DEVELOPMENT AND APPLICATION OF AN OBJECTIVE METHOD FOR CLASSIFYING LONG-FINNED SQUID, *LOLIGO PEALEI*, INTO SEXUAL MATURITY STAGES

WILLIAM K. MACY III[1]

ABSTRACT

An objective method of classifying long-finned squid, *Loligo pealei*, by their sexual maturity was developed using cluster and discriminant analysis techniques. The resulting system recognizes four developmental stages and employs a maximum of only five easily measured morphometric parameters. Such a system is easy to use and is suitable for large-scale field studies with relatively untrained help. The value of determining clearly recognized reproductive stages is demonstrated by an application of the method.

Each summer schools of long-finned squid, *Loligo pealei* Lesueur, 1821, move into shallow coastal waters from Delaware Bay to Cape Cod to spawn (Verrill 1882; Haefner 1964; Summers 1968, 1971). During the colder months, however, the squid are found only offshore, concentrated in canyon mouths along the continental slope (Summers 1967, 1969; Vovk and Nigmatullin 1972; Serchuk and Rathjen 1974). While the size/age composition of the species has been relatively well described by Verrill (1882), Summers (1967, 1968, 1969, 1971), Tibbetts (1975, 1977), and Mesnil (1977), many details of its reproductive cycle, particularly during the offshore period, remain unclear (see Summers 1969, 1971; Vovk 1972; Arnold and Williams-Arnold 1977; Mesnil 1977). From such studies it appears that *L. pealei* lives only 12-18 mo on average, and that like most squid it dies after spawning (Arnold and Williams-Arnold 1977). Details of the reproductive biology and population structure of such a short-lived species, with only two year classes at most, are especially important in the development of prudent stock management programs. Unfortunately, no single method for characterizing the reproductive state of individuals of this species has been employed to date.

A number of classification methods have been employed for a variety of squid species, which reflect both interspecific differences and differing requirements of the investigators using them (Tinbergen and Verwey 1945; Mangold-Wirz 1963; Fields 1965; Hayashi 1970; Vovk 1972; Holme 1974; Ikehara et al. 1977; Durward et al. 1978; Juanicó 1979; Hixon 1980). Many of these methods are slow because of the large number of variables required or the need for sample weighing or microscopic examination. Some methods also rely mainly on subjective distinctions.

From 1975 through 1978, ecological studies concerning the population structure, movement patterns, and feeding habits of *L. pealei* were conducted (Macy 1980). During the first year of the study, the Vovk (1972) method for classifying squid into one of five stages of sexual maturity was used with length-frequency data to characterize changes in the population reproductive structure over time. While the Vovk method was useful, in practice squid were often encountered which could not be readily classified. This report concerns the development and application of a new, faster, and more objective maturity classification system. The method was then successfully employed to classify large numbers of squid throughout the remainder of the study referred to above.

METHODS

From late April through November each year, squid were collected on an approximately biweekly basis throughout the inshore study area located in the southern part of the West Passage of Narragansett Bay, R.I. (inset Fig. 1). A balloon-type otter trawl (Oviatt and Nixon 1973), towed at ca. 4.6 km/h, was used. To insure randomness and adequate size-class representation, samples of at least 100 squid each were randomly selected from the pooled catch of duplicate 20-

[1]Narragansett Mussel Co., 146 Main Street, North Kingstown, RI 02852.

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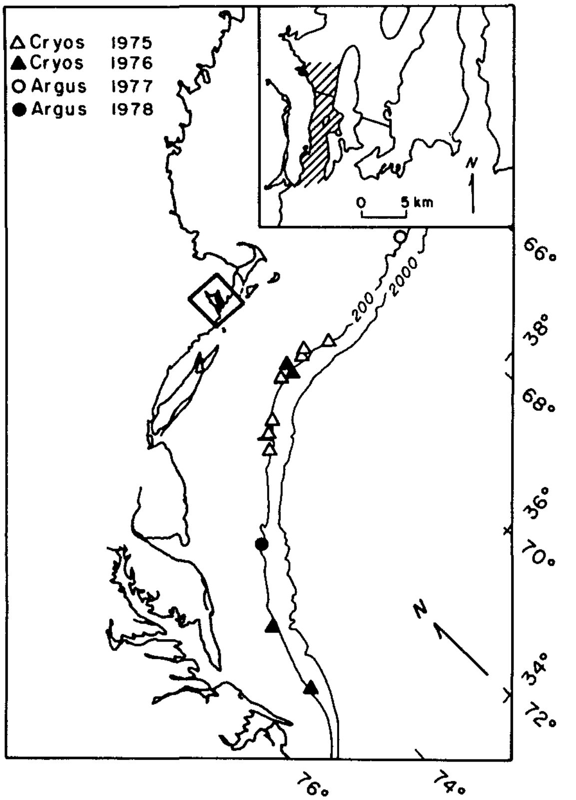


