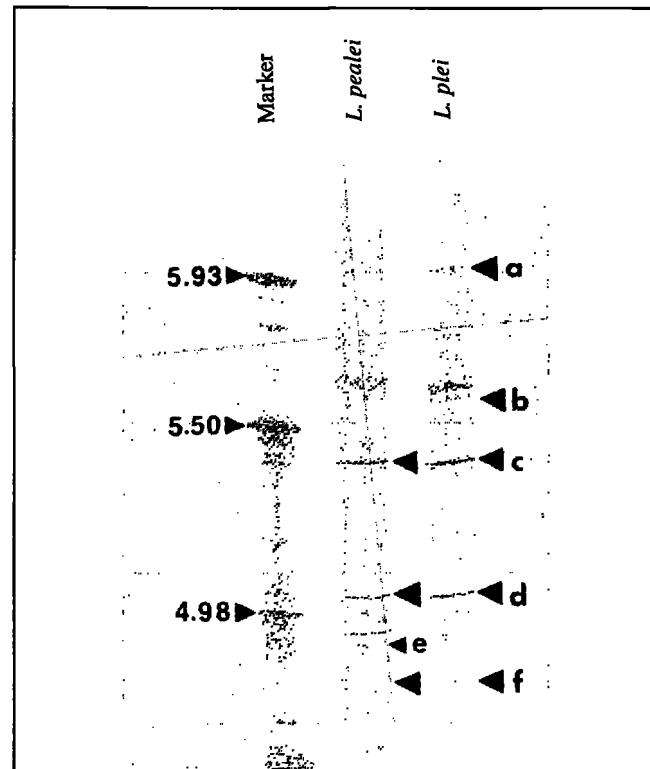


Figure 3

Protein electrophoretic patterns of mantle tissue from *Loligo pealei* and *Loligo plei* (numbers=pH, letters=protein bands).

Table 2

Isoelectric points (pH) used to separate *Loligo pealei* and *Loligo plei*.

Band	pH	Species
<i>a</i> ¹	5.97	<i>L. plei</i>
<i>b</i> ¹	5.60	<i>L. plei</i>
<i>c</i>	5.41	<i>L. plei</i> , <i>L. pealei</i>
<i>d</i>	5.02	<i>L. plei</i> , <i>L. pealei</i>
<i>e</i> ²	4.89	<i>L. pealei</i>
<i>f</i>	4.76	<i>L. plei</i> , <i>L. pealei</i>

¹ Unique to *L. plei*.

² Unique to *L. pealei*.

were easily separated: bands *a* and *b* were unique to *L. plei* and band *e* to *L. pealei*. Bands *c*, *d*, and *f* were shared by both species.

General description of juveniles and subadults

In fresh specimens, the chromatophores of the mantle generally are red in color in *L. plei* and brown in *L.*

pealei. Color, however, is not a reliable means of separating the species. Well-defined lines of chromatophores ("flame stripes") occur on the ventral surface of the mantle in large males of *L. plei*; however, specimens of *L. pealei* also may have a similar, though less defined, color pattern. The mantle is long, slender, and cylindrical in both *L. pealei* and *L. plei*, but the posterior end of the mantle is more acutely pointed in *L. plei*. Juvenile and subadult *L. pealei* are more robust than *L. plei* of similar mantle length: the head and eyes of the former species are moderately large and the arms and tentacles are thick, muscular, and long. The more robust character of *L. pealei* is particularly evident when compared with *L. plei* of the same mantle length. The fins are rhomboidal in shape in both species.

Fin length as a percentage of mantle length is variable in individuals of both species, but it is generally greater in *L. pealei* than in *L. plei* in specimens of similar mantle length (Table 3). Mean fin length in relation to mantle length of *L. pealei* was 50% in the size class 90.0 to 99.9 mm, and the mean ratio increased as mantle length increased. However, fin length to mantle length ratio of individual *L. pealei* could be 50% in specimens as small as 70.0 mm ML; the ratio of FL to ML was not consistently 50% or greater until the individual mantle length reached

110.0 mm. For *L. plei*, the ratio of fin length to mantle length was less than 50% for individual squid smaller than the 120.0 to 129.9 mm ML size class. Mean fin length to mantle length did not exceed 50% until size class 140.0 to 149.9 mm ML.

Morphometric analyses

Individual measurements were taken of 304 juvenile and subadult squids (*L. pealei*, $n=151$; *L. plei*, $n=153$). All specimens were immature according to the maturity classification scheme of Macy (1982). The relationships between fin length and mantle length, fin width and mantle length, and rachis width and mantle length were best represented by the linear model. The relationships between weight, head width, funnel cartilage length, nuchal cartilage length, gladius width, arm length, and tentacle length, with mantle length as the independent variable, were best represented by the multiplicative model. All indices were best represented by the linear model.

Sexual dimorphism

Loligo pealei Sex could be determined for only 36% of the specimens identified as *L. pealei* ($n=62$). Males

Table 3
Fin length in relation to mantle length in *Loligo pealei* and *Loligo plei*.

Mantle length (mm)	Fin length as % of mantle length							
	<i>L. pealei</i>				<i>L. plei</i>			
	<i>n</i>	Mean	±SD	Range	<i>n</i>	Mean	±SD	Range
20–29.9	0				1	20.7		
30–39.9	1	37.6			9	27.2	±3.3	18.1–29.5
40–49.9	27	37.6	±2.6	31.7–42.6	22	31.5	±3.1	26.3–37.6
50–59.9	47	40.6	±2.8	33.3–45.3	44	34.6	±2.3	26.9–38.7
60–69.9	16	43.2	±1.9	40.5–46.4	41	36.7	±2.1	33.1–40.3
70–79.9	9	46.2	±2.4	43.5–50.8	12	39.2	±2.0	35.8–42.9
80–89.9	7	47.0	±2.9	41.7–52.2	9	41.7	±1.3	39.9–43.9
90–99.9	13	50.0	±3.9	42.5–57.1	5	44.3	±2.9	40.0–47.5
100–109.9	5	50.4	±1.8	47.8–53.2	3	46.0	±2.1	43.5–48.7
110–119.9	4	52.0	±1.9	50.4–55.2	0			
120–129.9	8	53.4	±3.1	49.7–58.6	3	47.8	±1.3	46.6–49.6
130–139.9	4	55.8	±1.6	53.4–57.6	1	47.5		
140–149.9	3	54.9	±0.9	54.1–56.1	1	50.5		
150–159.9	1	54.3			1	51.7		
160–169.9	0				1	53.4		
170–179.9	2	56.2	±1.7	54.5–57.9	0			
180–189.9	0				0			
190–199.9	2	59.5	±0.5	59.0–60.0	0			
≥200	2	59.8	±1.5	58.3–61.3	0			

used in statistical analyses ranged in size from 50.0 to 147.0 mm and females from 64.0 to 140.0 mm ML. Results of statistical analyses of tests of sexual dimorphism for characters and indices are shown in Table 4.

Coefficients of determination (r^2) were high for both males and females for AL, FCL, FL, GW, NCL, RW, and W when regressed on mantle length. The slopes of the regression lines were similar between sexes for W and GW, but females were heavier than males (Fig. 4) and had a broader gladius (Fig. 5). There were also no significant differences between males and females in the slopes of the regression lines for AL, FCL, FL, NCL, and RW, and the 95% confidence intervals of the regression intercepts overlapped. Fin width was highly correlated with ML only in females. Overlap occurred in all measured characters.

