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Biological, Oceanographic, and Acoustic Aspects of The Market Squid, Loligo Opalescens Berry

Edited By
Conrad W. Recksiek
Moss Landing Marine Laboratories
Moss Landing, California
and
Herbert W. Frey
California Department of Fish and Game
Long Beach, California
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ABSTRACT

This report describes the results of 3 years of research on the market squid, Loligo opalescens. A number of questions concerning this species were addressed by this research program; particularly those concerned with fisheries management. Areas of study included spermatogenesis and oögnesis; age and growth; feeding dynamics and prey of market squid; marine fish, bird, and mammal predators of this species in Monterey Bay; assessment of total population size and structure (number of stocks), and possible morphological indicators of stocks; the properties of market squid with respect to acoustic identification and quantification; and the relationship of the availability of this species to the commercial fishery in relation to the physical oceanographic conditions in Monterey Bay.

IN MEMORIAM

This publication is dedicated to the memory of Thomas William Thompson, 1936–1977.

Tom was an initial Principal Co-Investigator of the Sea Grant/California Department of Fish and Game Squid Research Program. Without his abilities, enthusiasm, hard work, knowledge, and determination, this program would not have existed. Tom was one of those rare individuals who could ascertain problems in a field of applied science, in this case fishery management, and develop a research program at the academic level to help solve these problems. In addition, he had the talent and dedication to find funding for such research projects, and the administrative talent to see they were initiated.

ACKNOWLEDGMENTS

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We take this opportunity to express our thanks and appreciation to the members of the Squid Program Scientific/ Technical Panel for their suggestions, guidance, and support. Members of this panel are John Radovich, California Department of Fish and Game; John Royal, Fishermen's Union ILWU—Local 33, San Pedro, California; Susumo Kato, National Marine Fisheries Service; William Summers, Western Washington State College; and Gilbert Voss, University of Miami.

There are so many individuals to whom we are indebted for their help that it is not possible to list them without committing the sin of omission. Our thanks to members of the San Jose State University Foundation; the University of California Sea Grant Program; the Moss Landing Marine Laboratories; the California Department of Fish and Game; the Sea Grant Marine Advisory Program; the Marine Research Committee; the CalCOFI Committee; San Francisco State University; and California State College, Stanislaus.

Conrad W. Recksiek and Herbert W. Frey

1. BACKGROUND OF MARKET SQUID RESEARCH PROGRAM, BASIC LIFE HISTORY, AND THE CALIFORNIA FISHERY

CONRAD W. RECKSIEK
Moss Landing Marine Laboratories
Moss Landing, California
and
HERBERT W. FREY
California Department of Fish and Game
Long Beach, California

1.1. INTRODUCTION

The market squid, Loligo opalescens, has been the object of a fishery in California since at least 1863 when Chinese fished for this species near Monterey. While there has been a continuous harvest of this species, it has been relatively small and the market squid has been considered the last, readily marketable, underutilized resource in the California Current System. Fisheries experts have estimated the California fishermen could expand their harvest of this species, presently about 15,000 tons (Frey, 1971), to as much as 300,000 tons (Gulland, 1971). For many years fishery scientists have advocated an expanded fishery for the market squid (Baxter *et al.*, 1968; Voss, 1973). Increased interest in squid fisheries in recent years has reinforced their belief.

While there was little question that increased catches of market squid could be taken on a sustained basis, the quantity of this increase had not been realistically estimated. Fields (1965) provided a good basic understanding of the biology of market squid; however, many questions remained unanswered. In order to manage the fishery for this animal on a sustainable basis as well as insuring adequate quantities being available to maintain predator populations, some of these questions had to be answered. More knowledge of squid spawning areas and the ability to predict spawning on the basis of oceanographic conditions was needed. A method for estimating total size of the market squid population had to be developed and its structure (i.e. number of stocks involved) determined. We needed to know the longevity of L. opalescens, the age at which it matures sexually, and the number of times it is capable of reproduction. It was necessary to determine the impact of an increased squid harvest upon other living marine resources by investigating what L. opalescens consumes, with what it competes, and to what extent other species are dependent upon this species as a food source.

In order to provide some of the needed answers, the Moss Landing Marine Laboratories and the California Department of Fish and Game

initiated a cooperative research program during the summer of 1973. In addition to basic funding (salaries, vessels, etc.) by both organizations, this research was funded by the Marine Research Committee of California, the State of California (Tideland oil revenues), and the University of California Sea Grant Program. Five interdisciplinary research teams were established to attack the problems outlined above from the viewpoint of: squid productivity in the California Current System, chemical and physical oceanography as related to squid spawning and larval distribution, population biology of L. opalescens, reproductive biology, and food chain analysis. In addition to scientists representing the basic areas of experimental biology, invertebrate and vertebrate zoology, plankton analysis, and physical and chemical oceanography, expertise in fisheries management was also represented in this team approach. Research proceeded in the five basic areas of productivity studies, population biology, reproductive biology, descriptive oceanography, and food chain analysis.

The results of this research are reported in this bulletin.

1.2. BASIC LIFE HISTORY

The market squid, Loligo opalescens Berry, is a small coleoid cephalopod of the family Loliginidae (Order Teuthoidea, Suborder Myopsida), occurring from British Columbia to central Baja California. Adults may reach a length of about 12 inches (approximately 300 mm) including the eight arms and two tentacles. While this species may be found in offshore areas (Mais, 1974), it spawns in nearshore areas. Mating-spawning aggregations occur most frequently during winter in the southern part of the range and progressively later in the season northward. After copulation the female will extrude 20 to 30 gelatinous egg capsules, each containing 200 to 300 eggs (Fields, 1965). Each capsule, measuring 5 to 20 cm, is provided with an adhesive stalk. Several hundred egg capsules may be attached to the same spot to form a large cluster. These clusters may be scattered over several acres of inshore sea floor.

Larval squid hatch in 3 to 5 weeks (Fields, 1965; McGowan, 1954). The well developed hatchlings measure about 3 mm overall. Squid in varying stages of development have been encountered from the surface to bottom over the continental shelf and beyond. They are frequently observed in coastal waters, or over deep areas in proximity to the continental shelf or to offshore banks and islands. They are rarely encountered in estuaries but have been observed frequently in Puget Sound.

Principal prey items include small fishes, smaller squid, and planktonic crustacea, and in turn market squid are prey to a host of fishes, birds, and mammals.

1.3. THE CALIFORNIA MARKET SQUID FISHERY

Fishing for market squid was carried on in the southern bight of Monterey Bay around 1863 by Chinese who sun-dried and exported the catch primarily to China. These fishermen employed torches to attract schools, in conjunction with small purse seines. Around 1905 Italian fishermen began using lampara gear which is still the standard in the Monterey Bay fishery.

The fishery primarily is conducted throughout the Southern California Bight, including the channel islands, and in the inshore southern part of Monterey Bay between Pt. Pinos and Fort Ord.

At stated above, the Monterey fishery employs lampara gear almost exclusively. A seine skiff is used and fishing is carried out during the early morning hours from before first light to several hours after dawn. Use of lights is not permitted in Monterey Bay. Squid generally are on the spawning grounds between April and November. The catch peaks around June, but occasionally a noticeable "fall run" occurs which reaches its maximum around November.

In southern California squid are taken by several types of fishing methods and gear. One fishery relies primarily on attracting the squid by night-light. Upon anchoring on a suitable ground and/or locating a concentration of squid, incandescent lamps in the rigging of the fishing vessel are switched on to attract and concentrate the animals so they can be brailed or pumped aboard. The southern California fishery, in contrast to the Monterey fishery, is spread over a vast area. In general the grounds are inshore, of good water quality, often sheltered somewhat from the prevailing weather, and/or in proximity to marked submarine topographic features (e.g. La Jolla Submarine Canyon). Areas consistently fished include waters about Santa Rosa, Santa Cruz, and Santa Catalina Islands and various spots near Pt. Dume, Pt. Mugu, La Jolla, etc.

The time of night that squid are landed depends upon if and when the squid "float" close enough to be loaded aboard easily. This can occur between sundown and sunrise, but the hours before dawn seem most productive. Fishing is generally best on the darkest nights. In southern California the fishing season is generally during the winter months. Most fishing takes place December through April.

Squid in southern California are often taken by purse seine. Although purse seiners are normally dedicated to other fisheries such as northern anchovy, jack mackerel, Pacific mackerel, etc., some boats will pursue squid when conditions, economic and otherwise, are suitable. Some squid are taken for live bait for use by the commercial passenger fishing vessel (partyboat) industry. They are considered outstanding bait for many of the fishes taken by recreationalists. Kato and Hardwick (1975) have published a detailed decription of the California squid fishery.

Demand for Loligo opalescens is rising, but certain factors may inhibit or slow demand in the future. While Monterey fishermen presently can market most or all of their catch, southern California fishermen are probably influenced in some degree by the processors' ability to accept available fish. This is likely due to processors' being involved in fisheries other than squid. The fishermen themselves may also regard squid fishing as supplemental to other fisheries, e.g. swordfish and albacore. Foreign interest in squid is strong, but Loligo opalescens may not be as attractive as other squid species to frozen squid markets because of its small size.

If squid importing countries begin to accept smaller frozen squid, then there appears to be a considerable potential for an expanded fishery for Loligo opalescens.

2. A STUDY OF SPERMATOGENESIS IN THE SPAWNING POPULATION OF THE SQUID, LOLIGO OPALESCENS

THOMAS M. GRIEB*
and
ROBERT D. BEEMAN
San Francisco State University
San Francisco, California

2.1. INTRODUCTION

The reproductive systems of male cephalopods have long been studied because of their morphological and functional intricacies. The reproductive system consists of a single testis and accessory organs. Sperm produced in the testis pass through accessory organs and are packaged into spermatophores, each of which contains millions of densely packed spermatozoa. During copulation the male uses a specialized, hectocotolized arm to pass spermatophores into the mantle of the female, where they ejaculate and release the male gametes.

The anatomy of reproductive structures and functional morphology of spermatophores has been well documented (Marchand, 1907; Williams, 1909; Drew, 1919a,b; Blancquaert, 1925; Austin, Lutwak-Mann, and Mann 1964; Mann, Martin, and Thiersch, 1970).

Past studies of cephalopod spermatogenesis have been limited to the light microscope level. Franzen (1955, 1967) reviewed spermiogenic events and sperm structure in several cephalopods and discussed the significance of spermiogenesis as a systematic tool within the class. Austin *et al.* (1964) gave a brief description of sperm of the squid Loligo pealei using phase-contrast microscopy. Fields (1965) described the structure of living, mature sperm of Loligo opalescens.

The objective of our work was to provide information concerning spermatogenesis in the market squid, Loligo opalescens. As a result of these studies, an electron micrographic study of the spermiogenic process has been presented (Grieb, 1976). Study results presented in this paper describe the maturation process in a spawning population of L. opalescens.

* * Present Address: Tetra Tech, Inc.
Lafayette, California

2.2. MATERIALS AND METHODS

Market squid used in this study were collected from waters just off Catalina Island, California, and from Monterey Bay, California (Table 1). All specimens were taken by either handline and jig or by dip net.

Testicular tissue used for study of structure was fixed in sea-water Bouin's solution and embedded in paraffin. Sections were cut at 5.0 [u]m, and stained with haematoxylin and eosin.

TABLE 1
Collection Data for *Loligo opalescens* Examined in this Study

Maturity group	*Dorsal mantle length (mm)*	*Collection date*	*Collection area*
I	96		
II	115		
II	143	August 1974	Monterey Bay
III	130		
I	118		
I	135		
I	138		
II	115		
II	120		
II	128	February 1975	Catalina Island
II	139		
II	145		
II	145		
II	149		
II	149		
I	139		
II	151	June 1975	Monterey Bay
III	101		
III	137		
III	131		
III	135	February 1976	Catalina Island
III	159		
im	75		
im	80		Monterey Bay
im	81		
im	85		

*TABLE **1***
Collection Data for** Loligo opalescens **Examined in this Study

For all other optical and transmission electron microscopic work, testicular tissue or spermatophores were cut into small pieces approximately 1 mm^3 in size and fixed in cold 3.0% glutaraldehyde buffered with 0.1 M Sorensen's phosphate buffer, pH 7.3, with 0.4 M sucrose added. The tissue was allowed to remain in chilled glutaraldehyde buffer for periods ranging from 1 hour to 2 weeks. Once removed from glutaraldehyde solution, the tissue was rinsed three times in buffer and sucrose solution and post-fixed 90 minutes at 4 C in 1.0% osmium tetroxide with 0.1 M buffer and 0.45 M sucrose. The tissue was then rinsed in cold water, dehydrated in a graded alcohol series, and placed in three changes of propylene oxide for 10 minutes each. It was then placed in a 1:1 mixture of propylene oxide and

Epon 812 for an infiltration period of 24 hours. Finally, the tissue was embedded in Epon 812 and cured under vacuum for 36 hours at 60 C.

Testicular tissue from 26 specimens was examined using the light microscope to determine the degree of sexual development. This tissue was sectioned at 0.5 [u]m with glass knives on a Reichert Om U2 ultramicrotome. Sections were transferred to glass slides and dried. They were then stained for 1 to 5 minutes in a 0.5% Toludine Blue, 1.0% sodium borate (borax) solution.

For transmission electron microscopy, thin sections were cut with both diamond and glass knives on a Reichert Om U2 ultramicrotome. The sections were mounted on 300 mesh copper grids and stained for 30 minutes in 2% aqueous uranyl acetate, and for 1.5 minutes in lead citrate. The grids were examined on a Zeiss EM 10 electron microscope.

2.3. RESULTS

2.3.1. Structure of the Reproductive System

The reproductive system of the mature male squid fills up the largest portion of the coelomic space, and occupies approximately the entire posterior third of the animal's body. Reproductive organs of large, mature males account for 5 to 7% of the total body weight of L. opalescens.

The testis of L. opalescens is a long white organ which appears flattened and ovate in cross section (Figures 1 and 2). The testis is medially located and suspended from the upper wall of the posterior portion of the coelom. The other organs of the reproductive system, the vas deferens, spermatophoric organ, vas efferens, spermatophoric or Needham's sac, and penis, are laterally located on the left side of the body (Figure 1).

The testis is composed of hundreds of separate tubules which are arranged with their axes approximately perpendicular to the surfaces of the organ (Figure 2). The individual tubules empty into the longitudinal lumen of the testis, which in turn opens into the coelom. The testicular tissue is bathed by the genital aorta which passes from the systemic heart into the dorsal portion of the organ where it becomes branched.

Mature sperm are drawn from the longitudinal lumen of the testis and coelom, via a ciliated funnel, into the vas deferens. The mature cells then enter the ampulla whose walls are muscular and bear large numbers of cilia and microvilli. After leaving the ampulla, the sperm continue anteriorly through the vas deferens to the spermatophoric organ, where they are encased into spermatophores by the secretory action of the organ's walls. These spermatophores are complicated structures consisting of the sperm mass and an ejaculatory apparatus; both are surrounded by several membranes and tunics. From the spermatophoric organ, the spermatophores enter the vas efferens and pass posteriorly to the spermatophoric or Needham's sac, where they are neatly stored before being passed from the penis to the female during copulation by the hectocotylized left ventral arm.