FIGURE 1.—Inshore and offshore sampling locations on the coast and continental shelf of New England. The study area in lower Narragansett Bay is indicated by shading in the inset. Depths in meters.

min trawl tows. The selected squid were bagged and placed in chilled containers for transportation to the laboratory, where they were either immediately examined or were quick-frozen at −25°C for storage. Additional frozen samples of 100-500 squid each were obtained from the National Marine Fisheries Service, Woods Hole, Mass.; from offshore surveys by the French vessel *Cryos* conducted during November-December 1975 and 1976; and from the Russian vessel *Argus* during November 1977 and March 1978 (Fig. 1). Only the 1976 Narragansett Bay and *Cryos* samples were used to develop and verify the classification system described here. The new system was then used to classify the 1977 and 1978 samples.

For analysis, squid were opened midventrally to expose internal organs and allow sexing. Twenty characters were then determined (Table 1). Length measurements were made to the nearest millimeter using a ruler along the greatest dimension, taking care not to stretch the organ or tissue. Frequently, the length of typical spermatophores can be determined while still within Needham's sac or the penis. Mantle width (MW, Table 1), a measure of mantle circumference, was defined as the distance across the widest part of the mantle when opened flat to form a rough isosceles triangle. Mantle thickness (MT) was measured at the thickest portion along the midventral incision. All weight measurements were blotted fresh or live weights (e.g., wet weight, WW, and gonad weight, GW) and were recorded to the nearest 0.01 g if <120 g or to the nearest 0.1 g if >120 g, on a top-loading digital balance. The relative abundance of spermatophores in Needham's sac (ASN), in the penis (ASP), of eggs in the ovary (AEO), or in the oviduct (AEOV) were scored on the basis of "none," "a few," or "many" present. In females, a distinction was also made between immature and mature eggs (Table 1). The four variables—ASN, ASP, AEO, and AEOV— were purposely scored on this nonquantitative basis to avoid time-consuming enumeration. After completing the above length measurements and abundance estimates, the complete reproductive tracts, including gonadal products (Table 1), were dissected out and weighed (GW). From the tabulated raw data the various proportional indices (GI, MWI, MTI, TLI, SPI, NGI, and AGI (see Table 1)) were then computed.

TABLE 1.—The initial 20 input parameters tested for classifying squid into maturity stages. See Figure 3 for organ identification.

Mantle length, cm = DML = dorsal mantle length
Wet weight, g = WW = total live weight
Mantle width, cm = MW
Mantle thickness, cm = MT
Gonad weight, g = GW[1]
Gonad index = GW/WW = GI
Mantle width index = MW/DML = MWI
Mantle thickness index = MT/DML = MTI
Males:
Testis length, cm = TL
Spermatophore length, cm = SPL[2]
Abundance of spermatophores in Needham's sac = ASN[3]
Abundance of spermatophores in penis = ASP[3]
Testis length index = TL/DML = TLI
Spermatophore length index = SPL/DML = SPI
Females:
Nidamental gland length, cm = NGL
Accessory gland length, cm = AGL
Abundance of eggs in ovary = AEO[4]
Abundance of eggs in oviduct = AEOV[3]
Nidamental gland index = NGL/DML = NGI
Accessory gland index = AGI/DML = AGI

[1]For females: (nidamental + accessory + oviducal glands) + ovary/oviduct + eggs.
For males: testis + (Needham's sac + spermatophoric organ + penis).
[2]Length of an average spermatophore excluding tail (= cap thread), scored as "0" if not present or if visible only as a round speck.
[3]Scored on a 1-3 scale, where 1 = none, 2 = "a few," 3 = abundant.
[4]Scored on a 1-5 scale, where 1 = none, 2 = "a few" immature, 3 = many immature, 4 = "a few" mature, 5 = many mature.

Statistical analyses were performed using the Biomed Computer Programs P-Series (BMDP) (Brown 1977) on an Itel AS-5[R] computer[2] of the University of Rhode Island Academic Computer Center. The initial data matrix consisted of the 20 variables listed in Table 1, from 675 males and 693 females randomly selected from the 1976 *Cryos* offshore and 1976 Narragansett Bay inshore samples. After standardization of the variables, principal components analysis (BMDP program 4M) (Morrison 1976) was employed to group variables and to determine their importance in accounting for observed variance. Cluster analysis (BMDP 2M) was then used with the Euclidean-distance metric as the amalgamation algorithm to group cases (Anderberg 1973). Finally, stepwise linear discriminant analysis (BMDP 7M) (Anderson 1958) was used with different variable combinations to generate a series of functions which best discriminated between the groups identified in the cluster-analysis stage. A goal of 95% or better overall correct classification of individuals was set.