Significant differences were found in the slopes of the regression lines only for W/ML. With the exception of W/ML in females, all indices had low coefficients of determination. Means of the indices of FW/ML, GW/ML, GW/RW, and RW/ML were significantly higher in females than in males; however, overlap was high.

Loligo plei Sex could be determined for 51% of the specimens identified as *L. plei* ($n=73$). Males used in statistical analyses ranged in size from 52.0 to 107.0 and females from 46.0 to 101.0 mm ML. Results of statistical analyses of tests of sexual dimorphism for characters and indices are shown in Table 5.

No significant differences were found between the sexes in the slopes of the regression lines for any of the characters. Weight (Fig. 6) and GW (Fig. 7) were highly correlated to ML in both males and females; however, there was no significant difference in the slopes of the regression lines, and the 95% confidence intervals of the regression intercepts overlapped. Females were heavier than males of similar length, and the gladius was broader in female specimens. Fin length and RW were also highly correlated with ML but, as with W and GW, there was no significant difference in the slopes of the regression lines and the 95% confidence intervals of the regression intercepts overlapped. Fin width, FCL, and NCL were highly correlated with mantle length only in males.

With the exception of FCL/ML in males, all indices had low coefficients of determination. There were significant differences in the means of FW/FL,

Table 4

Statistical tests of sexual dimorphism in *Loligo pealei* (sample sizes are given in parentheses). Abbreviations are defined in Table 1. NS = not significant.

	AL	FCL	FL	FW	GW		
Male (r^2)	0.85 (27)	0.92 (28)	0.97 (28)	0.81 (28)	0.95 (28)		
Female (r^2)	0.88 (18)	0.93 (19)	0.96 (19)	0.90 (19)	0.93 (19)		
Slope test	NS	NS	NS	NS	NS		
	HW	NCL	RW	TL	W		
Male (r^2)	0.62 (28)	0.95 (28)	0.90 (28)	0.50 (22)	0.96 (28)		
Female (r^2)	0.67 (19)	0.96 (19)	0.92 (19)	0.62 (15)	0.98 (19)		
Slope test	NS	NS	NS	NS	NS		
	AL/ML	FCL/ML	FL/ML	FW/FL	FW/ML	GW/ML	GW/RW
Male (r^2)	0.00 (27)	0.64 (28)	0.64 (28)	0.56 (28)	0.00 (28)	0.17 (28)	0.03 (28)
Female (r^2)	0.15 (18)	0.74 (19)	0.53 (19)	0.20 (19)	0.05 (19)	0.00 (19)	0.05 (19)
Slope test	NS	NS	NS	NS	NS	NS	NS
<i>t</i> -test of means	NS	NS	NS	NS	$P=0.0097$	$P<0.0001$	$P=0.0076$
Means (M, F)	0.58, 0.60	0.15, 0.16	0.50, 0.51	1.08, 1.14	0.53, 0.58	0.15, 0.17	2.99, 3.17
	HW/ML	NCL/FCL	NCL/ML	RW/ML	TL/AL	TL/ML	W/ML
Male (r^2)	0.50 (28)	0.49 (28)	0.14 (28)	0.33 (28)	0.21 (22)	0.21 (22)	0.87 (28)
Female (r^2)	0.41 (19)	0.29 (19)	0.56 (19)	0.08 (19)	0.09 (15)	0.25 (15)	0.95 (19)
Slope test	NS	NS	NS	NS	NS	NS	$P=0.0145$
<i>t</i> -test of means	NS	NS	NS	$P=0.0241$	NS	NS	NS
Means (M, F)	0.21, 0.22	1.22, 1.20	0.18, 0.19	0.050, 0.052 ¹	2.67, 2.68	1.55, 1.61	0.38, 0.41

¹ Difference between means is small but *t*-test is significant.