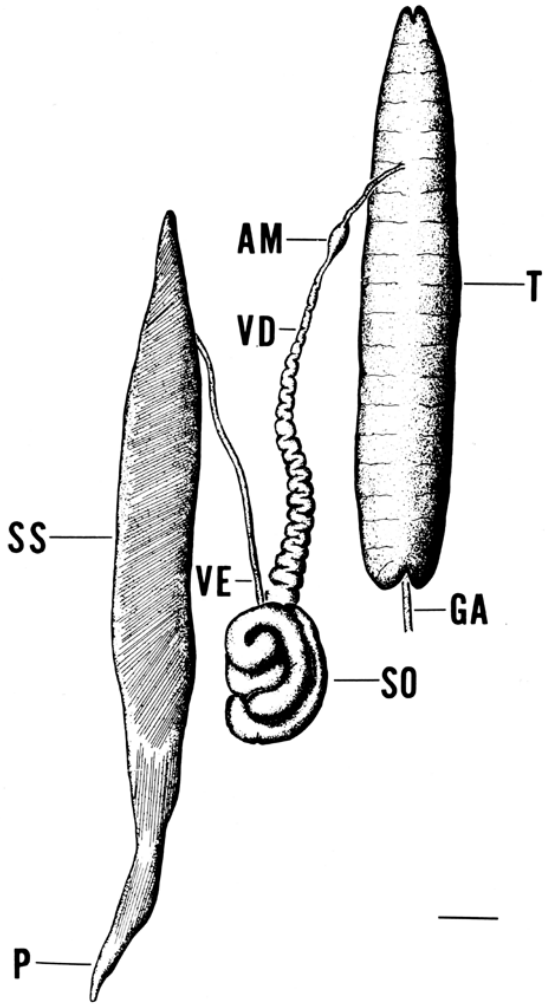

FIGURE 1. Reproductive system of the mature male squid. The testis (T) is medially located and suspended from the upper wall of the posterior portion of the coelom. The other organs of the reproductive system, the ampulla (AM), vas defferens (VD), spermatophoric organ (SO), vas efferens (VE), spermatophoric or Needham's sac (SS), and penis (P) are laterally located on the left side of the squid's body. The testicular tissue is bathed by the genital aorta (GA). Scale line 2 mm.

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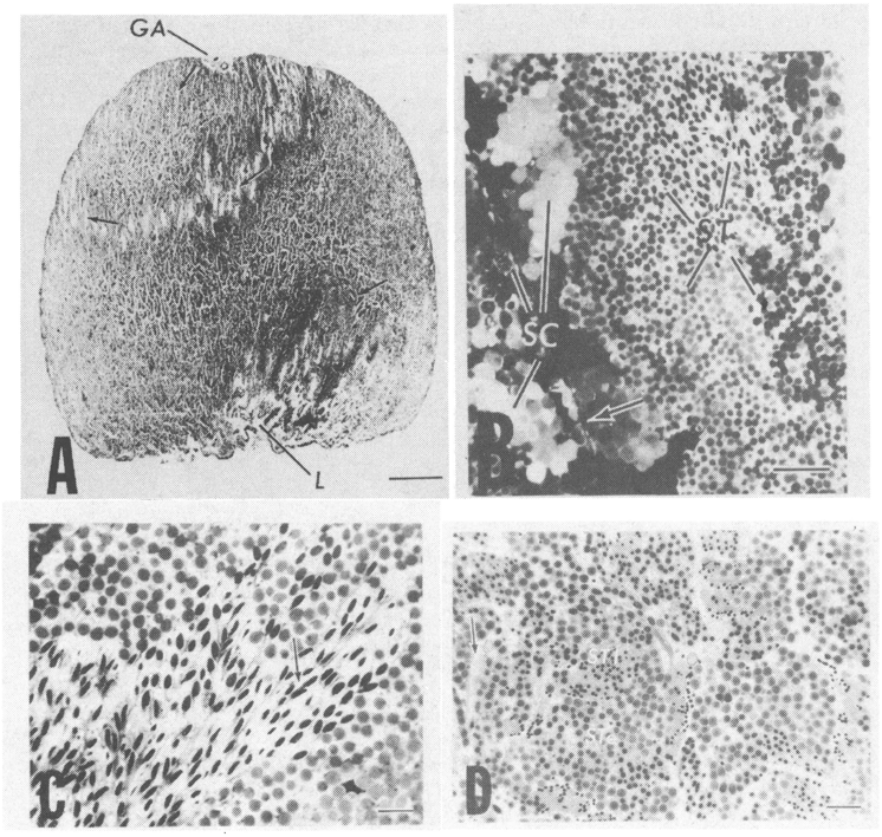

FIGURE 2. **Testicular tissue. *A.* Cross section approximately midway along the length of the organ. Genital aorta (GA). Individual tubules (arrows) empty into the longitudinal lumen (L). Haematoxylin and Eosin. Scale line 1 mm. *B.* Longitudinal section through an individual seminiferous tubule of a Group I animal. Secondary spermatocytes (SC) and spermatids (ST). Toludine Blue. Scale line 10 μ. *C.* Lumen of seminiferous tubule of a Group I animal showing mature and maturing spermatids (arrow). Toludine Blue. Scale line 4 μ. *D.* Group II tissue. Early and late spermatids. Arrow denotes tubular wall. Scale line 5 μ.**

FIGURE ***2. Testicular tissue.*** *A.* ***Cross section approximately midway along the length of the organ. Genital aorta (GA). Individual tubules (arrows) empty into the longitudinal lumen (L). Haematoxylin and Eosin. Scale line 1 mm.*** *B.* ***Longitudinal section through an individual seminiferous tubule of a Group I animal. Secondary spermatocytes (SC) and spermatids (ST). Toludine Blue. Scale line 10 [u]m.*** *C.* ***Lumen of seminiferous tubule of a Group I animal showing mature and maturing spermatids (arrow). Toludine Blue. Scale line 4 [u]m.*** *D.* ***Group II tissue. Early and late spermatids. Arrow denotes tubular wall. Scale line 5 [u]m.***

2.3.2. Spermatogenesis in Spawning Animals

Spermatogenesis takes place within the individual seminiferous tubules, which are the functional units of the testis (Figures 2, 3, 4) Generally, the immature gametogenic cells lie along the edges of the tubular walls, while the mature spermatids occupy the innermost regions of the lumen (Figures 2 and 4). In the animals examined, walls of the seminiferous tubules appeared thin and elastic, and seemed structurally to serve only a deliminating function, simply facilitating the movement of mature sperm to the

longitudinal collecting lumen of the testis. No germinal cell growth was observed. Only cells representing the late stages of spermatogenesis were found within the seminiferous tubules. No primary spermatocytes were distinguished. Secondary spermatocytes were the most immature cells found; the testicular tissue of many specimens contained only maturing and mature spermatids within the tubules.

Secondary spermatocytes were found to exist in several distinct maturation stages. In the light-microscope preparations (Figure 2), three distinct cell types were distinguished based upon their staining reaction: 1) large,

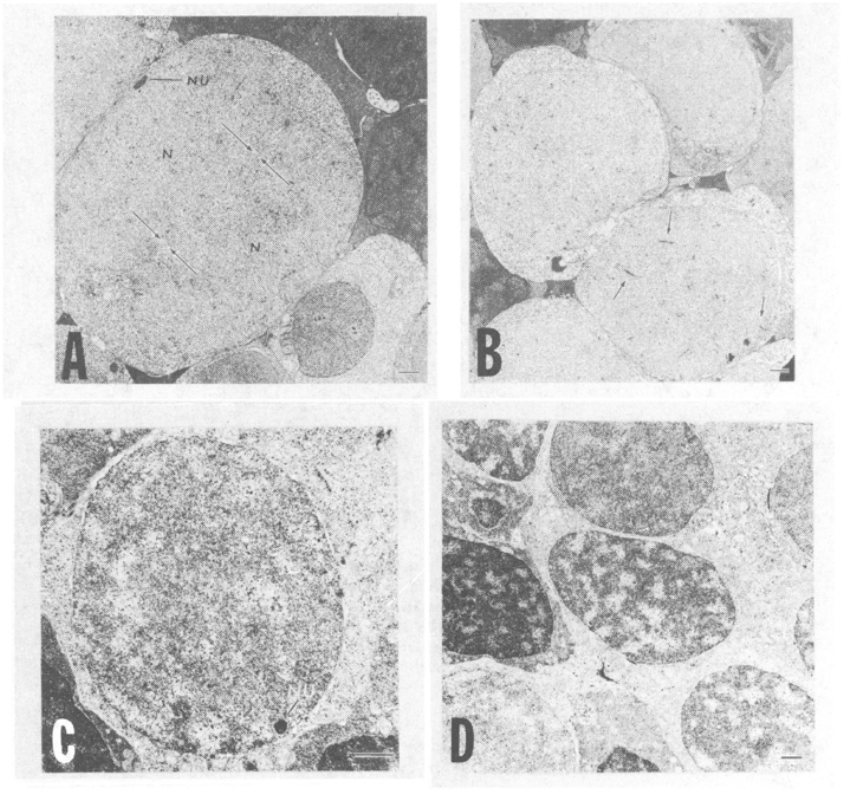

FIGURE 3. Electron micrographs of various spermatocyte stages. *A.* Spermatocyte which has just completed Telophase I. Arrows indicate the space separating the two nuclei (N). Nucleolus (NU). Scale line 1 μ. *B.* Spermatocytes after cytokinesis. Arrows indicate chromatin fibers. Scale line 1 μ. *C.* Secondary spermatocyte in early prophase II. Nucleolus. Scale line 1 μ. *D.* Secondary spermatocyte at a slightly later stage of chromatin condensation than shown in C. Scale line 1 μ.

FIGURE ***3. Electron micrographs of various spermatocyte stages.*** *A.* ***Spermatocyte which has just completed Telophase I. Arrows indicate the space separating the two nuclei (N). Nucleolus (NU). Scale line 1 [u]m.*** *B.* ***Spermatocytes after cytokinesis. Arrows indicate chromatin fibers. Scale line 1 [u]m.*** *C.* ***Secondary spermatocyte in early prophase II. Nucleolus. Scale line 1 [u]m.*** *D.* ***Secondary spermatocyte at a slightly later stage of chromatin condensation than shown in C. Scale line 1 [u]m.***

spherical cells in which the nuclei were only lightly stained, and hence indistinguishable from the cytoplasm; 2) spherical or elongated cells in which the nuclei stained more intensely and took on a mottled appearance; and 3) cells that stained so intensely that they appeared totally opaque, with all cellular structures masked.

Electron micrographs (Figure 3) of similar testicular tissue have elucidated the maturation stages of the secondary spermatocytes, and have helped to clarify the light microscope observations.

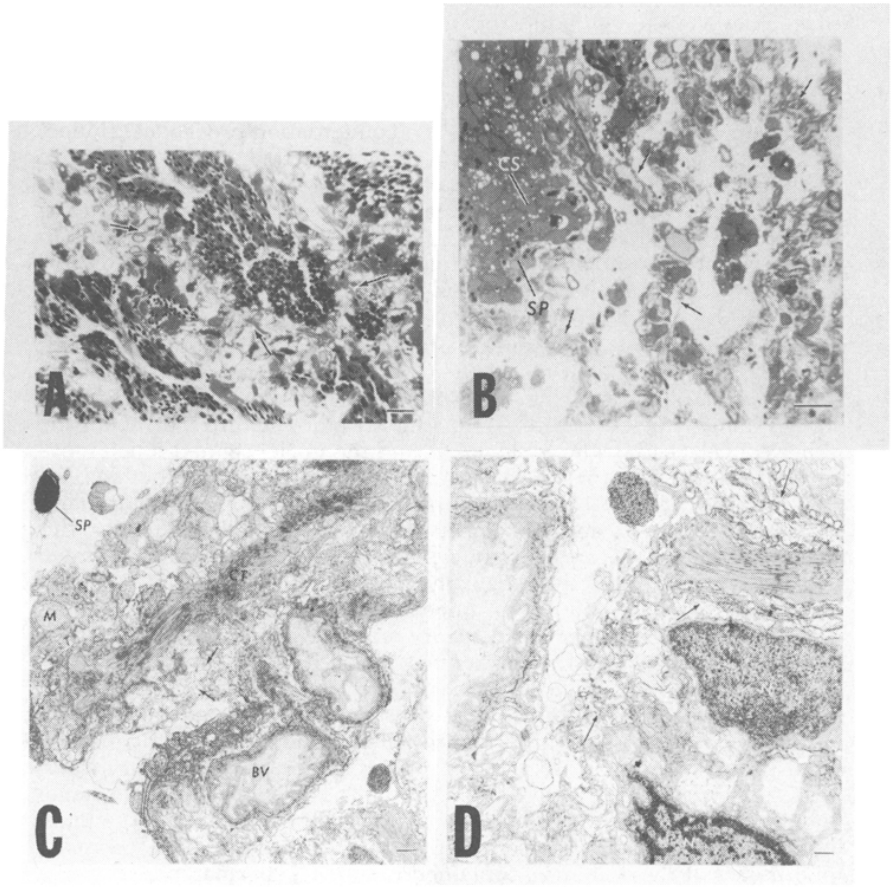

FIGURE 4. Section through the testicular tissues of Group III animals. *A.* Late spermatids near the lumen of a single tubule. Arrows indicate tubule walls. Scale line 5 μ. B. Further deterioration of seminiferous tubules which contain only a small number of relict spermatozoa (SP) surrounded by what appears to be sloughed cytoplasm (CS). Arrows indicate tubule walls. Toludine Blue. Scale line 5 μ. *C.* Electron micrograph. Seminerous tubule walls. Arrows indicate vacuoles. Blood vessels (BV). Connective tissue (CT). Mitochondria (M). Sperm (SP). Scale line 1 μ. *D.* Electron micrograph. Seminiferous tubule walls. Arrows indicate endoplasmic reticula. Nucleus (N). Scale line 0.5 μ.

*FIGURE **4. Section through the testicular tissues of Group III animals.** A. **Late spermatids near the lumen of a single tubule. Arrows indicate tubule walls. Scale line 5 [u]m. B. Further deterioration of seminiferous tubules which contain only a small number of relict spermatozoa (SP) surrounded by what appears to be sloughed cytoplasm (CS). Arrows indicate tubule walls. Toludine Blue. Scale line 5 [u]m.** C. **Electron micrograph. Seminerous tubule walls. Arrows indicate vacuoles. Blood vessels (BV). Connective tissue (CT). Mitochondria (M). Sperm (SP). Scale line 1 [u]m.** D. **Electron micrograph. Seminiferous tubule walls. Arrows indicate endoplasmic reticula. Nucleus (N). Scale line 0.5 [u]m.***

The large, lightly stained, spherical cells seen in light-microscopic preparations represent the earliest or least mature secondary spermatocytes. Electron micrographs of these cells (Figures 3A and 3B) show that they are in meiotic interphase. A binucleate arrangement was commonly seen in large aggregates of secondary spermatocytes (Figure 3A). At this stage, and at a slightly later stage when cytokinesis was completed (Figure 3B), the uncoiled chromatin was found to be diffuse, and existed in long, thin fibers characteristic of meiotic interphase chromosomes. Following cytokinesis, the interphase nuclei were approximately 9–11 [u]m in diameter and accounted for a disproportionately large share of the cell's volume.