RESULTS

Development of the Discriminant Functions

The initial cluster analysis revealed only two major groupings for each sex. Further examination, however, suggested that the major clusters consisted of different size-based groupings of mature and immature individuals. Spent squid (using the Vovk (1972) scale) did not group together. Weight variables—WW, GW, and GI (Table 1)—were then dropped from the data matrix because it was known that length measures correlate well with their respective weight counterparts and because principal components analysis had not indicated any particular advantage to using one variable type or the other. Cluster analysis was then rerun using the remaining 17 variables. Four clusters of developmental stages could then be recognized, corresponding to "ripe/spent," "nearly mature," "advanced immature," and "immature" (barely sexable). Several size-based subgroups were still evident within the major clusters. After scoring the squid on a 1-4 scale based on the cluster results, subsequent discriminant analysis produced moderately good separation of the four groups.

Efforts were then focused on improving class separation and reducing the number of variables required. First, those cases which were suspected to be misclassified based on posterior probability and Mahalanobis D^2 statistics (Lachenbruch and Mickey 1968) were corrected. By this time a rather clear picture of the characteristics of each stage had been formed, and thus inspection of the raw data was often sufficient to determine if reclassification was warranted. Using different combinations of variables in the discriminant analyses, the number of variables was further reduced by retaining only those which improved classification accuracy, as indicated by a pseudojackknife test (see BMDP documentation; Lachenbruch and Mickey 1968).

Best results were obtained with the following input variables: MW and MWI, SPL or AGL, API or AGI, TLI or NGI, and ASP or AEOV (Tables 1, 2). In males 94.6% correct classification (Table 2) was obtained using only the three most important variables, SPI, MWI, and TLI (determined by their order of entry into the stepwise analysis), while 96.6% of the females were correctly classified using the first four variables—AEO, AGI, MWI, and NGI. Stage 2 squid were incorrectly classified 19% in males and 10.7% in females, but other squid were correctly grouped in at least 90% of the cases. A plot of the

TABLE 2.—Classification of *Loligo pealei* into stages of sexual maturity using linear discriminant functions. The variables to be measured are listed below with their coefficients or weighting factors for each maturity stage. To classify an individual, construct four linear equations, one for each stage, using the measured values and the appropriate coefficients and constant and solve. The equation resulting in the largest value indicates the stage into which the individual has been assigned.

	Stage			
	1	2	3	4
Variable:				
Males:				
SPI	−949.083	−838.030	−972.007	596.250
MWI	112.680	88.635	92.161	16.472
TLI	7.445	65.620	133.984	130.547
Constant	−49.822	−39.961	−60.416	−35.921
			94.6% correctly classified	
Females:				
AEO	9.695	9.440	13.297	28.915
AGI	−71.476	82.104	233.148	282.733
MWI	98.606	83.025	67.263	76.922
NGI	−26.426	−10.377	54.122	35.443
Constant	−46.310	−37.240	−49.256	−105.526
			96.6% correctly classified	

Example: Assume squid is male.
Then,
$Y(1) = -949.083 \times SPI + 112.680 \times MWI + 7.445 \times TLI - 49.822$

$Y(4) = 596.250 \times SPI + 16.472 \times MWI + 130.547 \times TLI - 35.921$,
where SPI, MWI, TLI are the actual values for the squid in question.

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

mean values of the first and second canonical variates for each stage (Anderson 1958) (Fig. 2) shows that stages 1-4 follow a logical sequence from immaturity to ripeness. The first two canonical variates are the orthogonal pair of linear combinations of the variables which best discriminates between the groups. Mean values for the final discriminant function variables are listed in Table 3. Typical DML's for each of the sex-stage groups have also been included for reference.

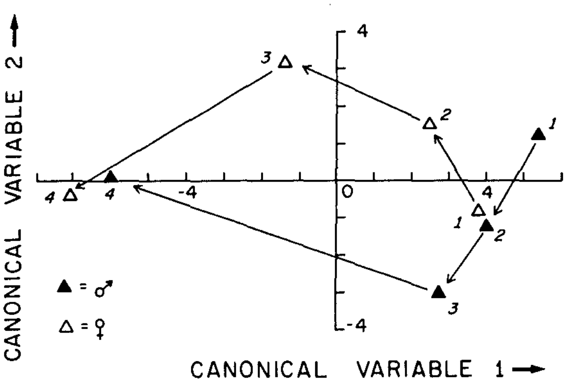


FIGURE 2.—Canonical variates for each stage of sexual maturity evaluated at the stage or group means for each sex. Trajectories between subsequent stages are indicated by arrows.

The Classification Process

To determine the stage of maturation of a squid, the sex must first be determined. If this cannot be done by visual inspection, the squid is considered juvenile (stage 0). Otherwise, DML and MW, plus TL and SPL for males, or NGL, AGL, and EOV for females, should then be determined. From these four or five parameters the appropriate indices needed (Table 2) can be calculated. As indicated in Table 2, a set of four linear equations is then constructed by combining the measured parameter values with the appropriate discriminant function coefficients (weighting factors) and constant listed for each stage. The equation with the largest solution indicates the stage into which the squid should be placed. Thus, once the raw data have been measured and recorded, the actual classification can be done at a later date using even a simple hand calculator. If the raw data are stored on a computer-readable medium, as was done in this study, the process is particularly efficient.