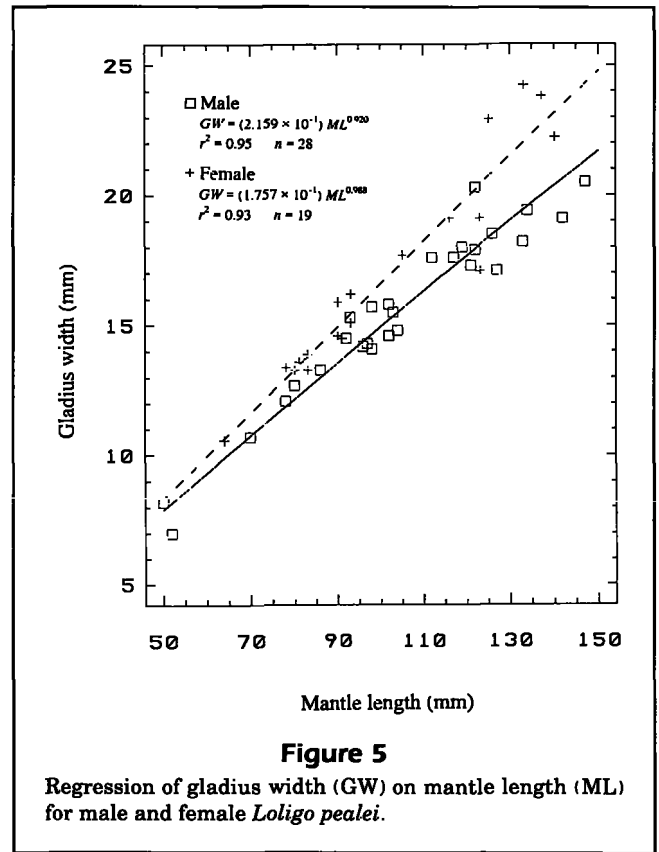
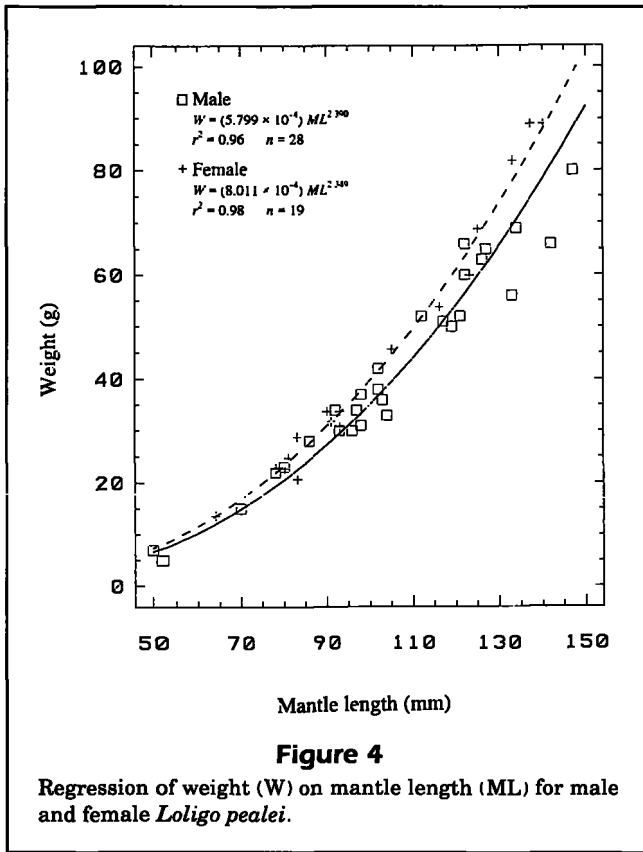
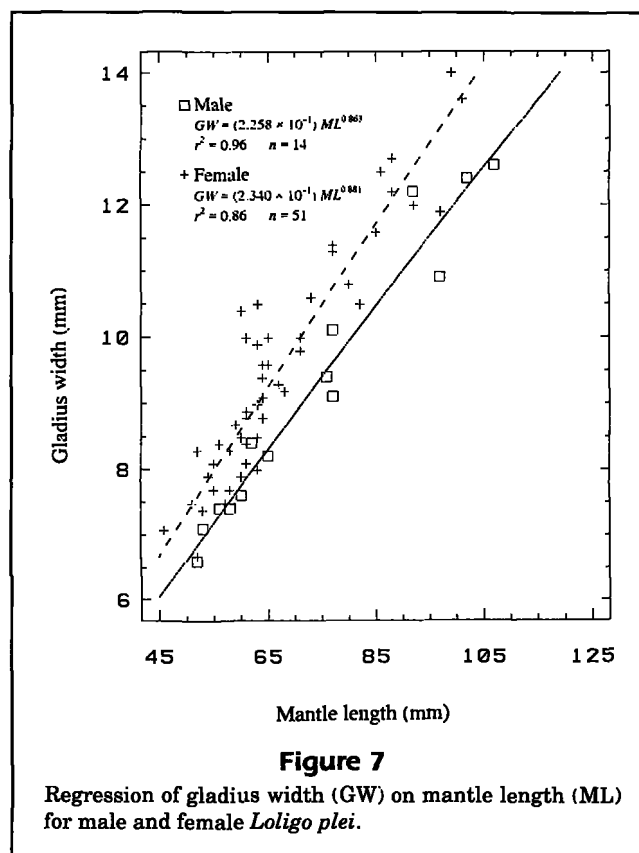
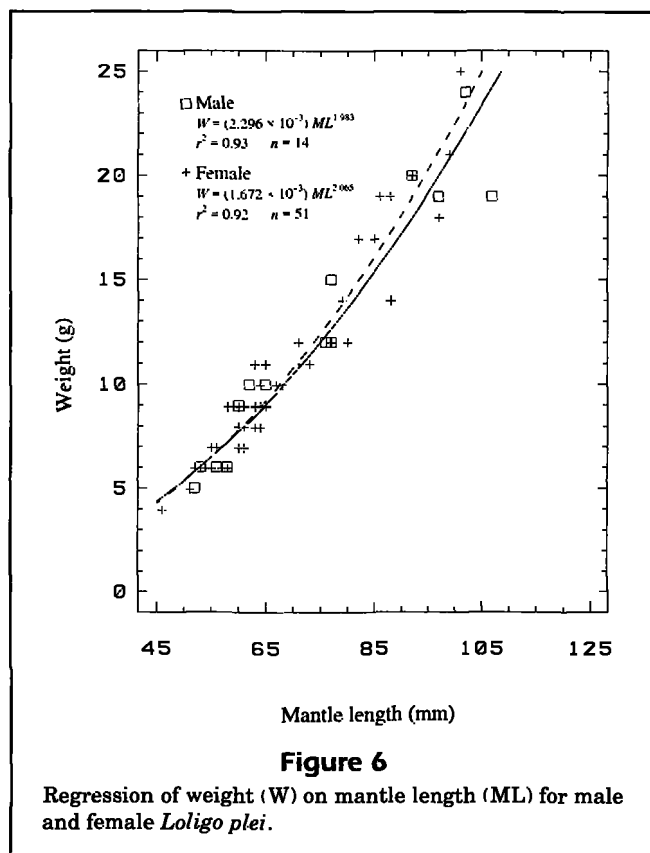


Table 5

Statistical tests of sexual dimorphism in *Loligo pealei* (sample sizes are given in parentheses). Abbreviations are defined in Table 1. NS = not significant.

	AL	FCL	FL	FW	GW		
Male (r^2)	0.73 (14)	0.88 (14)	0.97 (14)	0.90 (14)	0.96 (14)		
Female (r^2)	0.60 (51)	0.53 (51)	0.97 (51)	0.83 (50)	0.86 (50)		
Slope test	NS	NS	NS	NS	NS		
	HW	NCL	RW	TL	W		
Male (r^2)	0.59 (14)	0.91 (14)	0.90 (14)	0.39 (9)	0.93 (14)		
Female (r^2)	0.28 (51)	0.66 (51)	0.86 (50)	0.58 (36)	0.92 (51)		
Slope test	NS	NS	NS	NS	NS		
	AL/ML	FCL/ML	FL/ML	FW/FL	FW/ML	GW/ML	GW/RW
Male (r^2)	0.59 (14)	0.91 (14)	0.80 (14)	0.49 (14)	0.01 (14)	0.39 (14)	0.06 (14)
Female (r^2)	0.03 (51)	0.61 (51)	0.74 (51)	0.50 (50)	0.01 (50)	0.10 (50)	0.09 (50)
Slope test	NS	NS	NS	NS	NS	NS	NS
<i>t</i> -test of means	NS	NS	NS	$P=0.0322$	NS	$P<0.0001$	$P=0.0012$
Means (M, F)	0.50, 0.49	0.14, 0.14	0.39, 0.38	1.13, 1.22	0.44, 0.46	0.13, 0.14	2.08, 2.24
	HW/ML	NCL/FCL	NCL/ML	RW/ML	TL/AL	TL/ML	W/ML
Male (r^2)	0.69 (14)	0.51 (14)	0.61 (14)	0.41 (14)	0.19 (9)	0.54 (9)	0.73 (14)
Female (r^2)	0.45 (51)	0.21 (51)	0.50 (51)	0.36 (50)	0.00 (36)	0.10 (36)	0.77 (51)
Slope test	NS	NS	NS	NS	NS	NS	NS
<i>t</i> -test of means	NS	NS	NS	NS	NS	NS	NS
Means (M, F)	0.20, 0.20	1.17, 1.17	0.16, 0.17	0.06, 0.06	3.08, 3.18	1.52, 1.58	0.16, 0.15



GW/ML, and GW/RW, and means were higher for these indices in females. Overlap occurred in all indices.

Species comparisons

The specimens of *Loligo pealei* used in species comparisons ranged in size from 37.0 to 147.0 mm ML, whereas specimens of *L. plei* ranged in size from 29.0 to 148.0 mm ML. Results of statistical analyses of tests of species comparisons for characters and indices are shown in Table 6.

There were significant differences in the slopes of the regression lines for all characters except HW and RW. Variability in W was low in both species and overlap occurred in only the smaller size classes (Fig. 8). Coefficients of determination also were high for FL, FW, GW, NCL, and RW in both species; however, overlap occurred in all characters (Figs. 9–13, respectively). Arm length and FCL were highly correlated with mantle length only in *L. pealei*.

Significant differences occurred in the slopes of the regression lines for the following indices: AL/ML, FCL/ML, FL/ML, FW/FL, GW/ML, NCL/ML, RW/ML, TL/AL, and W/ML. With the exception of W/ML for *L. pealei*, the coefficients of determination were low. Although little of the variability was explained by the regression lines for GW/RW regressed on

mantle length, a scatter plot of the GW/RW ratios showed minimal overlap; thus this index may have predictive value in separating juveniles and subadults of the two species (Fig. 14). Significant differences were found between mean indices for all but FW/FL and NCL/FCL. Mean indices were greater in *L. pealei* for all ratios with the exception of RW/ML and TL/AL.