At a slightly more mature stage, the chromatin of secondary spermatocytes had begun to condense. The condensation process continued, and Prophase II nuclei (Figure 3D) took on a mottled appearance due to the mixture of heterochromatin and dispersed euchromatin. This accounted for the mottled appearance of secondary spermatocytes in the light-microscope preparations (Figure 2B). Condensation of the chromatin continued, and presumably accounted for the very intensely stained spermatocytes seen in light-micrographs.

Within the seminiferous tubules developing spermatids were found to exist in various forms representing progressive stages of maturation (Figures 2C and 2D). In light-microscopic preparations, the early spermatids were seen as spherical cells ca. 4–5 [u]m in diameter with homogeneous, lightly stained nuclei (Figure 2D). In slightly more mature spermatids the spherical cells had begun to elongate, and the nuclei stained much more heavily due to condensation of chromatin. More mature spermatids (Figures 2C and 4A) continued the process of elongation; the chromatin became very dense, and cytoplasm began to be sloughed. Finally, this process produced mature sperm which passed into the longitudinal lumen of the testis and entered the coelomic space.

The 22 spawning animals examined were divided into three groups according to degree of sexual maturity or, more precisely, to the degree of development within their seminiferous tubules.

Group I consisted of five specimens of L. opalescens in which significant numbers of secondary spermatocytes were found within the seminiferous tubules (Figure 2B). This group represented the first stage in the maturation process found in spawning animals. Although secondary spermatocytes accounted for the major portion of the spermatogenic stages found within this group, seminiferous tubules also contained large numbers of maturing and, to a lesser extent, mature spermatids. The spermatophoric sacs of these animals contained functional spermatophores.

Group II consisted of 11 specimens in which maturing and mature spermatocytes represented the preponderant gametogenic stages (Figure 2D). Secondary spermatocytes were rarely found in these animals. The seminiferous tubules were typically filled with all stages of maturing spermatids. Immature spermatids were normally found aggregated in peripheral regions of the individual tubules, while mature spermatids were found near the center of the tubules (Figure 2D). The spermatophoric sacs were characteristically filled with spermatophores.

The remaining six specimens constituted Group III which represents the final stage of the maturation process in spawning animals. This group included animals in which only mature or very late, maturing spermatids were found within the seminiferous tubules (Figures 4A and 4B). Squid within this group were undergoing a degenerative process. While the seminiferous tubules of some animals contained relatively large numbers of mature spermatids (Figure 4A), the testicular tissue of other specimens was composed of empty or collapsed tubules which contained only a very small number of relict sperm (Figure 4B). In this latter stage, the lumen of tubules sometimes contained large amounts of what appeared to be sloughed cytoplasm and spherical, vacuolate bodies.

The walls of the seminiferous tubules of Group III animals were, for the most part, made up of connective and vascular tissue (Figure 4C). Except for long, thin, muscular fibers and the thick walled blood vessels which run through the seminiferous walls, the cellular structure of the tubules appeared rather nondescript.

Several nuclei could be distinguished in the cells comprising the tubular walls; many large mitochondrial bodies were dispersed throughout the cytoplasm, and relatively large amounts of rough endoplasmic reticula were present near the nuclei and surrounding the connective tissue (Figures 4C and 4D). However, the remainder of the cytoplasm was pervaded by small vacuoles.

In all Group III animals the seminiferous epithelium was found to be thin, and no visible signs of epithelial growth could be detected. Furthermore, the mantles of these animals were flaccid, and their general condition was best described as emaciated. Finally, the spermatophoric sacs of animals of this stage contained very few spermatophores.

The examination of testicular tissue from four nonspawning squid (Table 1), ranging from 75–85 mm DML, revealed a condition within the seminiferous tubules remarkably similar to those of Group I animals. In one squid there were no spermatophores present in the spermatophoric sac. Early spermatids were the most abundant cells found in the seminiferous tubules of the testis, and the only other cells observed were secondary spermatocytes. Spermatophores were noted in the spermatophoric sac and, as anticipated, developing spermatids were the only cells distinguished in the seminiferous tubules.

2.4. DISCUSSION

2.4.1. Spermatogenesis in Spawning Animals

Although it has been reported that market squid spawn once and subsequently die, no histological data exist in the literature to give credence to this generally accepted belief. This assumption has been based upon population data concerning growth rates and size frequencies, behavioral observations, and observations concerning changes in the external morphology of the mantle and reproductive organs of the spawning animals.

There currently exists some doubt as to the fate of spawning male squid. Summers (1969) has suggested that some specimens of male Loligo pealei

might not be subject to spawning related mortality after first spawn, and that they might survive to reach an age of 3 years. Fields (1965) deduced that female specimens of L. opalescens spawn once and die. Since he found a 1:1 sex ratio among immature and spawning populations, he concluded that males, even precociously mature individuals, also spawn once with death following.

Using information obtained from the study of spermiogenesis in spawning L. opalescens and observations of testicular tissue of nonspawning individuals, an alternative explanation of the maturation process and sexual cycle has been constructed. According to this hypothesis the three groups in which the spawning males were placed represent steps in the transition which takes place within the seminiferous tubules of an individual during the spawning process. Accordingly, Group I consisted of squid that had recently reached sexual maturity and were just beginning to spawn. The seminiferous tubules of these animals contained secondary spermatocytes (the earliest of the gametogenic cells found in any animals) as well as maturing spermatids. These animals were shown to be producing mature gametes, as noted by the presence of viable spermatophores within the spermatophoric sac.

According to the proposed explanation, all the secondary spermatocytes soon complete Telophase II, and these animals become members of Group II. This group or stage represents the apex of the maturation process, and squid in this group account for the greatest percentage and the most active members of the spawning population. In these animals, mature sperm produced by maturation of the spermatids is being rapidly packaged into spermatophores; the spermatophoric sacs of Group II animals were always found to be filled with viable spermatophores.

Group III animals represent the final stage in the spermatogenic process. The seminiferous tubules contain only late spermatids of mature spermatozoa, and there are usually very few spermatophores within the spermatophoric sac. Thus, with respect to the spawning population, Group III individuals represent functionally "spent" organisms. There were no spermatogonia or spermatocytes found within the seminiferous tubules of these animals. Further electron microscopic examination of the seminiferous epithelia revealed no signs of renewal of the gametogenic process. Instead, it suggested a degenerative process similar to senescence in testicular tissue of mammals (Bishop, 1970).

The renewal of seasonal or annual reproductive cycles in males has been reported for a wide range of organisms: echinoderms (Holland and Giese, 1965), molluscs (Ropes, 1968), birds (Engels, 1962), and mammals (Gier and Marion, 1970). In these organisms, spermatogenesis terminates seasonally or annually, the the seminiferous tubules may become tremendously reduced, yet in each case there exist primitive spermatogonia within the seminiferous epithelium.

Within the seminiferous epithelia of L. opalescens there was no indication of germinal cell growth or spermatogonia. This lack of any sign of a renewed gametogenic cycle, the degenerative condition of the seminiferous epithelia, and the emaciated condition of Group III animals suggests that males spawn only once and die soon afterward.

The 22 spawning male squid examined in this study represent a small sample; however, they represent a wide size range (Table 1) in which all of the animals examined displayed identical maturation and degenerative processes. In this context Group II specimens are of special interest. These animals varied in dorsal mantle length from 101–159 mm. According to Fields (1965) and Evans (1976), at least two age classes are represented in this group. Since the condition of testicular tissue was identical in each animal, the maturation process for all males, regardless of age class, appears to be the same. This indicates there is no possibility that some males mature precociously, survive the initial spawn, and spawn again the following spawning period or year.

A functional analogue to the pineal-hypothalmic-pituitary-gonad system for the control of sexual maturity in vertebrates has been described for cephalopods. Wells and Wells (1959, 1969, and 1972) have shown that the onset and maintenance of sexual maturity in octopods is controlled by a hormonal secretion of the optic glands, which are in turn controlled by the central nervous system. *In vitro* experiments (Richard, 1970) have shown that the testes of Sepia, a decapod, develop normally when cultured together with actively secreting optic glands, but when deprived of the optic gland material, division of the spermatogonia is halted, even though spermatocyte division continues. Thus, in a short period of time, spermatogenesis is effectively terminated. Experiments by Richard (1967) and Wells and Wells (1959) suggest that day length is ultimately involved in the control of optic glands by the central nervous system, and that increased temperature also accelerates the onset of sexual maturity.

A model of the observed spawning activity of L. opalescens can be constructed, which includes a mechanism for the regulation of sexual maturity, and which is consistent with histological data presented in this study. According to this model: 1) the initiation of sexual maturity is the result of a hormonal pulse regulated by environmental factors, and 2) individual squid are not fully mature over the entire spawning season, but only for relatively short periods of time.

Histological data presented in this study demonstrate that the cell divisions leading to production of mature sperm in individual L. opalescens are almost synchronous. This suggests that the mechanism controlling sexual maturity functions for only a short period of time during the initiation of mitotic divisions leading to production of primary spermatocytes, or more likely, the intiation of the meiotic phase of sperm production. In any case, there is no maintenance of the spermatogenic process, and since the spermatophoric sacs of Group III animals contained substantially reduced numbers of spermatophores, it follows that males have a finite period of sexual productivity, which is directly related to the time it takes for spermiogenesis to be completed and the period of time spermatophores can be stored in the spermatophoric sac.

Environmental constraints, such as day length and water temperature, working upon the hormonal control mechanism insure the simultaneous sexual maturation of a large portion of the population. According to this model, these factors account for the observed peaks in spawning activity.

3. HISTOLOGICAL OBSERVATIONS ON OÖGENESIS IN LOLIGO OPALESCENS

JAMES H. KNIPE
and
ROBERT D. BEEMAN
San Francisco State University
San Francisco, California

3.1. INTRODUCTION

The general structure of the female reproductive system in the market squid, Loligo opalescens Berry, is well known (Fields, 1965); however, some details of physiology and histology of oögenesis are not currently understood.

The reproductive system in female squid consists of an ovary that is suspended by connective tissue mid-laterally and dorso-posteriorly in the body cavity. Mature oöcytes leave the ovary, enter the internal membranous oviduct, and then pass to the external glandular oviduct on the left side of the body where they exist. Nidamental and accessory nidamental glands are located dorso-anteriorly in the body cavity, and function in conjunction with the external glandular oviduct to produce the egg case in which fertilization takes place at spawning.

Oögenesis in a species of Loligo was studied by Lankester (1875) in Naples, Italy. Prior to 1875, studies were hampered by primitive techniques and microscopes of low resolution. Since then, oögenesis in various species of cephalopods has been studied by Bergmann (1902) and Yung-Ko-Ching (1930). These authors described ovarian structure and the stages of oögenesis. Recent investigations concerned with maturation in squid included those of Cowden (1968) on Loligo brevis; Burukoskii and Vovk (1974), and Selman and Arnold (1977) on Loligo pealei; Takahoshi and Yahata (1973) on Todarodes pacificus; and Bottke (1974) on Alloteuthis subulata. Raven's (1966) review of molluscan reproduction also included material on this subject.

Early authors describing oögenesis did not identify divisions between various events. Various researchers, using light microscopy, have divided these events, but justification for stage division did not exist until the study of Selman and Arnold (1977) using electron microscopy. In that paper, the authors stated their belief that earlier studies included an excessive number of maturation stages, and thus, they limited their division of maturation stages to five.

A fundamental question concerning cephalopod reproductive biology is whether squid are capable of spawning more than once. Previous research has primarily emphasized studies of mature animals. It has become clear from a review of the literature that in order to fully resolve the question of subsequent spawnings, juveniles must be sought and examined and the findings compared with data derived from adults.

3.2. MATERIALS AND METHODS

The work presented here was carried out on L. opalescens using 35 animals ranging from 11 to 143 mm in dorsal mantle length that were collected off the California coast from Monterey Bay, and from the coastal waters of southern California between Point Conception and the Mexican border. Squid were collected using mackerel, squid jigs, dipnets, midwater trawls, and plankton nets. Collection was carried out almost exclusively on board the California Department of Fish and Game vessel ALASKA. Squid were collected at various times between June 1975 and June 1977. Dorsal mangle length (DML) was used as a standard for size determination and, unless otherwise noted, all measurements of gross body size given in this paper refer to dorsal mantle length. Measurements were made on both live and fixed specimens. Without exception ventrally excised ovaries and, in the case of smaller squid, whole animals were fixed immediately in seawater Bouin's at ambient air temperature.

Fixed tissue was prepared from 35 different animals. In animals over 70 mm, the tissue was randomly selected from the ovary. Clearing and embedding was carried out according to standard histological techniques. Specimens were embedded in Paraplast (56.5°C) and sectioned at 3–7 [u]m on a Leitz microtome. Sections were adhered to glass slides by drying on a slide warmer. Staining with Harris' hematoxylin and counter staining with eosin was carried out following Humason (1972). Additional slides were prepared utilizing Heidenhain's hematoxylin stain (Galigher and Kozloff, 1971) and others by Periodic Acid Schiff reagent for polysaccharides. All sections were examined and measurements made using a Wild M20 compound light microscope with an American Optical reticle calibrated with a Bausch and Lomb stage micrometer. Photomicrographs were taken utilizing a Zeiss Photomicroscope III and a Rechiert Photoautomatic mounted on a Wild M20 compound microscope.

3.3. RESULTS

3.3.1. Stages of Oögenesis

Oögenesis begins when primordial germ cells differentiate and become primary oögonia. Primary oögonia enlarge and become secondary oögonia which in turn become oöcytes. Oögenesis can be divided into six stages depending on the degree of follicular cell development in association with oögonia and oöcytes. These stages are briefly defined below and will be described later.

Stage Ia. Probable primordial germ cells or primary oögonia (Figure 5 A) are approximately 9 [u]m in length and appear to have no clearly defined cytoplasmic area.

Stage Ib. Secondary oögonia (ca. 16 [u]m in length) have a well defined nucleus with one or more nucleoli (Figure 5 B). The nucleus is surrounded by a thin layer of cytoplasm.

Stage Ic. Secondary oögonia are now larger (ca. 59 [u]m in length) and show an increased ratio of cytoplasmic to nuclear volume. Numerous nucleoli 1–5 [u]m in diameter are present (Figure 5 C).