To investigate the broader applicability of the classification, additional squid from the 1976 Narragansett Bay and *Cryos* samples were classified. The discriminant analysis was then performed on these new data to see if the same four stages were identifiable. Over 98% of the individ-

TABLE 3.—Typical mean values (1 SD) of selected maturity stage variables of *Loligo pealei*, listed by sex and stage of development. With the exception of mantle length values (DML), which are from a much larger pooled sample from 1976 to 1978, the reproductive character means are those of the 1976 Narragansett Bay and *Cryos* samples used to compute the final discriminant functions listed in Table 2. Variable coding follows that of Table 1.

	Stage			
	1	2	3	4
Variable:				
Males:				
TLI (%)	11.6 (3.62)	19.0 (3.70)	30.7 (3.73)	30.3 (4.45)
MWI (%)	85.8 (6.90)	71.7 (7.51)	78.0 (8.74)	53.2 (11.71)
SPI (%)	0	0	0	3.6 (0.93)
n	131	119	42	383
DML (cm)	7.1 (2.17)	11.1 (3.25)	10.8 (4.67)	18.6 (8.03)
n	506	610	159	708
Females:				
AEO	1.0	1.0	1.8	4.7
AGI (%)	0	2.6 (1.00)	5.2 (2.62)	5.7 (1.27)
MWI (%)	83.6 (7.98)	74.7 (8.09)	65.0 (11.12)	61.4 (9.25)
NGI (%)	7.8 (3.23)	11.4 (4.86)	24.0 (3.74)	25.8 (4.24)
n	243	124	64	262
DML (cm)	7.8 (2.24)	11.4 (3.09)	13.6 (4.47)	14.3 (3.99)
n	947	467	155	498
Juveniles:				
MWI (%)	97.3 (10.24)			
n	1,857			
DML (cm)	4.6 (1.13)			
n	1,887			

uals of both sexes were correctly grouped, and the new discriminant functions did not differ appreciably from the original ones. Thus the method was shown to be internally consistent (precise). Accordingly, during 1977 and 1978 only the four or five measurements necessary for classification purposes were routinely taken.

The Four Maturity Stages

A description of the general characteristics of each of the four maturity stages of *L. pealei* follows. Figures 3a and b illustrate the important morphometric changes indicated by the discriminant analysis results.

Stage 0: These juvenile squid lack visible gonads (Fig. 3a) and are very broad for their length.

Stage 1: In females (Fig. 3a) the nidamental glands appear as thin white streaks, averaging 8% of DML (Table 3). In males the testis appears as a pale oval body, about 11% of DML, and is frequently obscured by the stomach and caecum.

Stage 2: Both the nidamental glands and testis have almost doubled in length, to 11% and 19% DML, respectively, and the accessory and oviducal glands of females or the spermatophoric organ/Needham's sac complex of males becomes evident. The ovary appears as a fine-grained band of tissue.

Stage 3: This is a transitional stage between immaturity and maturity. The NGI and AGI have approximately doubled again (24% and 5% DML), and the translucent white immature ova give the ovary a distinctly granular appearance (AEO = 2 or 3). The TLI averages 31% DML (Fig. 3b; Table 3), and immature spermatophores may be visible as small white specks in Needham's sac (ASN = 2). No gametes can be found in the penis or oviduct, however.

Stage 4: In these mature squid (Fig. 3b), much of the mantle cavity is occupied by the bulging ovary and oviduct or by the testis. Ripe eggs fill the oviduct and appear as amber spheres 1-2 mm in diameter (AEO>3, AEOV>1). Elongate mature spermatophores (ca. 5 mm long) are visible both in Needham's sac and in the penis (ASN, ASP>1). Immature ova are also found beneath the ripe eggs in the ovary, and usually the spermatophore receptacle is packed with spermatophores. A few virgin females were seen, however.

An Application of the Method

Insights into the usefulness and relevance of the new classification system can be provided by a brief examination of several findings of the overall population study of which this was a part (Macy 1980). Vovk (1972) distinguished between ripe/mature squid and spent squid, but throughout 1975 when the Vovk method was routinely employed, no spent squid were positively identified. Subsequent statistical analysis also failed to make this distinction. Laboratory observations (Macy 1980), however, suggest a reasonable explanation: Isolated females spawned repeatedly over 2-3 wk, but still contained significant numbers of immature eggs; neither sex ceased feeding after mating; and no evidence of nutrient depletion was found. Thus no truly spent individuals might be expected.