Overlap was high for all indices with the exception of GW/RW. Both the scatter plot (Fig. 14) and frequency histogram (Fig. 15) showed relatively low overlap in the GW/RW indices. The GW/RW index was higher for *L. pealei* in most instances. Only 9% of the *L. pealei* specimens were in the range of overlap, and this overlap was more common in smaller squid, including a distinct mode of small *L. pealei* within the GW/RW range of *L. plei* (Fig. 15).

Discriminant function coefficients were highest for cartilaginous structures. Results of discriminant analyses showed that no character, index, or combination of the two, classified *L. pealei* with 100% accuracy (Fig. 16; Table 7). Rachis width in combination with NCL enabled separation at an accuracy level of 93%, and in combination with FCL or GW separated 95% of the specimens of *L. pealei*. Indices that allowed separation of *L. pealei* from *L. plei* at accuracy levels of 92% and above included the GW/RW

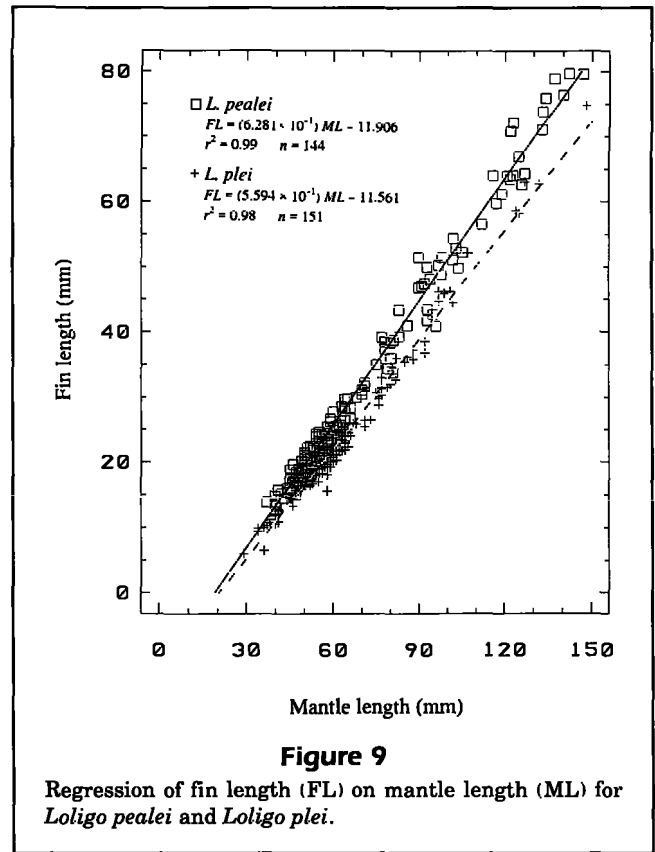
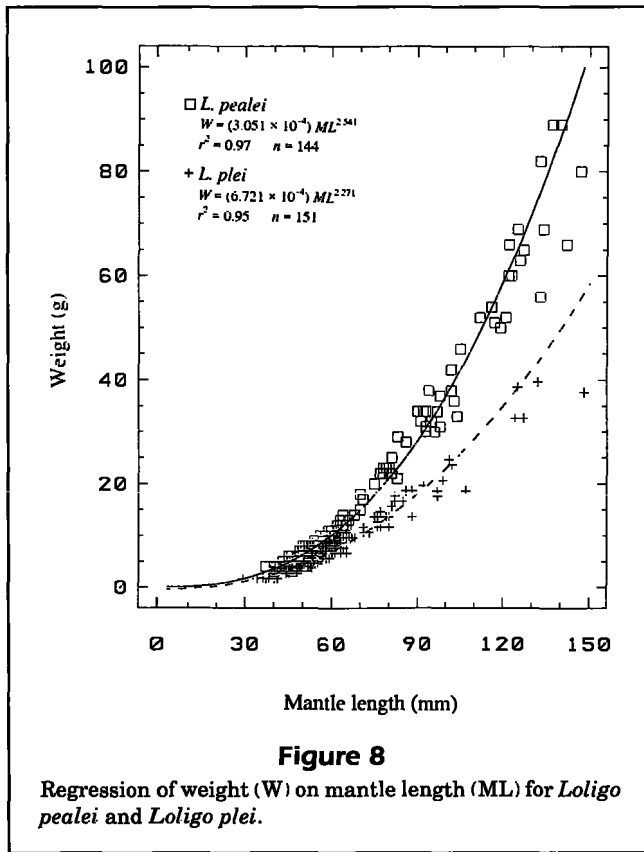
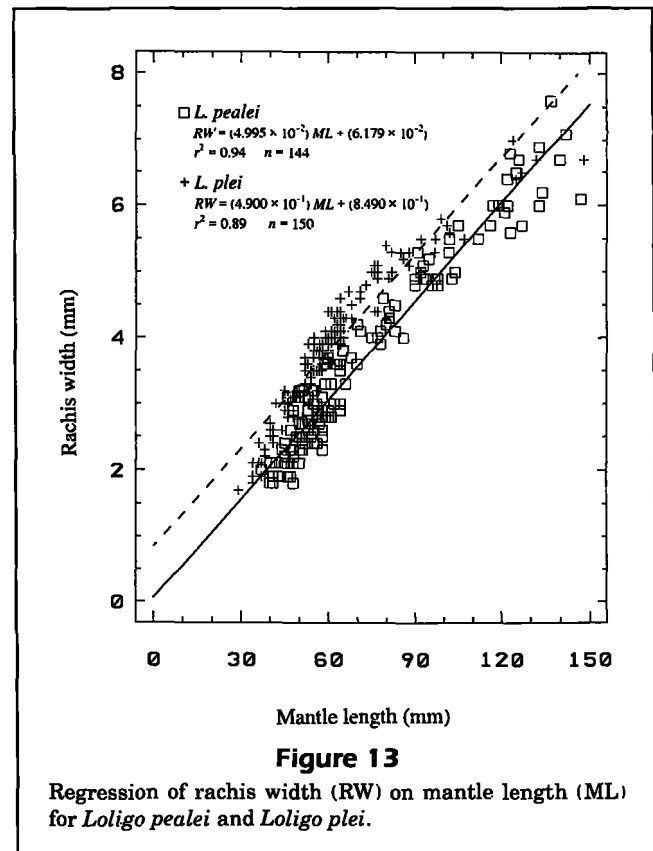
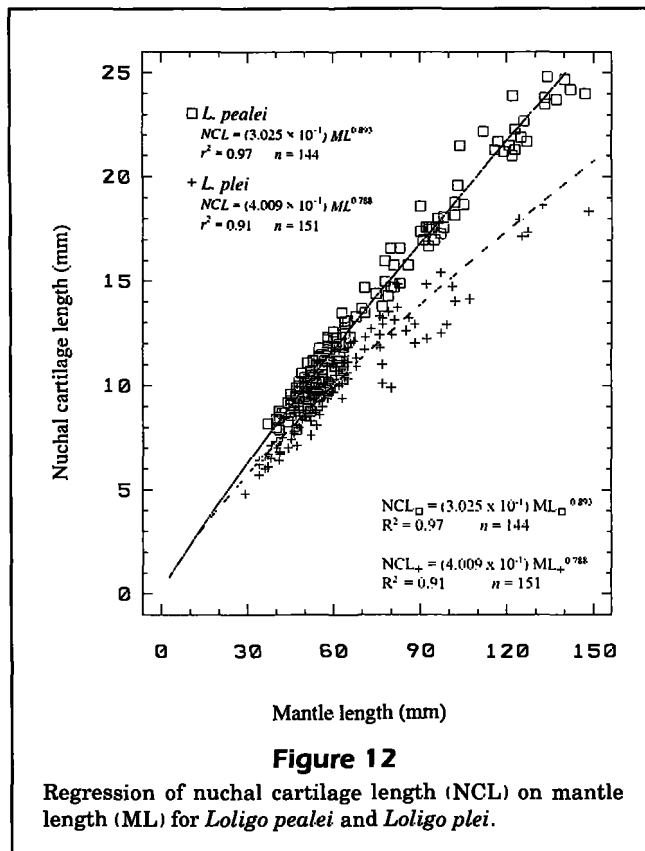
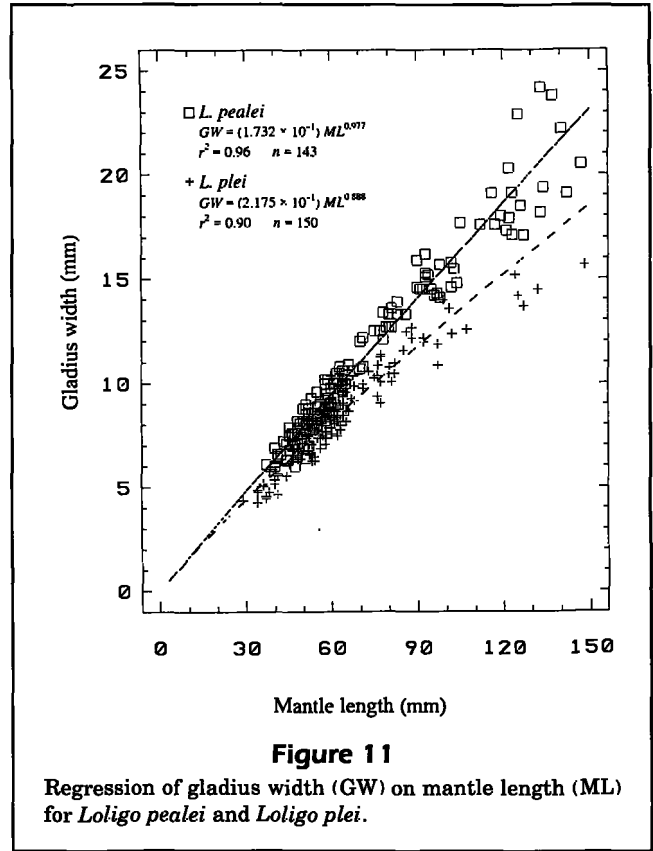
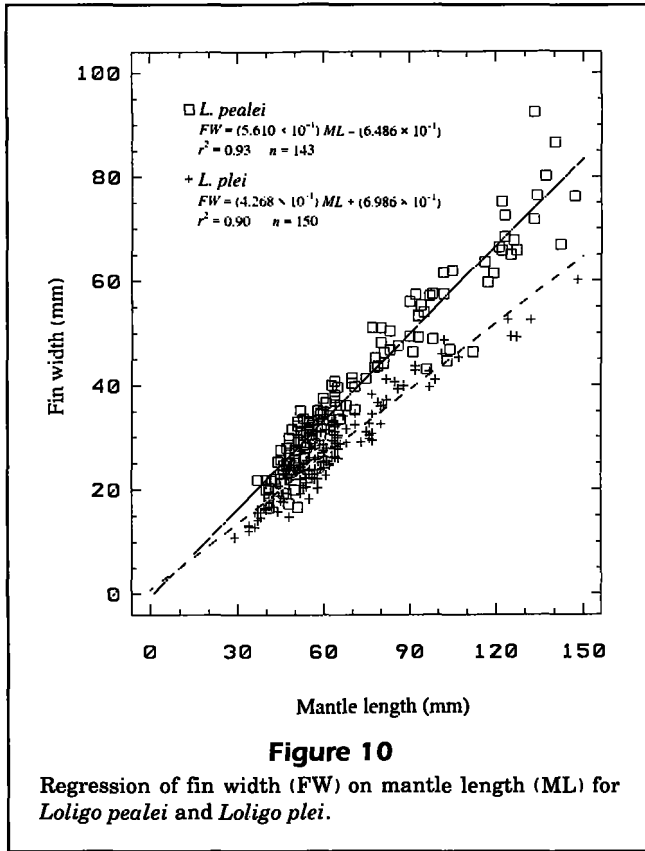