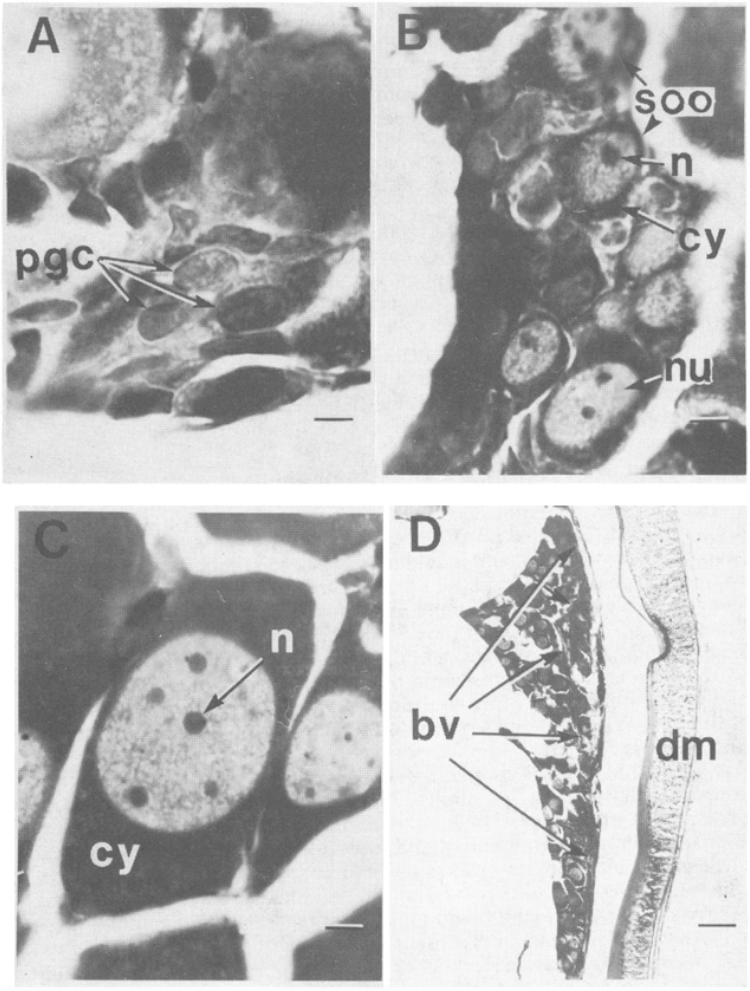

FIGURE 5. Preoöcytes in immature ovary. ***A.*** Probable primordial germ cells (oögonia) of State IA (pgc). Scale line 4.5 μm. ***B.*** Secondary oögonia (soo), cytoplasm (cy), nucleus (nu) and nucleoli (n) of Stage Ib. Scale line 5.3 μm. ***C.*** Enlarged secondary oögonia Stage Ic showing nucleoli and cytoplasm. Scale line 6.6 μm. ***D.*** Ovary from 11 mm DML squid with blood vessel (bv), oöcytes, dorsal mantle (dm). Scale line 56 μm.

FIGURE ***5. Preoöcytes in immature ovary.*** *A.* ***Probable primordial germ cells (oögonia) of State IA (pgc). Scale line 4.5 [u]m.*** *B.* ***Secondary oögonia (soo), cytoplasm (cy), nucleus (nu) and nucleoli (n) of Stage Ib. Scale line 5.3 [u]m.*** *C.* ***Enlarged secondary oögonia Stage Ic showing nucleoli and cytoplasm. Scale line 6.6 [u]m.*** *D.* ***Ovary from 11 mm DML squid with blood vessel (bv), oöcytes, dorsal mantle (dm). Scale line 56 [u]m.***

Stage II. The oöcytes contain a large germinal vesicle which is surrounded by an irregular corona. Follicle cells have attached to the oöcyte, and have begun to proliferate on its surface.

Stage III. The follicle cells have completely surrounded the oöcyte and are changing from squamous to cuboidal shaped cells.

Stage IV. Follicle cells continue to proliferate and begin to penetrate the oöcyte. Their morphology changes from cuboidal to columnar. Marking the end of their maximum penetration into the oöcyte is the formation of a syncytium.

Stage V. The syncytium formed by follicle cells is active in vitellogenesis and the formation of a chorion. The elaborated yolk forces the follicular syncytium to the periphery of the oöcyte. The end of vitellogenesis coincides with the end of this stage. The follicle cell-oöcyte complex appears laminar with the degenerating follicular syncytium located externally. The chorion, oöcyte cytoplasm, and yolk are present sequentially within.

Stage VI. Final degeneration of the follicular syncytium takes place and the mature oöcyte is ovulated.

3.3.2. Stages Found in Juveniles

An 11 mm specimen of L. opalescens contained an ovary that was well differentiated (Figure 5 D). Preoöcytes were in what is referred to here as Stage I development, and included primary and secondary oögonia. No synaptic stage oögonia were found. Some cytoplasm was observed in the late secondary oögonia but nuclei predominated. The cytoplasm of Stage I preoöcytes stained dark purple and the nuclei light purple, with Harris' hemotoxylin. In one median lateral section through the ovary, approximately 90 Stage I preoöcytes were counted. An ovarian blood vessel which had penetrated dorsally into the anterior portion of the ovary to about # of the ovary's depth before preceding posteriorly was also present in this section (Figure 5 D).

In a juvenile squid of approximately 24 mm the ovary was developed to approximately 2.2 mm in length. Numerous Stage I preoöcytes were present, but many Stage II oöcytes had also developed. Stage II (Figure 6 A) represents the attachment and multiplication of follicle cells on the surface of many oöcytes. This activity appeared to develop first at the vegetative pole of the oöcyte. The oöcyte nucleus was less dense than observed in previous stages and took up less than half the volume of the cell. An irregular corona was present around the nculeus (Figure 6 B) and was 10 [u]m at its greatest thickness. Nucleoli present from the beginning of this stage in immature squid, appeared to become larger and less dense with maturity. Their morphology was either in the form of a circle or a ring. Nuclei of large Stage II oöcytes (ca. 240 [u]m in diameter) were observed to shift toward the future animal pole.

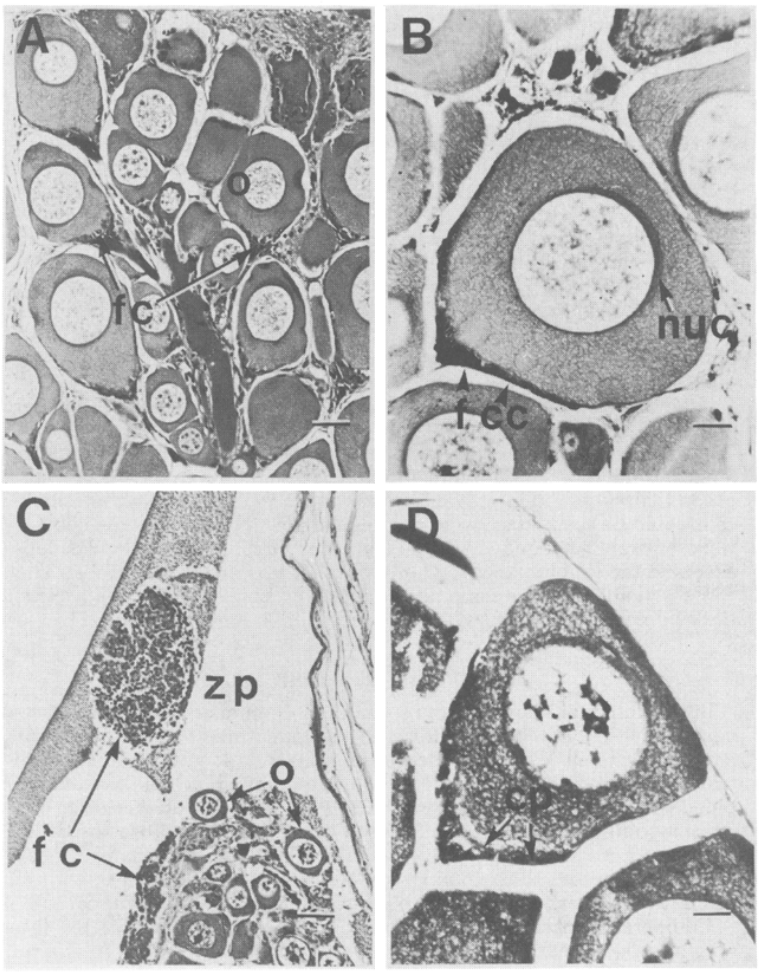

FIGURE 6. Stage II oöcytes and follicle cell proliferation. *A.* Attachment of follicle cells in Stage II. Follicle cells (fc), oöcytes (o). Scale line 137 μm. *B.* Stage II oöcyte with nuclear corona (nuc) and follicle cell cap (fcc) on vegetative pole. Scale line 64 μm. *C.* Zone of follicle cell proliferation (zp) displaced ventrally at the anterior portion of the ovary in a 35 mm DML squid. Scale line 61 μm. *D.* Capillaries (cp) between follicle cells in Stage II oöcytes. Scale line 27 μm.

FIGURE ***6. Stage II oöcytes and follicle cell proliferation.*** *A.* ***Attachment of follicle cells in Stage II. Follicle cells (fc), oöcytes (o). Scale line 137 [u]m.*** *B.* ***Stage II oöcyte with nuclear corona (nuc) and follicle cell cap (fcc) on vegetative pole. Scale line 64 [u]m.*** *C.* ***Zone of follicle cell proliferation (zp) displaced ventrally at the anterior portion of the ovary in a 35 mm DML squid. Scale line 61 [u]m.*** *D.* ***Capillaries (cp) between follicle cells in Stage II oöcytes. Scale line 27 [u]m.***

Proliferation of follicle cells appeared to originate in an area immediately anterior to the ovary. This zone of proliferation (Figure 6 C) was not located in the 24 mm specimen but was apparent in a 35 mm specimen. This oblong zone was ca. 247 [u]m in diameter. Contained in this specialized area were oblong cells with a long dimension of ca. 7 [u]m. The arrival and attachment of the follicle cells with each oöcyte appeared to coincide with the close proximity of a capillary. The capillaries could be seen between the follicle cells (Figure 6 D). The hemolymph filled capillary is formed as a result of the rebranching of the main blood vessel supplying the ovary. The capillaries may be necessary to maintain the follicle cells during oöcyte maturation. In subsequent stages, the oöcytes and associated capillaries came to be surrounded by supportive connective tissue. The ovary in this 24 mm animal contained Stage I and Stage II oöcytes which stained purple with Harris' hematoxylin. Blood and capillaries stained red with eosin and the Periodic Acid Schiff reaction was negative. As many as eight nucleoli were observed in some Stage II oöcyte sections.

Examination of a 70 mm animal revealed that oöcyte maturation remained in Stage II. The number of oöcytes in a midlateral section was 750. The greater number of oöcytes was associated with an increase in the length of the ovary to 12.5 mm. Oöcytes contained relatively more cytoplasm than observed in previous stages and in most cases a cap of follicle cells could be seen attached to the vegetal pole. However, some oöcytes were entirely surrounded by a single layer of squamous follicular cells except at the vegetative pole where the layer appeared to be double. The oöcytes maintained the same hematoxylin and eosin staining characteristics as described previously.

3.3.3. Stages Found in Adult Squid

Investigation of spawning and spent mature animals of 93–143 mm revealed ovaries cytologically different from immature ovaries. Since ovarian nests were all of the same degree of differentiation, the samples were taken from random locations. Nests are formed by branchlets of the main blood vessel supplying the ovary (Figure 7 A). Each branchlet leads to one preoöcyte in juveniles, or to an oöcyte in the case of mature squid. An extensive network of connective tissue was also visible in mature specimens and appears to surround each oöcyte in the ovary. In immature squid, the network appeared to be less extensive or was not apparent.

Comparative observations on spawning or spent squid revealed few Stage II oöcytes and no confirmed Stage I preoöcytes. However, the remaining stages of oöcyte maturation were seen. Stage III was characterized by continued proliferation of follicle cells which were seen to undergo a change in shape from squamous to cuboidal (Figure 7 B). Stage IV was reached (Figure 7 C) when the follicle cell complex appeared to penetrate the oöcyte, forming follicular folds. These were first formed on the vegetative pole. Connective tissue and blood vessels were incorporated into the follicular folds as they formed and penetrated the oöcyte.

The germinal disc of each oöcyte was displaced to the animal pole and the vegetative folds were seen to penetrate nearly to the disc surface

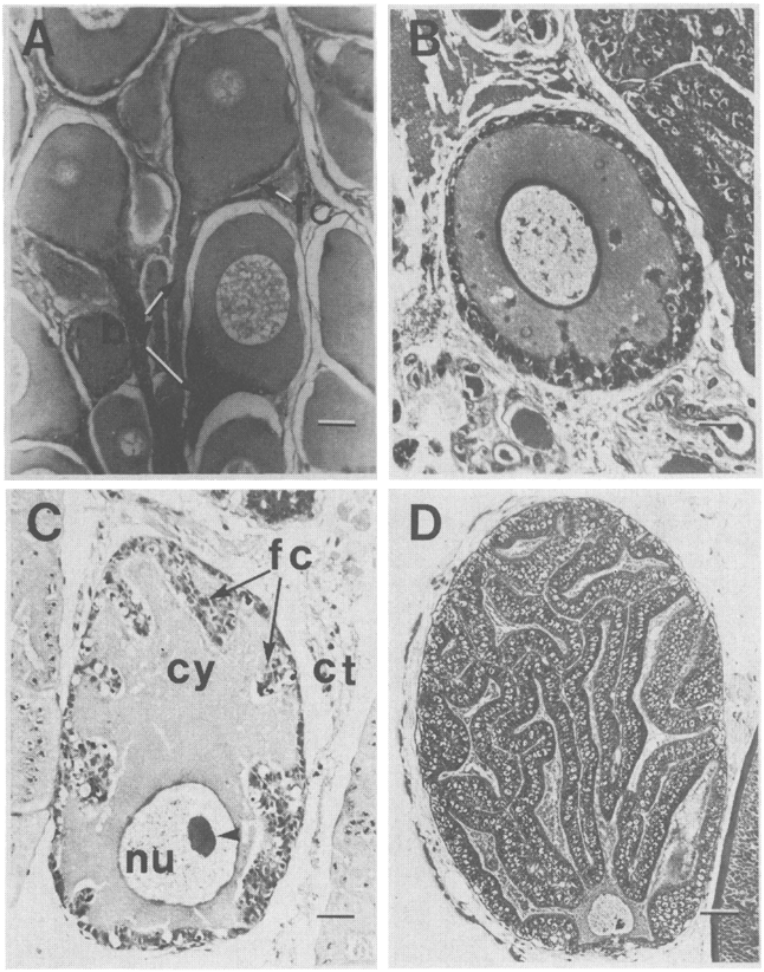

FIGURE 7. Stage II through Stage IV oöcytes and follicle cell proliferation. *A.* PAS negative oöcytes of Stage II with branching blood vessels (bv). Follicle cells (fc). Scale line 96 μm. *B.* Cuboidal follicle cells of Stage III. Section near animal pole. Scale line 34 μm. *C.* Initial penetration in Stage VI by follicle cells. Nucleus (nu), nucleoli (n), cytoplasm (cy), connective tissue (ct). Scale line 41 μm. *D.* Follicle cell displacement of nucleus to the animal pole. Scale line 127 μm.