Maturing squid, on the other hand, were abundant in late winter prior to the onset of inshore migrations. In the March 1978 *Argus* samples, stage-3 squid constituted 35-40% of the population, while only 8% of the females and 21% of the males had yet reached maturity (stage 4). About 1 mo later (late April) large mature squid began to arrive and spawn in Narragansett Bay. By late July most spawning activity was completed, but already stage-1 individuals from early spawnings were becoming numerically dominant. By the time of arrival offshore, in late fall and early winter (*Cryos* and *Argus* 1977 samples), fewer than 6% of either sex were mature or maturing, but over 50% of the population was composed of stage-2 squid.

These early winter stage-2 squid seem to represent two distinct age/maturity groups: Smaller developing young of the year, and larger and presumably older squid whose gonads appear to be resorbing or regressing. The 1976 *Cryos* sample, for example, consisted of three groups of males with modal lengths of 8.2, 11.6, and 19.1 cm. Only 4.5% of these males ($n = 287$) were mature (stages 3, 4), but 71.4% were at stage 2. The modal size of the stage-2 squid lay between 10 and 12 cm, but individuals ranged from 8 to 23 cm. The remaining stage-1 squid had a modal length of only 8-9 cm. Regressing stage-2 individuals belonged to both the 11.6 and 19.1 cm size classes. Their gonads had the coloration and approximate length of more mature individuals, but were distinctly thin and lacked eggs or spermatophores of any size.