Table 6

Species comparison of characters and indices for *Loligo pealei* and *Loligo plei* (sample sizes are given in parentheses). Abbreviations are defined in Table 1. NS = not significant.

	AL	FCL	FL	FW	GW		
<i>L. pealei</i> (r^2)	0.94 (141)	0.95 (144)	0.99 (144)	0.93 (143)	0.96 (143)		
<i>L. plei</i> (r^2)	0.75 (151)	0.84 (151)	0.98 (151)	0.91 (150)	0.90 (150)		
Slope test	$P=0.0130$	$P<0.0001$	$P<0.0001$	$P<0.0001$	$P=0.0022$		
	HW	NCL	RW	TL	W		
<i>L. pealei</i> (r^2)	0.71 (143)	0.97 (144)	0.94 (144)	0.84 (106)	0.97 (144)		
<i>L. plei</i> (r^2)	0.66 (151)	0.91 (151)	0.89 (150)	0.66 (109)	0.95 (151)		
Slope test	NS	$P<0.0001$	NS	$P=0.0133$	$P<0.0001$		
	AL/ML	FCL/ML	FL/ML	FW/FL	FW/ML	GW/ML	GW/RW
<i>L. pealei</i> (r^2)	0.00 (141)	0.65 (144)	0.78 (144)	0.44 (143)	0.01 (143)	0.02 (143)	0.02 (143)
<i>L. plei</i> (r^2)	0.10 (151)	0.61 (151)	0.76 (151)	0.48 (150)	0.00 (150)	0.17 (150)	0.00 (150)
Slope test	$P=0.0011$	$P=0.0001$	$P=0.0102$	$P=0.0017$	NS	$P=0.0006$	NS
<i>t</i> -test of means	$P<0.0001$	$P<0.0001$	$P<0.0001$	NS	$P<0.0001$	$P<0.0001$	$P<0.0001$
Means (<i>L. pealei</i> , <i>L. plei</i>)	0.59, 0.49	0.17, 0.15	0.44, 0.36	1.27, 1.24	0.55, 0.44	0.16, 0.14	3.13, 2.18
	HW/ML	NCL/FCL	NCL/ML	RW/ML	TL/AL	TL/ML	W/ML
<i>L. pealei</i> (r^2)	0.19 (143)	0.44 (144)	0.33 (144)	0.00 (144)	0.34 (105)	0.36 (106)	0.95 (144)
<i>L. plei</i> (r^2)	0.47 (151)	0.26 (151)	0.45 (151)	0.17 (150)	0.00 (109)	0.06 (109)	0.86 (151)
Slope test	NS	NS	$P<0.0001$	$P<0.0001$	$P=0.0005$	NS	$P<0.0001$
<i>t</i> -test of means	$P<0.0001$	NS	$P<0.0001$	$P<0.0001$	$P=0.0080$	$P<0.0001$	$P<0.0001$
Means (<i>L. pealei</i> , <i>L. plei</i>)	0.25, 0.20	1.15, 1.17	0.19, 0.17	0.05, 0.06	2.98, 3.13	1.75, 1.51	0.23, 0.13