FIGURE ***7. Stage II through Stage IV oöcytes and follicle cell proliferation.*** *A.* ***PAS negative oöcytes of Stage II with branching blood vessels (bv). Follicle cells (fc). Scale line 34 [u]m.*** *B.* ***Cuboidal follicle cells of Stage III. Section near animal pole. Scale line 34 [u]m.*** *C.* ***Initial penetration in Stage VI by follicle cells. Nucleus (nu), nucleoli (n), cytoplasm (cy), connective tissue (ct). Scale line 41 [u]m.*** *D.* ***Follicle cell displacement of nucleus to the animal pole. Scale line 127 [u]m.***

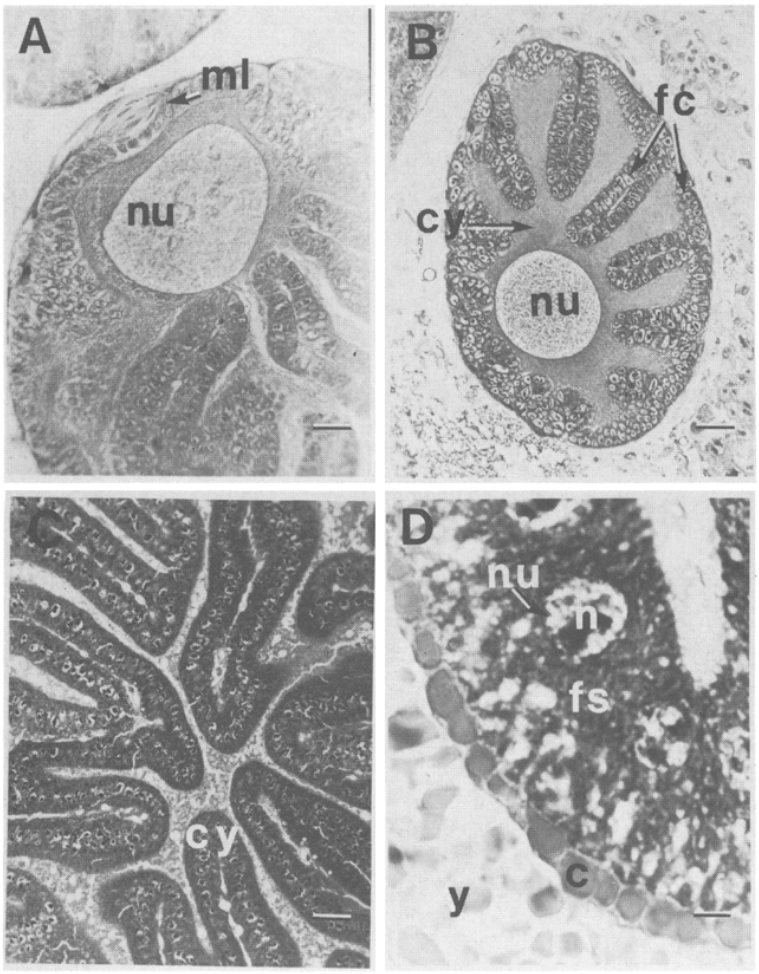

FIGURE 8. Stage IV and Stage V oöcytes. *A.* Micropyle lens (ml), nucleus (nu). Scale line 28 μm. *B.* Collumnar shaped follicle cells of Stage IV. Cytoplasm (cy), follicle cells (fc). Scale line 48 μm. *C.* Follicular syncytium (fs) at the end of Stage IV. Scale line 72 μm. *D.* Stage V follicular syncytium with the chorion (c) and yolk (y). Scale line 6 μm.

*FIGURE **8. Stage IV and Stage V oöcytes.** A. **Micropyle lens (ml), nucleus (nu). Scale line 28 [u]m.** B. **Collumnar shaped follicle cells of Stage IV. Cytoplasm (cy), follicle cells (fc). Scale line 48 [u]m.** C. **Follicular syncytium (fs) at the end of Stage IV. Scale line 72 [u]m.** D. **Stage V follicular syncytium with the chorion (c) and yolk (y). Scale line 6 [u]m.***

(Figure 7 D). The deeply penetrating folds were accompanied by numerous smaller folds that penetrated less deeply along the periphery of an oöcyte. On the surface of a given oöcyte, a depression was formed at the animal pole. In this depression a lens shaped thickening was present which would later include the micropyle (Figure 8 A). The nucleus appeared uniformly granular. From the beginning of Stage IV, follicle cells elongated and took on a columnar appearance (Figure 8 B). At their maximum height and penetration, follicle cells were observed to occupy at least 75% of the volume of the oöcyte. A Periodic Acid Schiff (PAS) staining procedure was carried out on Stages I through IV but proved to be negative. The end of Stage IV is characterized by the formation of a uniform follicular syncytium (Figure 8 C).

The follicular penetration of Stage IV was followed by the expulsion of the follicular syncytium in Stage V. An intimate contact exists between the follicular syncytium and the developing interior of the oöcyte (Figure 8 C). The expulsion of the syncytium coincided with formation of yolk platelets at the syncytium-oöcyte interface. Yolk platelets and the formation of a chorion appear to be functional products of the syncytium. The chorion is initially comprised of droplets (Figure 8 D). As the quantity of yolk increased in the oöcyte, the syncytium was forced to the periphery of the oöcyte (Figure 9 A). The yolk exhibits a vivid PAS reaction (Figure 9 B) as it is elaborated in Stage V. It also has greater staining differentiation with hematoxylin and eosin. Heidenhain's hematoxylin stained oöcytes of Stages I–IV clear to light grey, but with the presence of yolk a bluish color was apparent. The yolk granules generally appeared as contiguous polyhedra (Figure 9 C). The polyhedra were approximately 8 [u]m in diameter. The nucleus of the oöcyte was unchanged at the beginning of syncytial expulsion. Near the end of Stage V the syncytium was nearly at the periphery of the oöcyte and was degenerating. The nuclei of the follicle cells were distributed randomly (Figure 9 D). The chorion, now 20 [u]m thick, was almost complete and gave a positive PAS reaction. In Stage VI, final degeneration of the follicular syncytium took place and ovulation occurred. A mature fixed oöcyte had a long dimension of 1.5 mm.

Comparison of spawning squid with spent squid revealed that each had oöcytes present which represented Stages II–VI of maturation. Neither appeared to have Stage I preoöcytes. The ovary of spawning animals had more oöcytes present than spent squid, and their reproductive tract contained mature oöcytes. In spent animals, fewer oöcytes, or none were found in the reproductive tract.

3.4. DISCUSSION

The pattern of oöcyte maturation seen in L. opalescens agrees with descriptions in the literature, especially those of Raven (1962, 1966) and reported by Cowden (1968) and more recently by Selman and Arnold

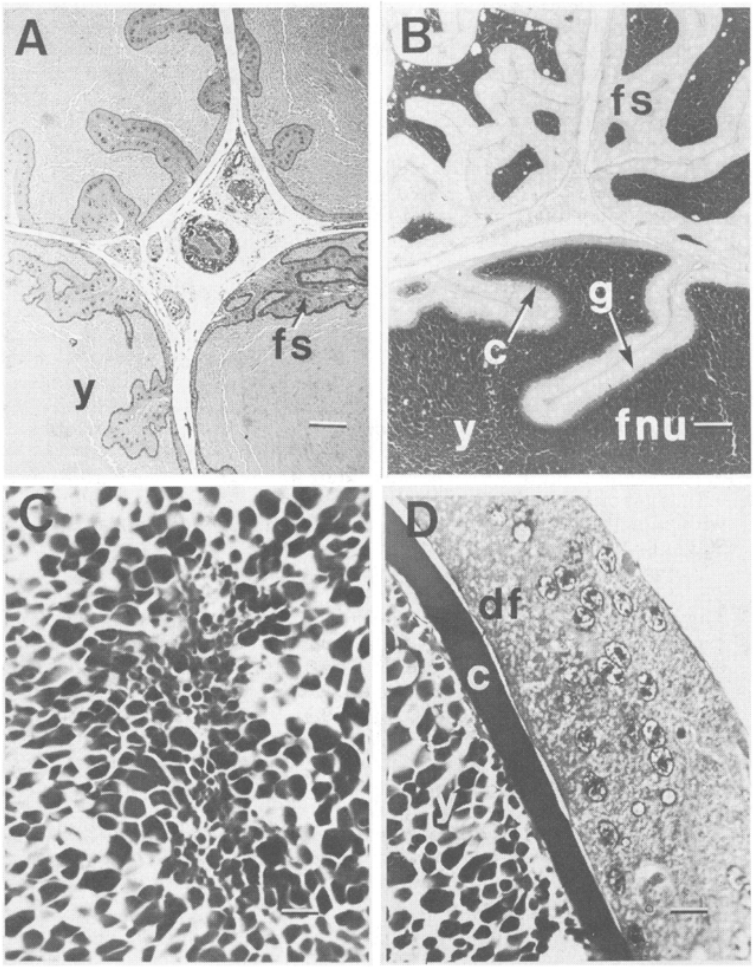

FIGURE 9. Stage V oöcytes. *A.* Stage V expulsion of follicular syncytium (fs) caused by the increase in yolk (y). Scale line 85 μm. *B.* Stage V positive PAS reaction in yolk and golgi apparatus (g). Chorion (c), follicular nucleoli (fnu). Scale line 65 μm. *C.* Yolk platelets. Scale line 17.3 μm. *D.* End of Stage V exhibiting the yolk, chorion and degenerating follicles (df). Scale line 20 μm.

*FIGURE **9. Stage V oöcytes.** A. **Stage V expulsion of follicular syncytium (fs) caused by the increase in yolk (y). Scale line 85 [u]m.** B. **Stage V positive PAS reaction in yolk and golgi apparatus (g). Chorion (c), follicular nucleoli (fnu). Scale line 65 [u]m.** C. **Yolk platelets. Scale line 17.3 [u]m.** D. **End of Stage V exhibiting the yolk, chorion and degenerating follicles (df). Scale line 20 [u]m.***

(1977). The question of multiple spawnings has only been partially resolved. Fields (1965) stated that L. opalescens spawn once and die. Summers (1971) concluded that a breeding induced mortality also occurred in both sexes of L. pealei; however, no histological evidence for this phenomenon was provided. In mature animals examined in the present investigation, including those animals which appeared to be spent, no oögonia nor a site for their production could be identified which would provide a basis for a future spawn. Cells were seen within the ovary of exhausted animals which are probably remnant follicle cells. Burukovskii and Vovk (1974) were unable to detect the existence of oögonia in mature L. pealei and concluded that the oögonia may have already entered the synaptic path. They hypothesize that the units found may be follicle cells which have yet to attach and become the follicular epithelium of the developing oöcytes. The present study agrees with this hypothesis, although oögonia may exist which for unknown reasons simply do not develop or are not utilized.

4. AGE AND GROWTH OF THE MARKET SQUID, LILOGO OPALESCENS BERRY, IN MONTEREY BAY

J. D. SPRATT
California Department of Fish and Game
Monterey, California

4.1. INTRODUCTION

Age and growth studies on squid, as a group, are very difficult. Few species have been taken in numbers large enough to allow any age and growth analysis. Market squid, Loligo opalescens (Fields, 1965); Illex illecebrosis (Squires, 1967); and Loligo pealei (Summers, 1971) have been aged by analyzing modal length frequencies collected through time. All of these researchers estimated age at maturity to be 2 or 3 years. In this type of an analysis several assumptions must be made which casts some degree of doubt on the validity of results obtained. Absolute age is not known because the age of the smallest specimens collected must be estimated. Random sampling is difficult and sampling probably introduces bias. Finally, movements or migrations of size classes make it extremely difficult to sample the same group or size class throughout its life span.

Tag and recovery studies on Todarodes pacificus (Otsuki and Araya, 1958) show that this species reaches maturity in 1 year, spawns, and dies. Tag and recovery studies could be conducted on L. opalescens, but it would require a method of holding specimens while experimenting with methods of tagging.

La Roe (1970) successfully cultured the loliginid squid, Sepioteuthis sepioidea. This species grew to 105 mm mantle length in 146 days and was sexually mature. Hurley (1976) has raised L. opalescens to an age of 100 days, her largest animal being approximately 13 mm TL at an age of between 70 and 80 days.

The technology to maintain juveniles and adults in long-term captivity studies would probably require considerable development effort. Even if squid could be raised for long-term studies, the effects of an artificial environment on growth would not be known and comparison with wild animals would be difficult. Finally, tagging juvenile market squid and recovering animals from the fishery present several logistical and sociologic limitations.

My intent in studying age and growth of market squid was to explore the possibility of using growth checks of hard body parts. The gladius, beaks, and statoliths offered the prospect of being indicators of age.

The gladius exhibits markings which could represent growth increments. Beaks of Moroteuthis ingens (Clarke, 1965) were found to have growth lines, but the time it took to form growth rings could not be determined. I gave a few statoliths from Loligo opalescens to Edward

Brothers (National Marine Fisheries Service, La Jolla), who found that statoliths of this species had many concentric growth rings much like fish otoliths.

It is my opinion that the gladius, beaks, and statoliths all are suitable for age and growth studies. However, I chose statoliths because of their similarity to fish otoliths, with which I have previous experience. This paper is concerned with the use of statoliths in aging Loligo opalescens, the techniques involved, and the validity of the results.

4.2. METHODS AND MATERIALS

4.2.1. Modal Length Data and Statolith Collections

All "wild" specimens of market squid used in this study were collected in Monterey Bay, California. The initial phase of this study was concerned with examining modal length frequency progressions in order to estimate growth. Squid were collected from July 1972 through July 1976. Length was recorded as dorsal mantle length (DML) in millimeters. Statoliths were collected as required. The commercial squid catch at Monterey was sampled March through July for adult animals. The commercial squid fishery takes spawning adults with lampara nets and does not provide samples of immature animals.

Purse seined northern anchovy catches landed at Moss Landing August through March provided samples of both juvenile and adult L. opalescens. Juvenile squid were sampled by small midwater trawl from Moss Landing Marine Laboratories' ARTEMIA July 1975 and June 1976.

Dorsal mantle length data were tabulated and pooled by week for graphical size class differentiation. For a given week's data, mantle length means and ranges were calculated for those animals judged to be from one size class. Sample statistics were calculated each for males, females, and unsexed animals (those less than 100 mm DML).

Age groups were identified graphically as clusters of length data means in a manner similar to that used by Summers (1971). I determined growth by following clustered means forward in time (see diagonal lines, Figure 10). This process amounts to qualitatively identifying length frequency modes and following their progression through the seasons.

4.2.2. Statolith Extraction and Preparation

A dissecting microscope with magnification up to 40X–50X was used to dissect 'larval' sized squid (< 10 mm TL). Larger sizes required progressively less magnification and adults over 100 mm DML did not require a microscope for dissection.

Statoliths are located just posterior and ventral to the eyes and are removed in the following manner: With the ventral size up, the funnel apparatus is removed. In large squid it is often necessary to split the mantle in order to remove the funnel. In small squid (75 mm DML) the two statoliths are usually visible, appearing as white opaque objects lying side by side under a thin layer of transparent tissue and cartilage. In larger

squid it is necessary to scrape the muscle tissue away with a scalpel until the ventral side of the cranial cartilage is exposed. The two visible statoliths are then removed with forceps after cutting the statocyst in half.

The statoliths are not fragile, but can be broken if handled roughly. Generally, statoliths require no cleaning but any adhering tissue should be removed. They can be stored indefinitely in gelatin capsules.

Statoliths must be ground in order to see growth rings and, due to their small size, handling is much easier if they are embedded. A Fullum mold which is reusable and has space for 24 statoliths is used. The statolith must be placed on its side in the bottom of the mold so that it will be parallel with the plane of grinding. Any clear fast drying resin may be used and is poured over the statolith filling the mold. The hardened resin block is a permanent mount and can be stored in a coin envelope.

The embedded statoliths should be just under and parallel to the surface of the resin block. Grinding is done using carborundum paper (200–600 grit), and requires frequent stops to check progress under a microscope. Polishing with aluminum oxide or diamond paste will help eliminate scratches in the statolith, but is not necessary to view growth rings.