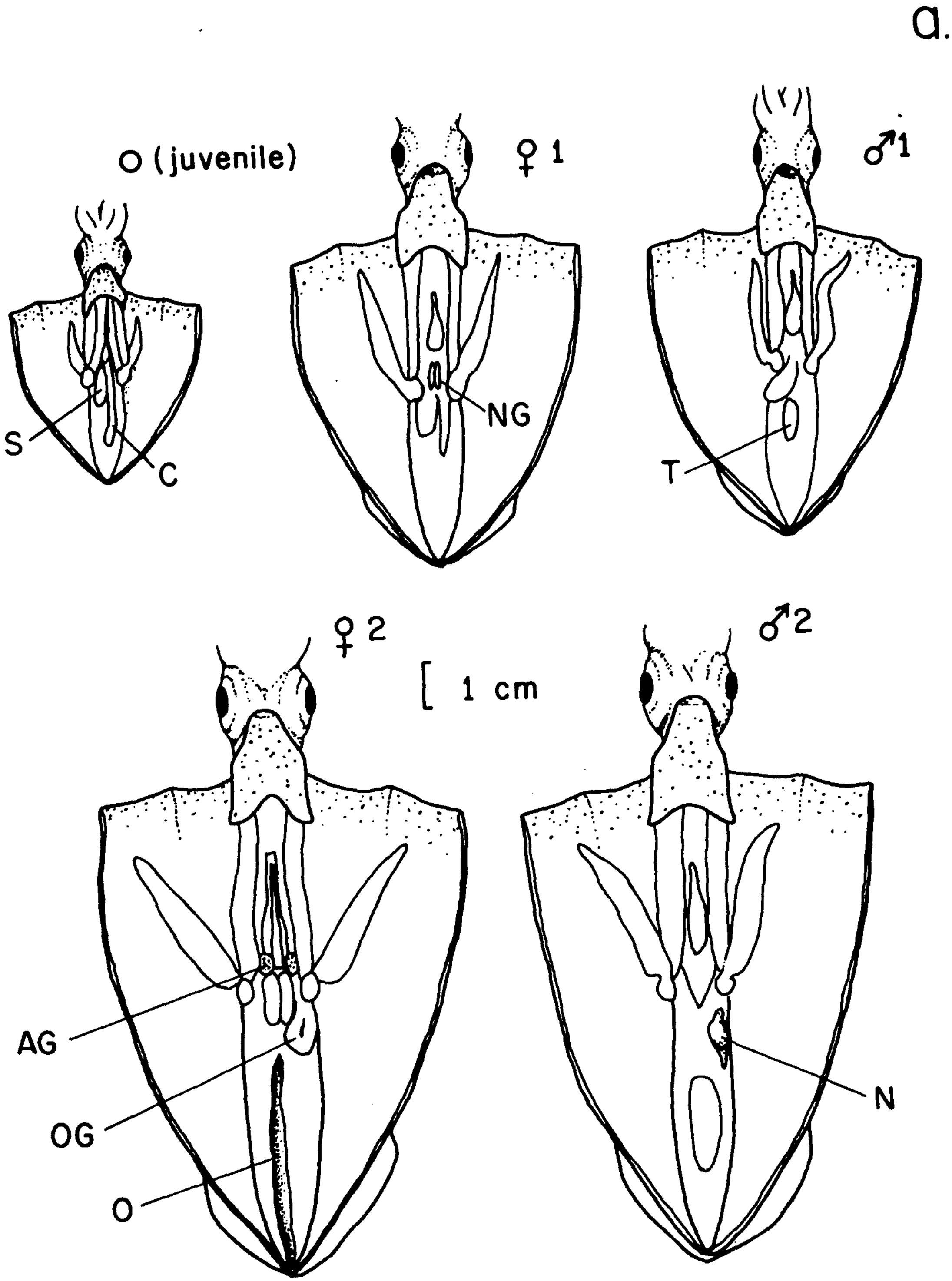
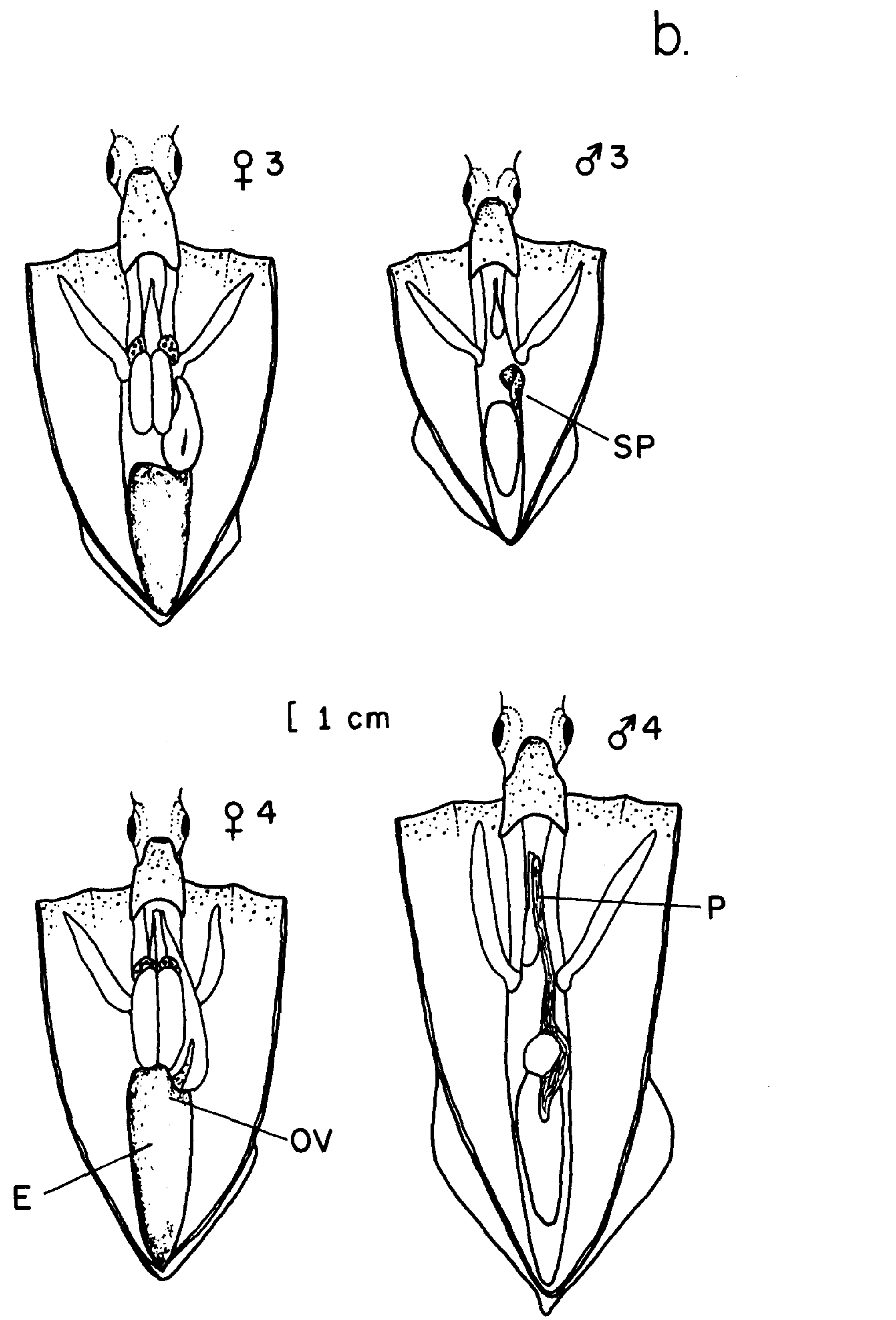


FIGURE 3.—Squid of each stage of maturity illustrating those morphometric changes which the discriminant analysis showed to be important. a, Juvenile and immature squid (stages 1, 2). b, Maturing and mature or ripe squid (stages 3, 4). The diagrams are drawn to scale using the mean values of the morphometric characters given in Table 3 for each sex-stage



combination. S = stomach; C = caecum; NG = nidamental glands; T = testis; AG = accessory glands; OG = oviducal gland; O = ovary; N = Needham's sac/spermatophoric organ complex; SP = spermatophores; E = egg mass (mature); OV = oviduct; P = penis. Digestive tracts are not shown in stages 3 and 4 (Fig. 3b).