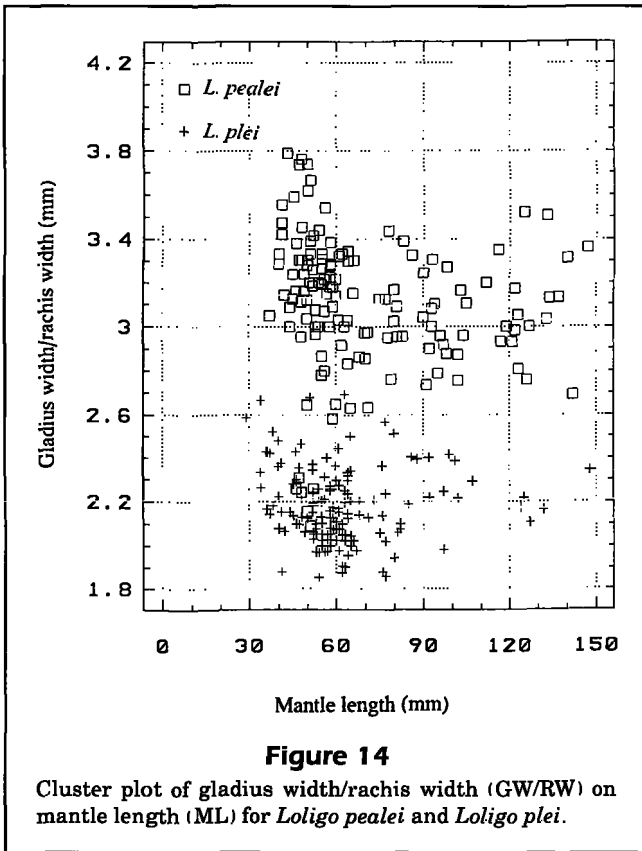


Figure 14

Cluster plot of gladius width/rachis width (GW/RW) on mantle length (ML) for *Loligo pealei* and *Loligo plei*.

ratio alone and this index in combination with either FCL/ML, GW/ML, NCL/ML or RW/ML. The RW/ML ratio in combination with FCL/ML, GW/ML, or NCL/ML provided for similar levels of accuracy. In contrast, *L. plei* could be correctly classified at 100% accuracy with the combination of FCL and RW (Table 8). Correct classification levels of 98% and 99% were obtained when RW was combined with GW or NCL, respectively. High levels of separation in *L. plei* also occurred with the indices GW/RW or RW/ML in combination with selected indices (Table 8). With the GW/RW index alone, 98% of the *L. plei* specimens were correctly classified.

Structure of the gladius

The gladius in *L. pealei* is broad and feather-shaped in all specimens (Fig. 17, A and B). The gladius is more rounded in females than in males. The greatest gladius width occurs at the mid-point of the vane. The ratio of the greatest width of the gladius to the greatest width of the rachis ranges from 2.26 to 3.36 in males and from 2.74 to 3.52 in females. The rachis has a single median rib with marginal ribs on each side that fuse in the posterior 1/3 of the rachis. The anterior and posterior tips of the rachis are pointed. The vane is broad and without ribs. The

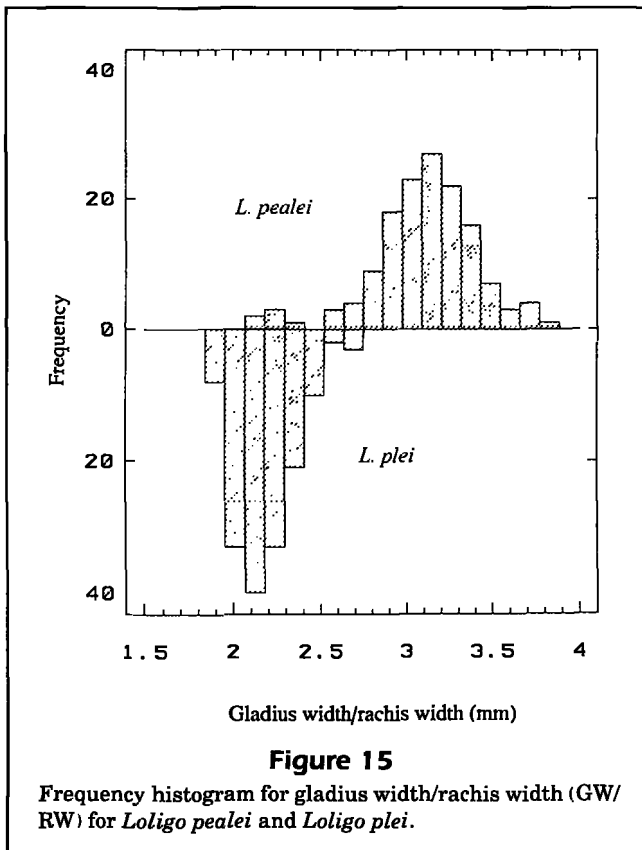


Figure 15

Frequency histogram for gladius width/rachis width (GW/RW) for *Loligo pealei* and *Loligo plei*.

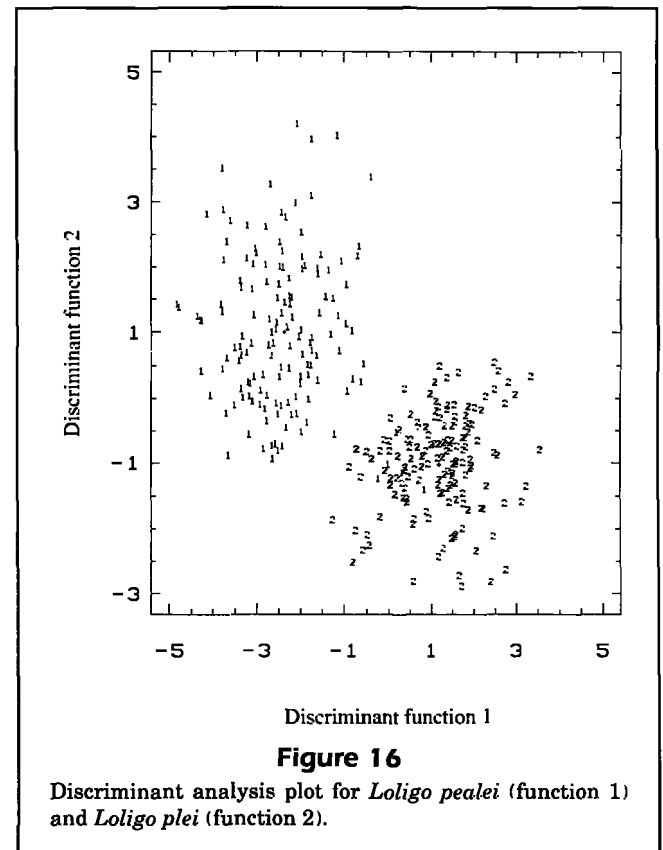


Figure 16

Discriminant analysis plot for *Loligo pealei* (function 1) and *Loligo plei* (function 2).

margins of the vane are curved, thin, and converge both anteriorly and posteriorly. The junction of the rachis and the vane is gradual and not distinct. The free rachis tapers anteriorly from the junction of the vane to the terminus of the marginal ribs. The ratio of AFR/RW ranges from 0.633 to 1.100 in males and from 0.684 to 0.946 in females.