4.2.3. Viewing Growth Rings

A compound microscope with substage illumination was used during the grinding procedure and for viewing growth rings. Magnification of 400x to 600x was adequate for counting growth rings. Higher magnification up to 1500x did not reveal any more detail.

An occular micrometer is useful in counting the uniformly spaced rings in juvenile squid. At 450x with a 10 mm occular micrometer, the growth ring widths nearly equal the graduations on the micrometer. These "first" uniform rings are best seen from the nucleus to the posterior margin of the statolith (Figure 11). "Larger" rings formed later in life can be counted at 100x to 400x without any special technique. On adult squid it is difficult to see the "first" uniformly spaced rings and counting must begin with the first "larger" ring. These "larger" growth rings formed later in life are best seen on the rostrum (Figure 11). When viewed under transmitted light, a ring or growth increment is defined as the interface between an inner light and outer dark band.

Ann C. Hurley, National Marine Fisheries Service, La Jolla, California, provided me with laboratory reared (Hurley, 1976) squid of up to 2 months in age. Statoliths from these animals were extracted and "aged" as described above. Growth increments were possible to count on six specimens. Counts were made without knowledge of age.

4.3. RESULTS

4.3.1. Modal Length Analysis

Mangle length data from 1,987 squid of 109 daily samples were tabulated. These squid ranged in size from 5 mm to 190 mm DML (Figure 10). I examined 100 and determined an "age" based upon growth rings in their statoliths. of the squid sent me by A. C. Hurley, I was successful in counting statolith growth rings in six (Table 2).

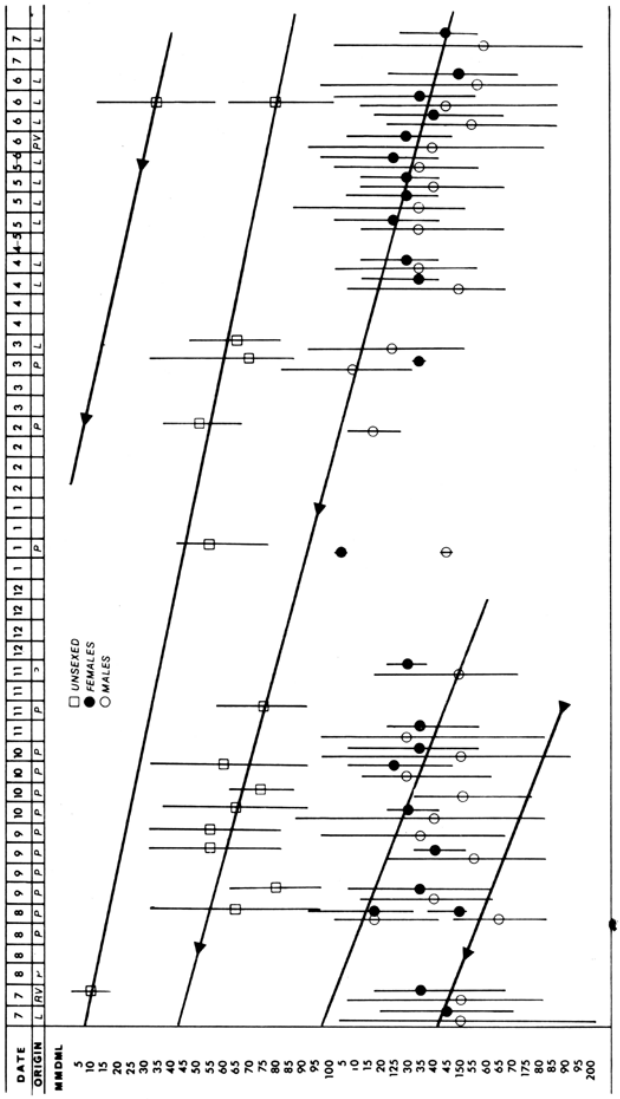

FIGURE 10. Weekly means (dots, squares) and ranges (lines) for 109 samples of 1,987 squid collected in Monterey Bay July 1972 through July 1976. Diagonal lines, fitted by eye to clustered means, follow brood growth through time. P = Purse seine sample. L = Lampara (squid fishery) sample. RV = ARTEMIA sample. MMDML = Dorsal mantle length in millimeters.

*FIGURE **10. Weekly means (dots, squares) and ranges (lines) for 109 samples of 1,987 squid collected in Monterey Bay July 1972 through July 1976. Diagonal lines, fitted by eye to clustered means, follow brood growth through time. P = Purse seine sample. L = Lampara (squid fishery) sample. RV = ARTEMIA sample. MMDML = Dorsal mantle length in millimeters.***

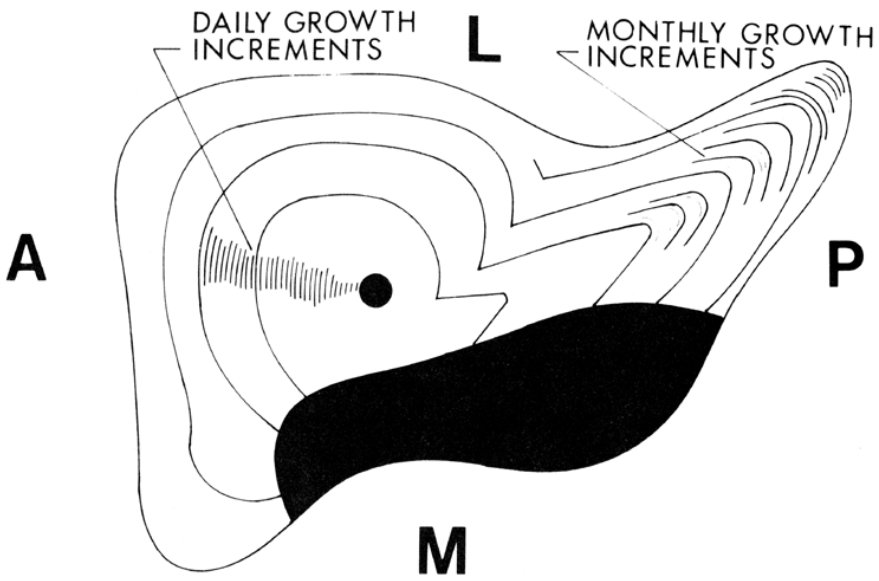

FIGURE 11. Ventral view of diagramatic statolith from an adult squid, as it appears under transmitted light. Anterior (A), posterior (P), median (M), and lateral (L) surfaces. Areas where daily and monthly growth increments are best viewed are indicated.

*FIGURE **11. Ventral view of diagramatic statolith from an adult squid, as it appears under transmitted light. Anterior (A), posterior (P), median (M), and lateral (L) surfaces. Areas where daily and monthly growth increments are best viewed are indicated.***

Visual inspection of clustered mean sizes suggested the existence of two and sometimes three separable size groups (Figure 10). Eye fitting a growth rate line to these data suggest that it takes market squid 2 years to reach maximum size.

TABLE 2.
Number Growth Rings in Statoliths from Squid of Known Age.

Specimen	*MMAge in days*	*Growth rings in stotoliths*
1	67–70	28
2	67–70	32
3	28–31	30
4	43–44	15
5	54–55	35
6	55–56	38

*TABLE **2.***
Number Growth Rings in Statoliths from Squid of Known Age.

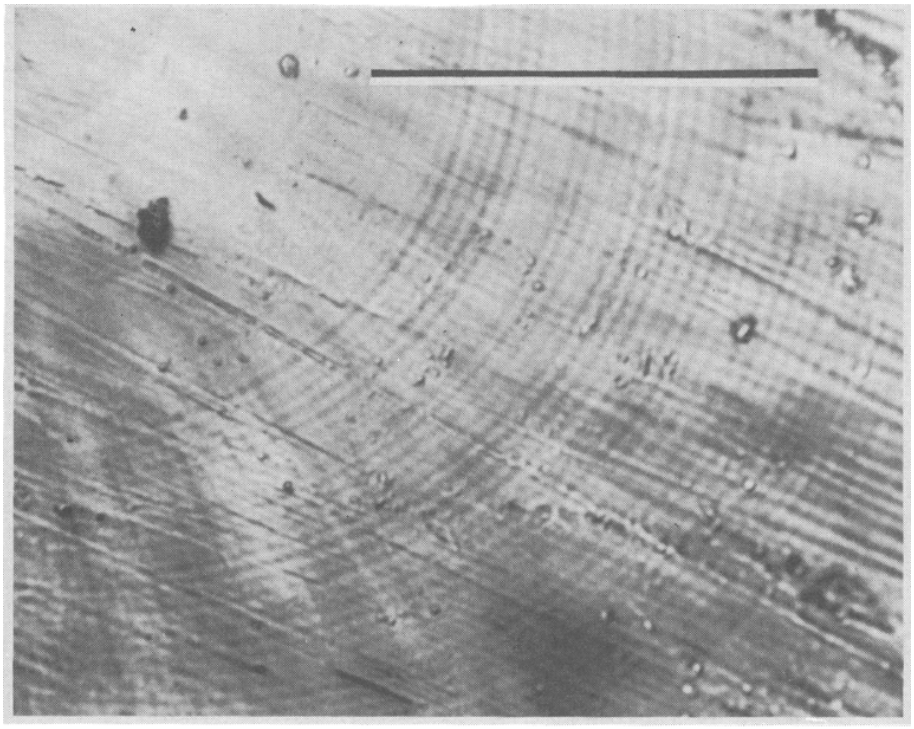

FIGURE 12. Small regularly spaced growth rings formed during juvenile life stages. This squid was 43 mm DML and was captured September 1975. Scale line approximately 25 μ.

*FIGURE **12. Small regularly spaced growth rings formed during juvenile life stages. This squid was 43 mm DML and was captured September 1975. Scale line approximately 25 [u]m.***

4.3.2. Aging with Statoliths

The growth increment or ring sequence found in market squid statoliths consists of two distinct patterns: 1. "First" rings which are discernable in young animals as uniform, regularly spaced layers (Figure 12); and 2. "Larger" rings which appear in older animals and which are irregular in size and spacing with large, prominent growth increments separated by five or six smaller ones (Figure 13).

I interpreted the above patterns as representing at first daily growth ("first" rings) followed by lunar monthly growth ("larger" rings). I found up to 150 daily and 18 lunar monthly growth increments. The validity of this scheme will be discussed later.

Using the daily—lunar monthly interpretation, the maximum age I encountered was 25 lunar months or almost 2 years. A growth curve (Figure 14), developed by combining age and length data into lunar quarters, shows growth is rapid from spring to summer and slows during the winter. Squid appear on spawning grounds at about 100 mm DML or at 14 lunar months of age. During the spawning season most market squid range in size from 100 to 145 mm DML and are, according to my aging calendar, 14–22 lunar months old. Squid up to 200 mm DML would probably have lived through two fast growth periods (summers) before they spawned.

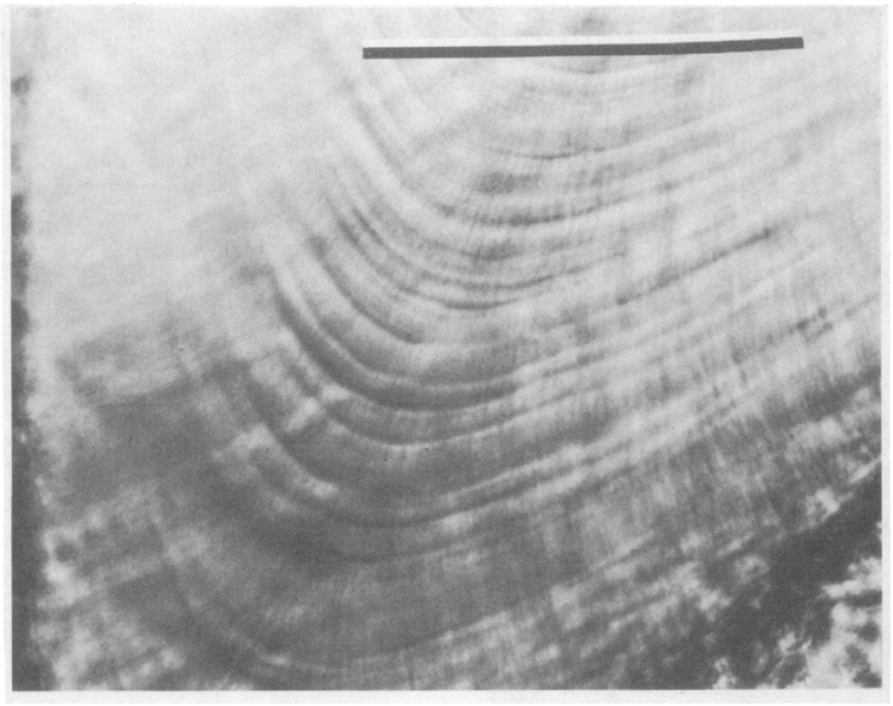

FIGURE 13. Large irregularly spaced growth rings formed during adult life stages. This squid was 140 mm DML and was captured June 1975. Scale line approximately 25 μ.

*FIGURE **13. Large irregularly spaced growth rings formed during adult life stages. This squid was 140 mm DML and was captured June 1975. Scale line approximately 25 [u]m.***

of the squid which were raised in the laboratory, one "age" determined from the statolith agreed with the animal's true age. However, the remaining five squid had markedly fewer growth increments than the days of age (Table 2). In general, statolith "age" was on the order of half the true age.

4.4. DISCUSSION

Sampling of commercial squid landings, anchovy catches, and midwater trawling in Monterey Bay from 1972–1975 provided length frequency data that indicate it takes market squid 2 years to reach maximum size (Figure 10). The spawning season extends from April to December in Monterey Bay, but usually peaks in May–June with an often noticeable peak in November–December. The long spawning season clouds length frequency data because squid of all sizes are present at all times of the year and it is difficult to follow each brood as it matures.

Fields (1965) analyzed modal length frequencies of market squid from Monterey Bay and concluded that most spawn at 3 years of age but that some animals mature at 1, 2, and 4 years of age. His basic hypothesis was that the growth rate was constant at about 4 mm per month. This could be unrealistic, since growth should be rapid initially and gradually slow as age progresses. Fields and I disagree, but growth increments found in statoliths tend to support my contention that the market squid spawns at between 1 and 2 years of age.

Conclusive evidence that the majority of market squid spawn at 1 to 1.5 years of age is very elusive. However, ageing by examining statolith rings was tested by two independent methods and both methods, i.e., ageing squid of known age and comparing ageing results with length frequency data, provide a measure of validity to my results.

Unfortunately, for my study, A. C. Hurley was unable to raise squid past 2 months of age. The growth rate of these squid was suspect because they were held in a near starvation state due to the difficulty in providing proper food organisms. The squid that lived 2 months were only 4–5 mm DML or about 2 mm larger than when they hatched. Based on my results, a squid 2 months old should be 15–20 mm DML. The statoliths from Hurley's squid did have small uniformly spaced growth increments. The fact that five of the six squid had substantially fewer growth increments than the days of age (Table 2) may be due to the near starvation situation. However, after examining the statoliths of these animals it is clear the growth increments were formed rapidly and at a uniform near daily rate. This provides a measure of confidence in suggesting that the rate of formation of the first 150 growth increments is daily.