DISCUSSION

A classification system should have two major attributes: It should be objective and easy to employ, and it should be biologically meaningful. The major impediment to satisfying both concerns appears to be the high degree of variability which exists within and between populations of *L. pealei.* This species has a wide geographic range, from the coast of South America to Nova Scotia (Cohen 1976), and thus populations living in different parts of the range are exposed to different and varying environmental conditions (temperature, salinity, photoperiod, and food availability) throughout their respective annual cycles. Such environmental variation is manifested by both spatially and temporally varying growth rates, which result in the presence of multiple cohorts within a year class (Summers 1968, 1971; Mesnil 1977; Lange and Johnson 1979), and by differences in the timing of inshore movement and gonad maturation (Hixon 1980; Macy 1980). Thus it is evident that samples used to construct a sexual development classification should at least reflect both the inshore and offshore portions of the range. This was done (Fig. 1). The Vovk (1972) classification, however, was based only on offshore collections, and as a result probably included relatively few spawning individuals and young of the year in early development. Summers (1968, 1969, 1971), on the other hand, sampled both inshore and offshore, but distinguished only between mature and immature individuals.

Objectivity and Utility of the Multivariate Approach

The multivariate approach to the classification problem is appropriate and objective when geographic (environmental) variability of unknown magnitude is superimposed on the usual random variation among individuals, producing the observed age or size and reproductive structure of the population or species. This is true because the analytical approach used here can effectively integrate the information and provide a simple numeric classification rule. Growth rates for *L. pealei* have only been estimated from modal size progressions (Summers 1971; Mesnil 1977), and hence the age of an individual or cohort can only be roughly estimated. Since multiple cohorts occur even within a small area (Narragansett Bay), mean and standard deviation values of morphometric characters, such as of DML or GW, have limited value for discrimination because of the large variability range of individuals (i.e., multimodality). It is known, for example, that considerable variation exists in the age or size of *L. pealei* at spawning (Haefner 1964; Summers 1971; Macy 1980). Standardization by the use of ratio parameters or indices may provide a partial solution to the variability problem.

In the interest of speed and ease of measurement, a set of nominal variables to assess the relative abundance of eggs or spermatophores (ASN, ASP, AEO, AEOV; Table 1) was used in addition to the ratio or interval variables (MWI, TLI, SPI, NGI). The "none," "some," "many" rating scale is not strictly objective, but such coarse evaluations can be done reliably with little training and have proved to be valuable discriminators. Theoretically it is possible to develop an entirely objective classification system. In practice, a somewhat more subjective approach usually proves necessary. What other investigators have found important in distinguishing different maturity groups, such as gonad to body weight ratios (Hayashi 1970), must certainly influence the initial selection of variables to be measured. The investigator must also decide how detailed a classification is desired and what additional parameters may be required. Repeated use of the exploratory technique of cluster analysis followed by stepwise discriminant analysis, as employed here, provides a way of learning how many groups may be present and how to "best" identify them using only those variables which can be shown to significantly aid discrimination. But, at this stage too, the researcher must at least roughly determine the basis for case clustering (by size, color, sexual development, etc.).

Biological Relevance and Accuracy

Statistically accurate and precise results may prove meaningless in reality. Thus the most important verification of the classification scheme is the demonstration of biological relevance in the appropriate context. In each of the 2 yr when the system was routinely employed, a predictable and logical progression of sexual development from hatching to spawning was observed. Moreover, the findings which resulted from this application of the method are reasonable and have significant, broader implications.

Laboratory studies confirm evidence from the field that spent squid cannot be reliably distinguished from spawning squid, even though the majority of samples (Narragansett Bay) were taken on the spawning grounds during the peak of reproductive activity. Hixon (1980) was also unable to find spent *L. pealei* or arrow squid, *L. plei*, in the Gulf of Mexico, and he too documented multiple spawning by *L. pealei*. Furthermore, it has been shown histologically (Burukovski and Vovk 1974) that egg development is highly asynchronous among individuals, and that a series of eggs at different stages of development in the ovary is typical. Prolonged spawning by poorly synchronized individuals of a population would tend to extend spawning over time, and may well account for the lack of reports of dead or dying squid of this species on the spawning grounds.

Stage-3 individuals, particularly males, were poorly sampled during 1976 (Table 3). This was expected since only fully mature individuals move inshore in large numbers, and reproductive development ceases or regresses in late fall. These animals show the first obvious signs of approaching maturity: Developing eggs and spermatophores may be visible, and the nidamental and accessory glands of females and the testes of males (Table 3) have reached sizes comparable to those of fully mature squid. The stage may be of short duration, since gonad development appears to be rapid offshore, with large squid, especially males, maturing faster than the smaller ones (Summers 1969; Macy 1980). In the March 1978 *Argus* samples, large numbers of stage-3 squid were identified, and it is unfortunate that other late winter or early spring offshore samples were not available to better document the latter stages of maturation.