The gladius in *L. plei* is slender and sword-shaped in all specimens (Fig. 17, C and D). The greatest gladius width in males occurs in the anterior 1/4 of the vane. In females, the greatest width occurs near the mid-point of the vane. The ratio of the greatest width of the gladius to the greatest width of the rachis ranges from 1.85 to 2.34 in males and from 1.98 to 2.69 in females. One median rib occurs on the rachis with marginal ribs on each side that fuse in the posterior 1/3 of the rachis. Anterior and posterior tips of the rachis are very pointed. The vane tapers both

anteriorly and posteriorly. The junction of the rachis and the vane is abrupt and distinct. The vane in both sexes has marginal ribs that gradually curve inward, anterior to the midpoint of the vane. The lateral margins of the vane are usually straighter in males than in females. The posterior tip of the rachis is less flexible than the anterior tip owing to the convergence and fusion of the ribs of the vane and the rachis. The thickness of the ribs in both the rachis and the vane increases with increasing size of the specimen. The ribs usually are more pronounced in males than in females. The ribs in the vane of small specimens are distinguishable only as a color variation when held up to the light. The free rachis tapers from the junction of the vane to the terminus of the marginal ribs. The ratio of AFR/RW ranges from 0.701 to 1.043 in males and from 0.829 to 1.360 in females.

Table 7

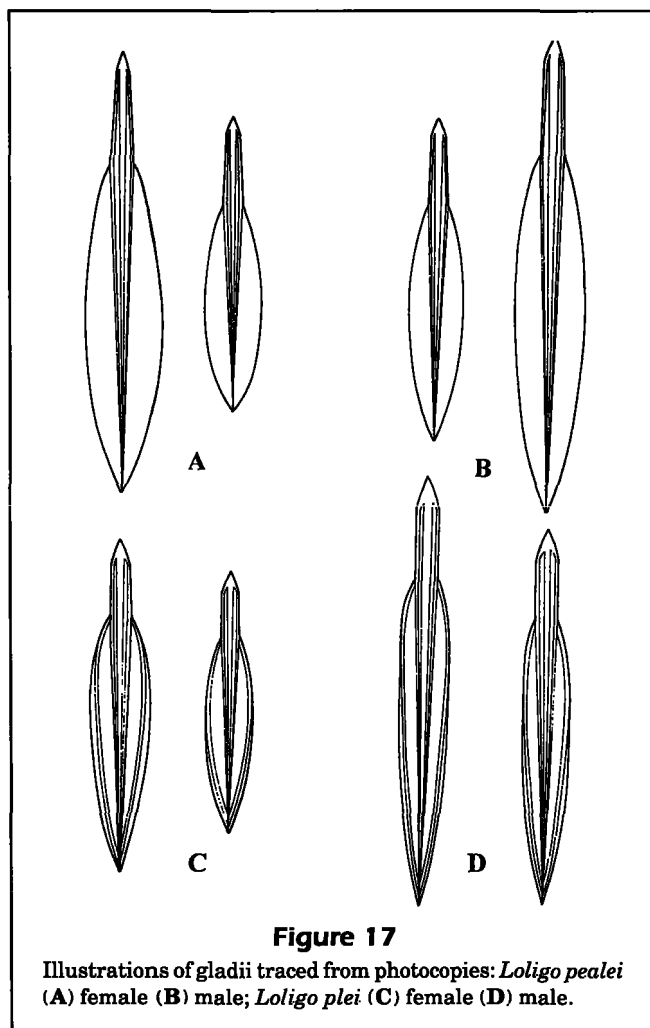
Percent *Loligo pealei* separated from *Loligo plei* by using cartilaginous structures (sample sizes are given in parentheses). Abbreviations are defined in Table 1.

	FCL	GW	NCL	RW	
FCL	49 (151)	61 (150)	69 (151)	95 (151)	
GW		46 (150)	46 (150)	95 (150)	
NCL			45 (151)	93 (151)	
RW				61 (151)	
	FCL/ML	GW/ML	GW/RW	NCL/ML	RW/ML
FCL/ML	72 (151)	81 (150)	95 (150)	79 (151)	93 (151)
GW/ML		83 (150)	92 (150)	86 (150)	95 (150)
GW/RW			92 (150)	93 (150)	93 (150)
NCL/ML				77 (151)	92 (151)
RW/ML					88 (151)

Table 8

Percent *Loligo plei* separated from *Loligo pealei* by using cartilaginous structures (sample sizes are given in parentheses). Abbreviations are defined in Table 1.

	FCL	GW	NCL	RW	
FCL	83 (153)	80 (152)	80 (153)	100 (152)	
GW		79 (152)	82 (152)	98 (152)	
NCL			82 (153)	99 (152)	
RW				53 (152)	
	FCL/ML	GW/ML	GW/RW	NCL/ML	RW/ML
FCL/ML	73 (153)	82 (152)	98 (152)	75 (153)	95 (152)
GW/ML		77 (152)	98 (152)	81 (152)	98 (152)
GW/RW			98 (152)	99 (152)	99 (152)
NCL/ML				75 (153)	97 (152)
RW/ML					87 (152)



Discussion

Isoelectric focusing of mantle proteins seems to provide a reliable means of distinguishing between the two species of *Loligo* in the Gulf of Mexico and to enable accurate identification of small, immature specimens. Garthwright et al. (1989) also biochemically separated *L. plei* and *L. pealei* but used the technique of horizontal starch gel electrophoresis. Morphometric analyses provided information on sexual dimorphism within the species and on species differences and similarities in specimens from the northern Gulf of Mexico, already identified to species by means of IEF.

Comparisons of morphometric characters and indices between males and females of each species were made in an effort to determine their value as a rapid means for fishery biologists to distinguish the sexes in the field. Although statistically significant differences were evident in many of the selected morphometric characters and indices, both between sexes within a species and between species, the differences

were small and overlap usually was high. The majority of squids examined in this study were between 40.0 and 80.0 mm ML, and greatest overlap in characters and indices occurred in this size range.