The second method of validation was to compare my lunar month growth rate (Figure 14) with the size frequency results (Figure 10). If length at age is superimposed on length frequencies (Figure 15), the results are encouraging. The estimated growth rate fits the length modal progressions quite well. My growth rate is for summer spawned squid, but

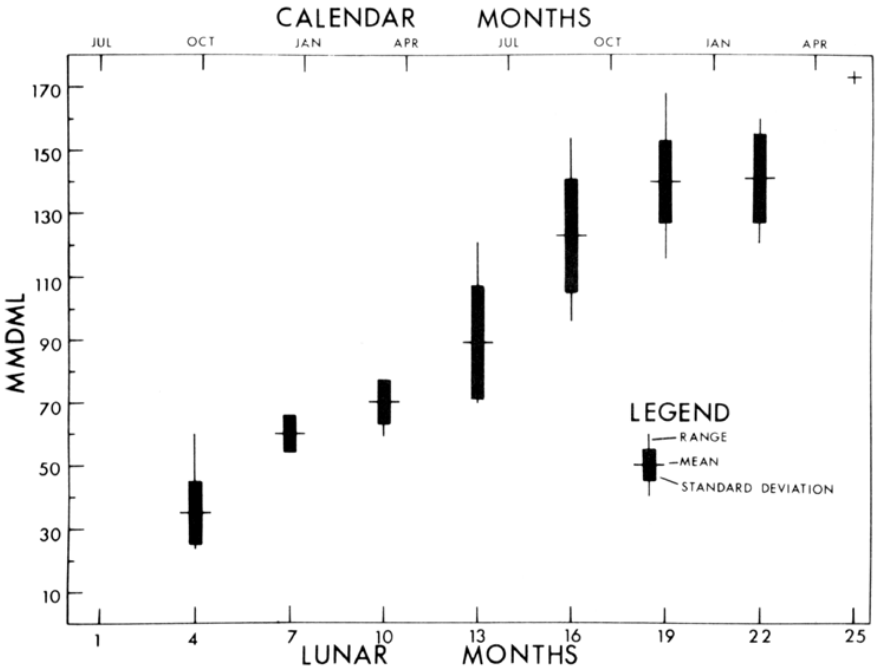

FIGURE 14. Growth rate of market squid from statoliths in millimeters per lunar quarter.

*FIGURE **14. Growth rate of market squid from statoliths in millimeters per lunar quarter.***

squid also spawn in the fall. If my growth curve is shifted 6 months to the right, very little adjustment is needed for it to pass through length modes not accounted for by the growth curve for summer spawned squid and could represent growth of fall spawned squid.

The majority of squid spawning in the spring and early summer could mask a probable different growth rate for fall spawned squid. Assuming the squid I have aged are all spring or early summer spawners, the resulting growth rate agrees well with monthly length frequencies.

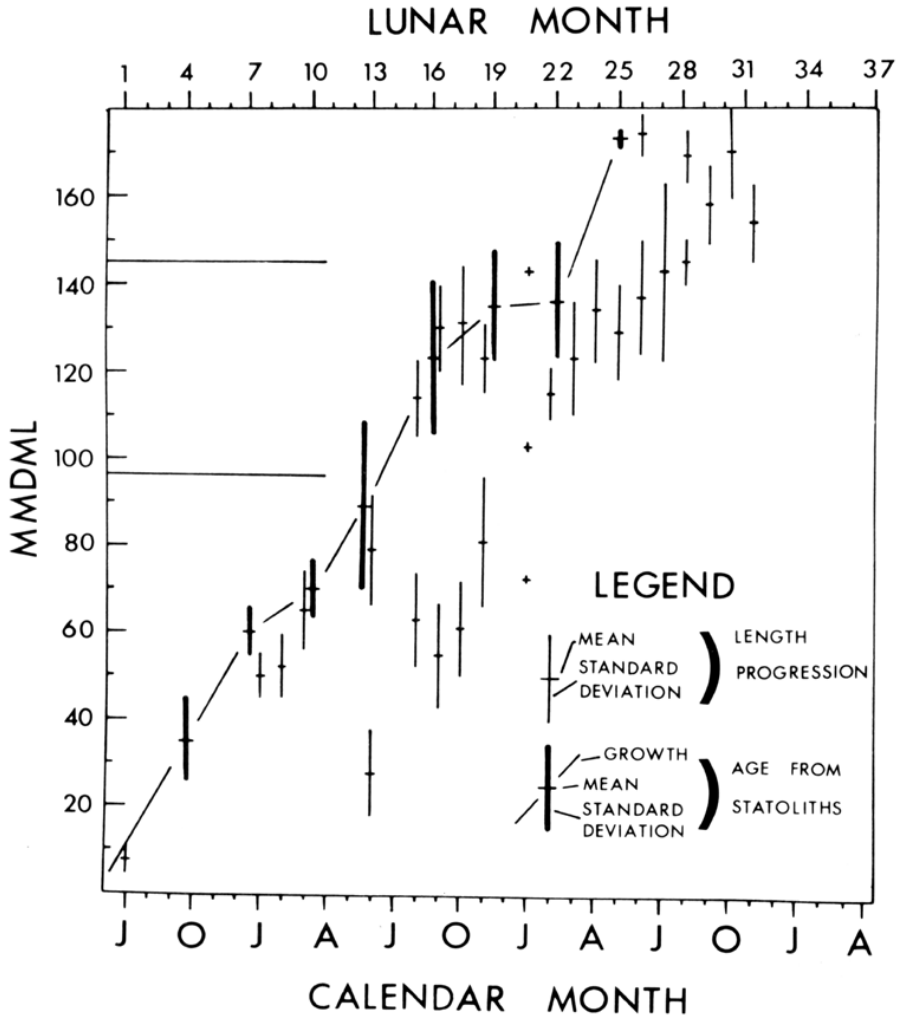

FIGURE 15. **Market squid growth rates from statoliths compared with modal length progressions. Bold lines represent quarterly growth rate for summer spawned squid from statoliths and light lines represent monthly modal length frequency progressions. By moving the growth rate 6 months to the right, the growth curve passes through length frequencies not accounted for by summer spawned squid and represents fall spawned squid.**

FIGURE ***15. Market squid growth rates from statoliths compared with modal length progressions. Bold lines represent quarterly growth rate for summer spawned squid from statoliths and light lines represent monthly modal length frequency progressions. By moving the growth rate 6 months to the right, the growth curve passes through length frequencies not accounted for by summer spawned squid and represents fall spawned squid.***

Squid are known to be phototactic and it has been shown that they feed during daylight hours (Karpov, 1977). This mechanism may cause the formation of daily growth increments. Contrasting growth increments could be formed during daily feeding and resting activities. At about 6 months of age, according to my ageing calendar, a change takes place in the type and rate of growth increment formation. This could represent a transition phase in the squid's life history. Why the growth increment formation becomes irregular is not clear. Prominent large growth increments are formed periodically that have up to five or six smaller increments between them (Figure 13). Spawning adults have six to 18 of these prominent growth increments. This number of adult growth increments correlates best with lunar periods, primarily since an annual rate of formation neither fits modal size progressions nor does it seem reasonable in light of what is known about age of other squid species. A prominent growth increment could be formed during heavy feeding activity associated with full moon. Prey organisms with a diurnal pattern would be more available on moonlight nights. At any rate, the large prominent growth increments are assumed to represent lunar growth.

The fact that other species of squid, e.g., Todarodes pacificus and Sepioteuthis sepiodea, reach adult size in 1 year or less, implies that market squid could also grow at a rapid rate. The modal length progressions and growth rings on statoliths support the hypothesis that market squid grow rapidly and are capable of reaching adult size in about 14 lunar months.

Upwelling normally begins about March–April in central California and the added nutrients in nearshore areas cause plankton blooms during the summer. Market squid spawned early in the summer (April–May) will grow rapidly during the summer growing season and are probably capable of reaching adult size in about 1 year. As spawning continues from June through September, newly hatched squid will have progressively less time available in the growing season. This will have the effect of slowing the growth rate. During the next summer (fast growth season) this late summer brood will increase in size and probably spawn from October to December at an age of 14–19 lunar months.

Late fall spawned squid (October–December) will grow slowly until late spring, when growth will accelerate. Some fall spawned squid may reach adult size in 1 year, but I suspect most fall spawners must live through part of a second growing season before they spawn. The majority of squid spawned in October to December would most likely return to spawn in their second summer when about 18 to 22 lunar months old.

The squid I aged at nearly 2 years probably represent slow growing squid that have lived through part of their second growing season. An individual squid depending on its growth rate could return to spawn at any time between 1 and 2 years of age.

My results indicate that the market squid is capable of spawning at 1 year of age and that all will spawn during their second year of life. A life span of 1 year has serious management implications. Spawning biomass can fluctuate dramatically from year to year because good recruitment is necessary each year to maintain the spawning population.

5. FEEDING DYNAMICS OF LOLIGO OPALESCENS

KONSTANTIN A. KARPOV*
and
GREGOR M. CAILLIET
Moss Landing Marine Laboratories
Moss Landing, California

5.1. INTRODUCTION

In addition to the important role that Loligo opalescens plays as prey for other marine organisms such as birds, mammals and fishes, it is important to understand its role as a predator in the pelagic nearshore waters of California. For this and other species of squid, some work has been done on prey composition and feeding chronology; however, rates of digestion, daily meal size, and the general role of these active predators in energy turnover at various levels of the pelagic food web are poorly understood.

Recent works by Fields (1965) and Loukashkin (1977) have determined the gross composition of Loligo opalescens' diet but failed to resolve specific problems such as how this diet is affected by habitat and biological state of the animal. Fields (1965) examined 106 squid stomachs with contents, obtained from subsampling commercial fish seiners and squid lampara boats in the Monterey Bay area. Most of these samples were taken from night catches and only from nearshore areas. Loukashkin (1977) examined 331 L. opalescens with contents. These samples were collected throughout the California coast but again from predominantly night catches. Loukashkin (1977) made no attempt to distinguish the samples caught on the spawning grounds from those caught in other nearshore and offshore areas.

Several authors have reported marked temporal and spatial fluctuations in stomach fullness for various species of squid. Kore and Joshi (1975) attributed these fluctuations in Loligo duvauceli stomach fullness to seasonal variation in diet. Loukashkin (1977), working with samples of Loligo opalescens collected at night, attributed such variation in fullness to location of capture along the California coast. Vovk (1972 b) found that the variation in Loligo pealei stomach fullness was both seasonal and diel in nature. He found that L. pealei fed most intensively during daylight hours, peaking at about 1600 hours, and fed least intensively during night hours.

Cephalopods are thought to have rapid digestion rates, but estimates of duration of digestion have widely varied. Octopus sp. was estimated to digest a meal in 18 hours (Bidder, 1950), and complete digestion in Sepia sp. was estimated to require 12 hours (Bidder, 1950). Bidder described the

two part digestive system of two European species of Loligo, comprised of a stomach and caecum, that can both act simultaneously and independently in breaking down and absorbing the meal. The caecum itself acts as a two part organ with its absorptive portion kept clear of undigestible debris by a ciliated sorting surface. Thus, absorption of the meal can proceed unimpeded by emptying functions. Bidder (1950) qualitatively estimated that it took only 4 to 6 hours for these Loligo to digest a meal.

In this paper we describe our studies on the effect of depth and location of capture, size of squid, and sex of spawners on the prey composition of L. opalescens. We also describe our determinations of diel feeding chronology and digestion rates.

5.2. MATERIALS AND METHODS

5.2.1. Prey Composition

Prey were identified from samples of Loligo opalescens taken from a variety of sources. Sampling was limited to Monterey Bay and its adjacent areas from Point Sur to Pigeon Point, California (Figures 16 and 17). Most of these were obtained from bottom and midwater trawls aboard three research vessels: the California Department of Fish and Game ALASKA, the National Marine Fisheries Service COBB, and PACIFIC RAIDER leased by the National Marine Fisheries Service. Samples were also taken from incidental research bottom trawl catches and commercial squid, anchovy, and groundfish catches.

The ALASKA used a large midwater trawl, as described by Mais (1974), with a ½ inch stretch mesh codend, during June 1976. Seven samples taken in the Monterey Bay area were used, with only two of these taken during daylight hours. All samples were taken at depths less than 40 fathoms. The PACIFIC RAIDER used a large 60 by 60 foot Herman Engel midwater trawl, with a 1½ inch mesh codend. The COBB used a 400 mesh Eastern bottom trawl, having a 94 foot ground rope, equipped with roller gear and a 71 foot head rope. The codend had a liner with 1¼ inch stretch mesh. A total of 14 samples was taken by both these vessels between Moss Landing Harbor and Pigeon Point, California, during August 1976. Samples were taken during daylight hours at depths greater than 40 fathoms (Figure 16).

Squid taken on the spawning grounds in Monterey Bay included two samples from the ALASKA and subsamples of commercial squid catches. These samples were taken within a few miles of Monterey Harbor at depths less than 20 fathoms (See Figure 57 McInnis and Broenkow). The commercial catches were subsampled while being unloaded between September 24 and October 28, 1975. During this period ten different samples were taken from seven different vessels. The squid were landed using lampara nets as described by Fields (1965). All catches were made between 2300 and 0800 hours.

The commercial anchovy fishery at Moss Landing, California, was also subsampled. L. opalescens can be taken directly from the conveyor belt while these boats are unloaded. Anchovies are fished in Monterey Bay using purse seines and lampara nets which have a maximum depth penetration

of 35 fathoms. Eighteen samples were collected between September 5, 1975, and March 10, 1976. The hauls sampled were all taken during night hours with none of these taken near the spawning grounds.

Subsampling commercial bottom trawlers yielded three samples of squid from two separate vessels. These were taken on September 24 and December 19, 1975, and January 9, 1976. Depths of sampling were between 47 and 80 fathoms. Two of the samples were taken off Point Sur and one was taken off Point Pinos, Monterey (Figure 16). Both vessels used large trawls with a 4½ inch stretch mesh codend.

A sample of L. opalescens was collected by the TAGE of Hopkins Marine Station, using a small otter trawl with a 24 foot head rope and a ¼ inch stretch mesh codend liner. This sample was taken north of Moss Landing Harbor (Figure 16) at a depth between 10 to 20 fathoms on March 9, 1976.