The relatively low classification accuracy of the method for stage-2 squid (81% for males) probably is due to the wide size range of these individuals in the fall and early winter, and to the unusual gonad development of the larger individuals. Sexual regression by gonad resorption to a neutral or inactive state, though relatively common in bivalve molluscs (Sastry 1979), appears to be rare in cephalopods. Regression has been suspected in *L. pealei* (Summers 1971; Vovk 1972; Arnold and Williams-Arnold 1977) and possibly also in European common squid, *L. vulgaris* (Tinbergen and Verwey 1945), however. The phenomenon could explain why only a few of even the largest squid (20 cm and larger DML), thought to be in their second year (Summers 1971; Mesnil 1977), are mature in the late fall and early winter offshore samples. Lacking a reliable means of aging this species, it is not currently possible to prove that the larger "regressed" squid are in fact older than their smaller developing stage-2 counterparts. It is also possible that sexual maturation merely halts at the onset of winter and resumes again in January or February prior to onshore migrations. This hypothesis does not explain why the gonads appear to be shrinking, nor would it account for the presence of both very large and small squid at the same stage of development. Unfortunately, *L. pealei* has not been held sufficiently long in captivity to confirm either supposition.

Comparisons with Other Classification Methods

The main assets of the classification method presented here are its objectivity and its ease and speed of use. To be sure, the development took considerable time, especially interpretation of early cluster analyses, but the basic strategy is relatively straightforward and does have internal accuracy checks. If a more detailed breakdown of the maturation process were desired, e.g., to examine details of gametogenesis, the same analysis techniques could be applied to objectively identify and separate the various phases. Thus the methodology should be applicable to a wide range of biological problems.

When compared with several other classification systems, the advantages of the present method become more evident. Two basic classes of systems exist. Those used by Tinbergen and Verwey (1945), Holme (1974), Juanicó (1979), and Hixon (1980) are mainly qualitative, in that the presence or absence of one or more characters, considerations of color or texture, and estimation of relative sizes or gamete abundance are used. The other group of classification schemes, typified by those of Mangold-Wirz (1963), Hayashi (1970), Vovk (1972), and Durward et al. (1978) are quantitative methods. These schemes may employ one or more subjective judgments or estimates, but rely mainly on objective characters such as relative organ lengths or weights, egg diameters, or spermatophore lengths (absolute or relative) to distinguish successive maturity stages. Both types of classification systems are of value, but only the latter group will be discussed further because their methods are more objective and perhaps more suitable for large-scale applications.

There are obvious similarities between this classification and that of Vovk (1972), and it appears that, except for stage-5 spent squid, the maturity stages are comparable in both systems. It should be reiterated, however, that the stage characteristics and average parameter values given in this report (Fig. 3a, b; Table 3) were identified after the classification functions had been determined, whereas in the Vovk system the stage indicators form the basis for classification. The offshore squid sampled by Vovk also appear to have been larger by at least 3-5 cm at each stage than those used in this study. Thus, NGL indices in the Vovk study are 6.4-25% greater than those given in Table 3. A TLI, surprisingly, was not used. Vovk did employ hectocotylus length, but in *L. pealei* the hectocotylized arm may be difficult to identify, particularly in small males, and, more important, may be lost or damaged during trawl capture of squid. These and other smaller differences between the methods appear to result mainly because Vovk did not sample the actively spawning inshore population. His method generally works well for experienced personnel, but dissection and weighing of the reproductive tracts and weighing the whole squid take more time and equipment than may be available in the field. At least two more characters must be recorded for each sex as well.

Two simple but objective classifications were developed for Japanese squid, *Todarodes pacificus* (Hayashi 1970) and for female short-finned squid, *Illex illecebrosus* (Durward et al. 1978). Hayashi computed a numerical index, M, equal to the weight of Needham's sac (NW) divided by NW plus the testis weight, or to the oviduct weight (odW) divided by odW plus the ovary weight. If the computed value of M is <0.5, equal to 0.5, or between 0.5 and 1.0, the squid is considered immature, mature, or spent, respectively. Since spent *L. pealei* are at least rare, the system reduces to a two-stage classification which offers no obvious advantage over the immature-mature distinction used by Summers (1968). The dissection and weighing of the two tissues are additional drawbacks.

Durward et al. (1979) used data initially obtained from six *I. illecebrosus* which matured in captivity but had not spawned to develop a five-stage maturity scale for females. These investigators showed (by scatter plots and regression analyses) that relative NGI's correlated well with identifiable stages of ovarian development. Thus only two parameters, DML and NGL, were needed for classification. As Durward et al. have pointed out, the critical values of the NGI for *Illex* for the first four stages are very similar to those for *Loligo* (Table 3). However, in *L. pealei* NGI ranked last of four variables in importance (Table 2), and even the most significant variable (AEO) alone yielded only 77.8% correct classification accuracy. This simple and objective classification for *Illex* is now widely used (Amaratunga and Durward 1979).

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