Tests of sexual dimorphism in measured characters regressed against mantle length for *L. pealei* indicated no significant differences between males and females in the slopes of the lines for W and GW. Females were heavier than males and the GW was broader in females than in males in all size classes. Weight and GW also were highly correlated to ML in both males and females of *L. plei*; however, no significant difference existed in the slopes of the lines, and the 95% confidence intervals of the regression intercepts overlapped. Differences in weight between males and females were more pronounced in *L. pealei*. Haefner (1964) noted little difference in the weight-length relationship between males and females of *L. pealei* below 95 mm ML; however, at mantle lengths greater than 95 mm ML, females began to outweigh males. Hixon (1980) also found that the rate of increase in weight per unit increase in mantle length was faster in females than in males. Both Haefner (1964) and Hixon (1980) attributed this faster rate of increase in females to sexual maturation and the increase in weight of the female reproductive structures. For northernmost populations of *L. pealei* off New England, females less than 170.0 mm ML generally weighed less than males (Lange and Johnson, 1981). Maturation at smaller sizes in southern populations of long-finned squid could account for this difference in the weight-length relationship between females and males in comparisons of data for northern and southern populations. Both Cohen (1976) and Hixon (1980) noted that *L. pealei* seems to mature at smaller sizes in the warmer portion of its range. Width of the gladius also has been linked to sexual maturation in female *Loligo*. Cohen (1976) noted that in mature females the broadest portion of the gladius, the vane, covers the full ovary and may provide support and protection for the eggs. In the present study, the gladius was broader in females of both species.

Although there were differences in morphometric characters and indices between sexes within a species, these differences were subtle and usually were more apparent in larger specimens. The high overlap both in measured characters and in indices precluded their use as a reliable means of distinguishing the sexes in juvenile and subadult specimens. Gender-specific morphological characters (e.g. hectocotylus or sexual organs) appear to be the only precise means of differentiating males and females of the two species. In those specimens in which these characters are undeveloped, no way was discovered to distinguish between the sexes.

Juvenile and subadult *L. pealei* and *L. plei* overlapped in all measured characters. For those characters in which there was a significant difference in the slopes of the regression lines and for which the coefficients of determination were high (FL, FW, GW, NCL, and W), the overlap generally was higher in the smaller size classes. Differences in W, NCL, and GW were more pronounced in larger specimens. Increases in measured characters per unit increase of mantle length were higher in *L. pealei*.

Both Cohen (1976) and LaRoe (1967) reported differences in fin lengths between *L. pealei* and *L. plei* and suggested that this character could be used to differentiate between the two species. Cohen (1976) found that fin length in relation to mantle length was similar in both species in small specimens. However, she noted that fin length was equal to or greater than half the mantle length of *L. pealei* when mantle length exceeded 55.0 mm, whereas fin length was equal to or greater than half the mantle length in specimens of *L. plei* only after mantle length exceeded 95.0 mm. Fin length/mantle length proportions in the present study corroborate these data for juvenile and subadult specimens from the northern Gulf of Mexico. Fin lengths were less than 50% of the mantle length in both species in all specimens under 70.0 mm ML. In *L. pealei*, the mean fin length of specimens from 90.0 to 99.9 mm ML was equal to 50% of the mantle length, and this percentage increased with increasing size. Fin length in relation to mantle length was 50% in *L. plei* only at mantle lengths of 120.0 mm and larger. LaRoe (1967) also listed width of the gladius and length of the funnel cartilage as useful characters to separate the species; however, Cohen (1976) noted that the overlap in values for these characters negated their usefulness. The present data show overlap in GW in all but the larger size classes. Overlap in FCL was more prevalent in smaller size classes, but in specimens greater than 90.0 mm ML, it may be a useful character.

The shape of the gladius and the area of the junction of the vane and free rachis were distinct in both species over the size range of individuals examined. The gladius in *L. pealei* was broader and more rounded than was the gladius in *L. plei*. It was feather-shaped in all specimens and the vane was without ribs. The junction of the vane and the free rachis was gradual and not distinct. The gladius in *L. plei* was more slender, elongated, and resembled a sword or dagger in shape. Ribs in the vane were more obvious in larger specimens. The lateral margins of the vane were usually straighter and the ribs in the vane were more pronounced in males than in females. The junction of the vane and free rachis was abrupt and distinct.

The best discrimination between the two *Loligo* species was based on combinations of measurements of the characters and calculated indices associated with cartilaginous structures. Funnel or nuchal cartilage lengths combined with rachis width measurements or indices enabled greatest percent separation of the species. Characters and indices based on gladius shape also had high percentage levels of separation. Highest correct classification of *L. pealei* (95%) by discriminant analysis was achieved by combining RW with either FCL or GW or by combining the GW/ML and RW/ML indices. *Loligo plei* were classified with 100% accuracy by combining FCL and RW.

Numerous studies cite the GW/RW ratio as a reliable index for species differentiation. Roper et al. (1984) summarized taxonomic data for the two species and reported GW/RW ratios from 2.4 to 3.7 for *L. pealei* and from 1.5 to 2.4 for *L. plei*. Cohen (1976), Whitaker (1978), and Hixon (1980) also used the GW/RW index to distinguish the two species of *Loligo* and used the ratio of 2.4 to differentiate between the species: below 2.4 for *L. plei* and above 2.4 for *L. pealei*. The present study is the first to use IEF to identify juvenile and subadult *L. pealei* and *L. plei* and thus provides a more accurate base from which to assess morphological variation between the species. The GW/RW ratios in the present study ranged from 1.9 to 2.7 for *L. plei* and from 2.1 to 3.8 for *L. pealei*. Separation of the species was best accomplished with a GW/RW ratio of 2.7. Applying the ratio of 2.4 to the present data resulted in overlap in both species; 10% of the *L. plei* had an index greater than 2.4, and 4% of the *L. pealei* had an index below 2.4. By using 2.7 as the critical value, all the *L. plei* were separated and 91% of the *L. pealei* could be identified. Hixon (1980) also found specimens of *L. plei* with GW/RW ratios above 2.4. He identified 21 mature females and one male *L. plei* from Texas waters with GW/RW indices ranging from 2.4 to 2.9. He attributed the greater index in the females to the wider vane associated with sexually mature individuals. This does not explain the higher GW/RW indices recorded for some specimens of *L. plei* in this study, because all individuals were immature. Also puzzling are the specimens of *L. pealei* with GW/RW ratios below 2.7. Because all specimens in the present study were identified biochemically, errors in identification should be negligible. According to Cohen (1976), *L. pealei* is composed of morphologically variable populations and greater variation occurs in areas where *L. pealei* co-occurs with *L. plei*. In these areas of sympatry, there is a closer resemblance between species. Cohen (1976) found a single specimen of *L. pealei* with a GW/RW ratio below 2.4, and this specimen was from an area where both species occur. The possibility of

genetic introgression was considered by Cohen (1976), but currently there is no evidence of hybridization between any of the loliginid species.

The original intent of this study was to examine selected morphometric characters of potential value in differentiating the two common species of *Loligo* in the field. Juvenile and subadult squids comprise a substantial portion of the invertebrate biomass in the northern Gulf of Mexico, and difficulty in separating the species of *Loligo* has been a contributing factor in our lack of knowledge of this group. No single character or index enabled 100% separation of the species in the size classes of squid under study. Overlap was high in many of the measured characters and indices and precluded their use as reliable determinants of species. LaRoe (1967) and Cohen (1976) also found high overlap in measured characters and indices for the two species and suggested that more than one character or index had to be used to separate the species. In the present study, combinations of characters or indices associated with cartilaginous structures (FCL, GW, NCL, and RW) enabled greatest percent separation.

The single GW/RW ratio of 2.7 enabled correct identification of 91% of *L. pealei* and 100% of *L. plei*. This percent separation was similar to that achieved in the discriminant analysis for the GW/RW ratio. The most practical method for separation of the species in the field is use of the 2.7 GW/RW ratio in conjunction with the overall shape of the gladius, including the presence or absence of marginal ribs.

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