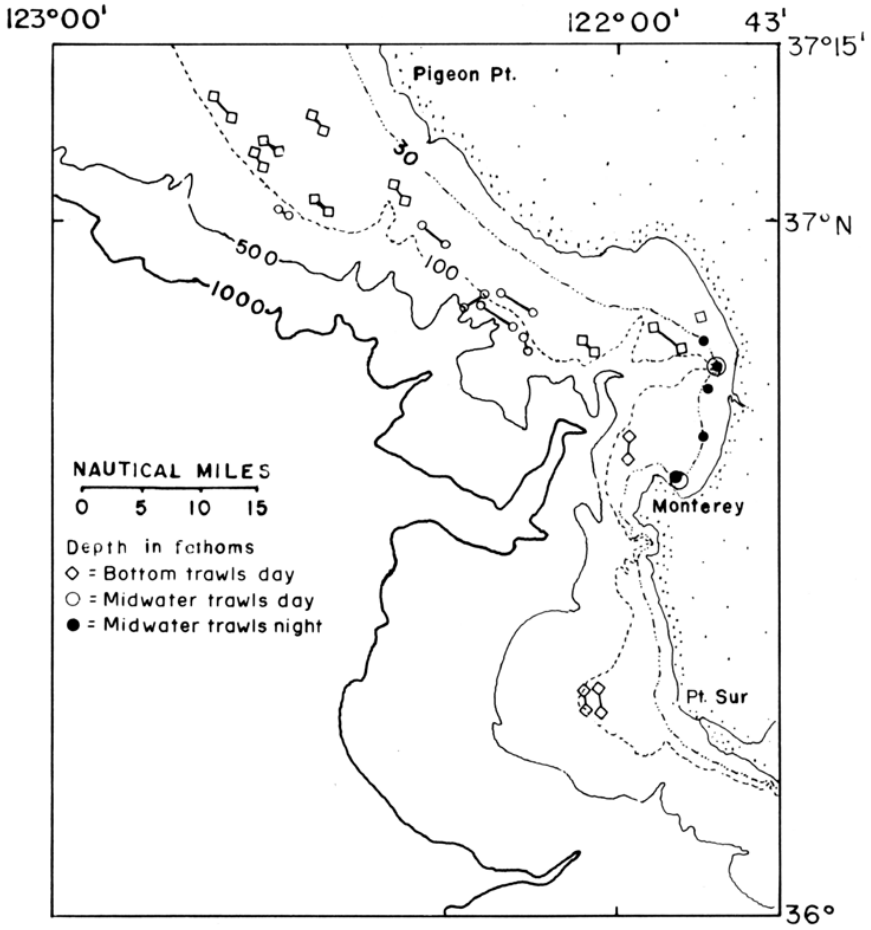

FIGURE 16. Locations of midwater and bottom trawls: Pt. Sur to Pigeon Pt.

*FIGURE **16. Locations of midwater and bottom trawls: Pt. Sur to Pigeon Pt.***

A maximum of ten squid with contents was analyzed in detail for prey composition from any one sample. These animals were first sexed and their dorsal mantle lengths were recorded. Then the stomachs were removed and the contents sorted, identified, and enumerated. Prey were identified to the lowest possible taxon. Rarely were whole organisms encountered in the stomachs examined, and for this reason key fragments had to play the major role in the identification process. Identification to the species level was not often accomplished. Some species of crustacea were identified from a reference collection compiled of common pelagic crustaceans taken in trawling operations in the Monterey Bay area. Fish found in the contents were identified from otoliths that were occasionally ingested and these were compared to a reference collection in the museum at Moss Landing Marine Laboratories. Squid were identified by using their beaks as a taxonomic tool. Recognition to the family level was possible using the key developed by Clark (1962), and some species could be identified using the beak drawings furnished by Pinkas, Oliphant, and Iverson (1971).

Most other identifications were more generalized. Crustaceans such as mysids, euphausiids, megalops larvae, amphipods, and shrimp possess distinctive eyes, mandibles, statoliths, and other parts that when taken together offer distinctive recognition. A collection of detailed drawings of such parts was assembled to aid in prey recognition.

To assess the number of stomachs needed to adequately reflect feeding habits of the squid population, plots (described in Karpov and Cailliet, in press, Calif. Coop. Ocean. Fish. Inv. Rept.) of cumulative numbers of taxa encountered per stomach were constructed for squid captured away from the spawning ground and from the spawning ground using 50 randomly selected squid stomachs for each category. Both plots leveled off at about 20 squid, indicating that this number of stomachs is sufficient to represent a valid comparison in any category. The smallest set of categories compared was between sexes on the spawning grounds with 24 females and 27 males sampled.

The fragmented and often well-digested state of the stomach contents made counts of individual prey difficult and relative volume determinations impossible. Therefore, counts were based on pairs of eye lenses, mandibles, statoliths, otoliths, or polychaete jaws. Counts were not based on paired soft parts, such a decapod eyes, that were subject to digestion. Occasionally, stomachs were largely distended and filled with numerous euphausiid mandible pairs. These stomachs were divided into approximate halves; one portion was enumerated and the other portion was qualitatively examined. In such cases, counts were doubled.

A modified form of the Pinkas *et al.* (1971) "index of relative importance" was calculated in each comparison of depth, location, and size of squid for the major prey types eaten. The index was modified by using numerical importance and frequency of occurrence. The numerical importance of a particular item was the percentage ratio of its abundance to the total abundance of all items in the contents. Its percent frequency of occurrence was the percentage of squid examined that contained at least one individual. The product in percents [(number) x (frequency)] is the

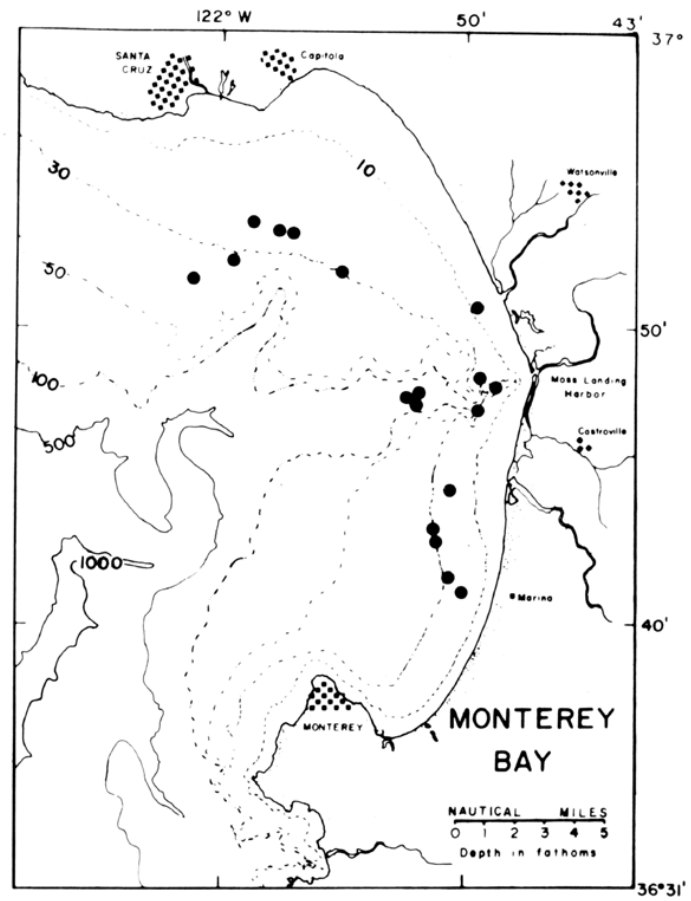

FIGURE 17. Locations of commercial anchovy purse seine sets sampled for prey analyses in Monterey Bay.

*FIGURE **17. Locations of commercial anchovy purse seine sets sampled for prey analyses in Monterey Bay.***

index of relative importance, which ranges from zero, when both values are zero, to 10,000, when both indices are 100% (a mono-diet).

In addition to indices of relative importance, we determined percent frequency of occurrence of prey categories for squid sizes, depths of capture, location on or off the spawning grounds, and sexes of spawning ground squid. Non-spawning squid were grouped into two size categories for comparison. Animals with 21 to 100 mm mantle lengths were compared to those with 101 to 180 mm mantle lengths. These two categories equally divided the number of animals, yet retained a significant number of both shallow water and deep water animals in each category.

Deep water samples were defined as those taken from depths of at least 40 fathoms or deeper. These trawls included most of the day midwater and bottom trawled samples. Samples were considered shallow regardless of bottom depth when they were taken from water depths of less than 40 fathoms. These included all anchovy hauls, ALASKA, and TAGE samples.

The two categories were somewhat arbitrary since the gear used did not sample at discrete depths. No samples taken near the spawning grounds were included in either category.

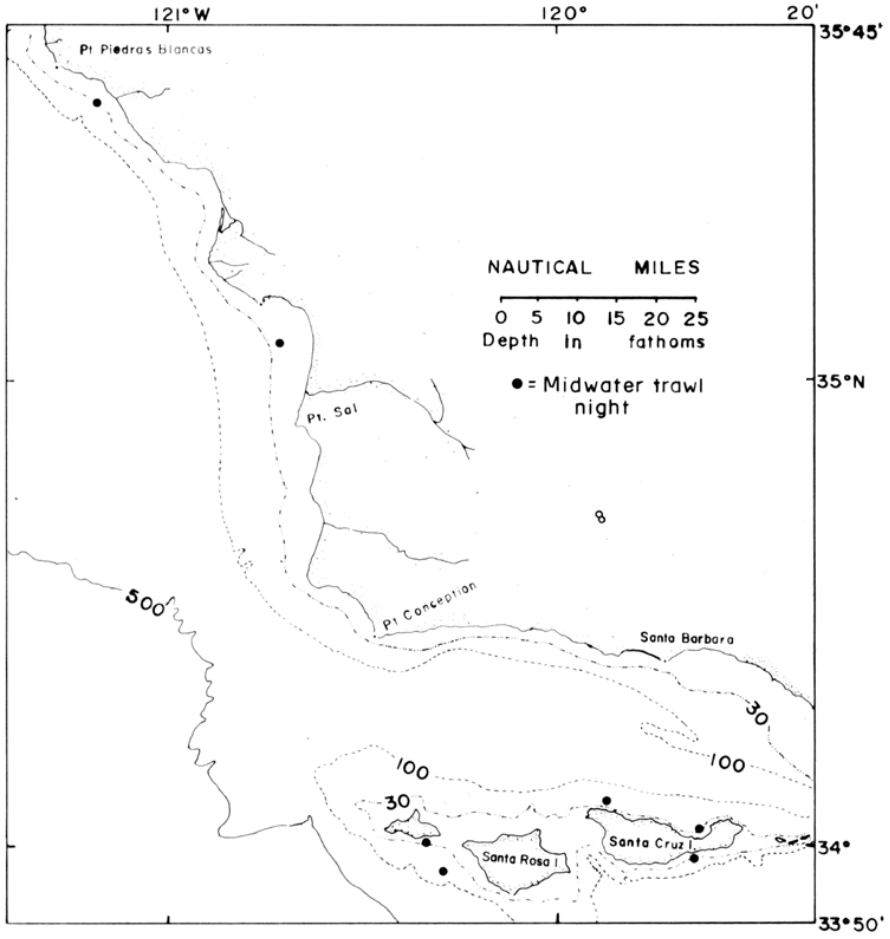

FIGURE 18. Locations of midwater trawls: Santa Rosa Island to Pt. Piedras Blancas.

*FIGURE **18. Locations of midwater trawls: Santa Rosa Island to Pt. Piedras Blancas.***

Rank correlation coefficients and indices of species similarity were calculated for comparisons. The Spearman rank correlation test (Fritz, 1974) was used to compare ranks of prey items, and the "percent similarity index" was used to examine the degrees of similarity for comparisons of percent by number (Silver, 1975). This index has no significance levels but serves to illustrate the relative similarities between comparisons.

5.2.2. Feeding Chronology Determination

Squid used to determine feeding chronology were captured using mid-water and bottom trawls. Twenty five of these samples were taken in areas away from spawning grounds over bottom depths ranging from 20 to 225 fathoms. ALASKA samples were taken primarily at night during June 1976 between Santa Rosa Island and Monterey, California (Figure 18), while COBB and RAIDER samples were taken in August 1976 between Point Sur and Pigeon Point during daylight hours at depths greater than 40 fathoms (Figures 16 and 17).

Trawl caught L. opalescens were subsampled. A maximum of 40 animals per sample was placed on thin plastic trays and frozen immediately.

In the laboratory the samples were thawed quickly using warmed seawater. State of digestion indices (Tables 3 and 4) and fullness indices were evolved in order to classify feeding states. Fullness was subjectively ranked before the stomachs were excised, from 0 to 4: 0. = empty stomach; 1. = stomach with fragments to # full; 2. = # to just full; 3. = full to somewhat distended; 4. = bulging. A similar index was developed by Kore and Joshi (1975) for the squid Loligo duvauceli. Their index incorporated a description of the caecal state which was too variable to be useful in this study.

In order to resolve major feeding states, the indices of digestion and fullness were combined in a 2x2 matrix (Cailliet, 1972) in which the major feeding states were: A. = not recently eaten or full, including empty stomachs (i.e., fullness states 0, 1 and 2 vs digestion states 1 and 2); B. = recent but not full (fullness states 0, 1 and 2 vs digestion states 3 and 4); C. = recent and full (fullness states 3 and 4 vs digestion states 3 and 4); and D. = full but not recently eaten (fullness states 3 and 4 vs digestion states 1 and 2).

Fullness-recency histograms were compiled for the frequency of the feeding states at 2 hour intervals over the 24 hour cycle. Due to cruise scheduling difficulties, no samples were obtained for the 0601 to 0800 hour and the 1801 to 2000 hour periods, but all other 2 hour periods were represented by one to four samples. Uniform dry body and stomach content weights were taken and percent body weights calculated. Drying was accomplished at 80°C by the method suggested by Paine (1971). Dry stomach contents as percent of dry body weight were plotted against time of day.

The validity of our fullness index was tested using 389 L. opalescens obtained from the feeding chronology portion of this study, covering a wide range of sizes. Using the method described above, the actual amount of dry stomach contents, as a percentage of dry body weight, was compared to index values for each animal. A graphical comparison was made assigning mean, 95% confidence interval, range, and one standard deviation around the mean for each index value (Figure 19). This comparison showed the two methods to be correlated. Index values 2 and 3 showed the least overlap in range and standard deviation, and the difference was highly significant using the Wilcoxon two-sample test (P [<] 0.001).

TABLE 3
Qualitative Description of State of Digestion Indices For Osteichthyes Eaten by the Market Squid, *Loligo opalescens*

Index	*Eyes*	*Gills*	*Flesh*	*Hard Parts*		*% Flesh/ Hard Part*
				Rays	*Vertebrae*	
4	Lens with socket pigments	Present undigested	Sharp lumps	With connective membrane	United in segments, flesh attached	50 +
3	Lens separate	Present undigested	Rounded lumps	Membrane digesting	United in segments, flesh attached	50 +
2	Lens separate	If present, well digested	Mostly connective tissue	Membrane gone	United in segments, little flesh	<50
1	Lens separate	Gone	Mostly gone	Separate	Separate, without flesh	<50

*TABLE **3***
Qualitative Description of State of Digestion Indices For Osteichthyes Eaten by the Market Squid, Loligo opalescens

TABLE 4
Qualitative Description of State of Digestion Indices For Crustacea Eaten by the Market Squid, *Loligo opalescens*

Index	*Eyes*	*Gills*	*Hard Parts*	*Flesh*		*% Flesh/ Chitin*	*Pigment*
				External	*Within Chitin*		
4	Intact; color normal	Present undigested	Fine bristles attached	Lumpy, discrete	Not digesting	≃70	Normal
3	Intact; surface wrinkled	Digesting or absent	Fine bristles attached	Becoming stringy	Not digesting	≃70	Less color
2	Fragmenting flesh present	Gone	Fine bristles, few attached	Aggregated, stringy	Digesting	70–30	Bleached
1	Flesh gone	Gone	Fine bristles, not attached	Stringy or gone	Gone	0–30	Bleached

*TABLE **4***
***Qualitative Description of State of Digestion Indices For Crustacea Eaten by the Market Squid,** Loligo opalescens*

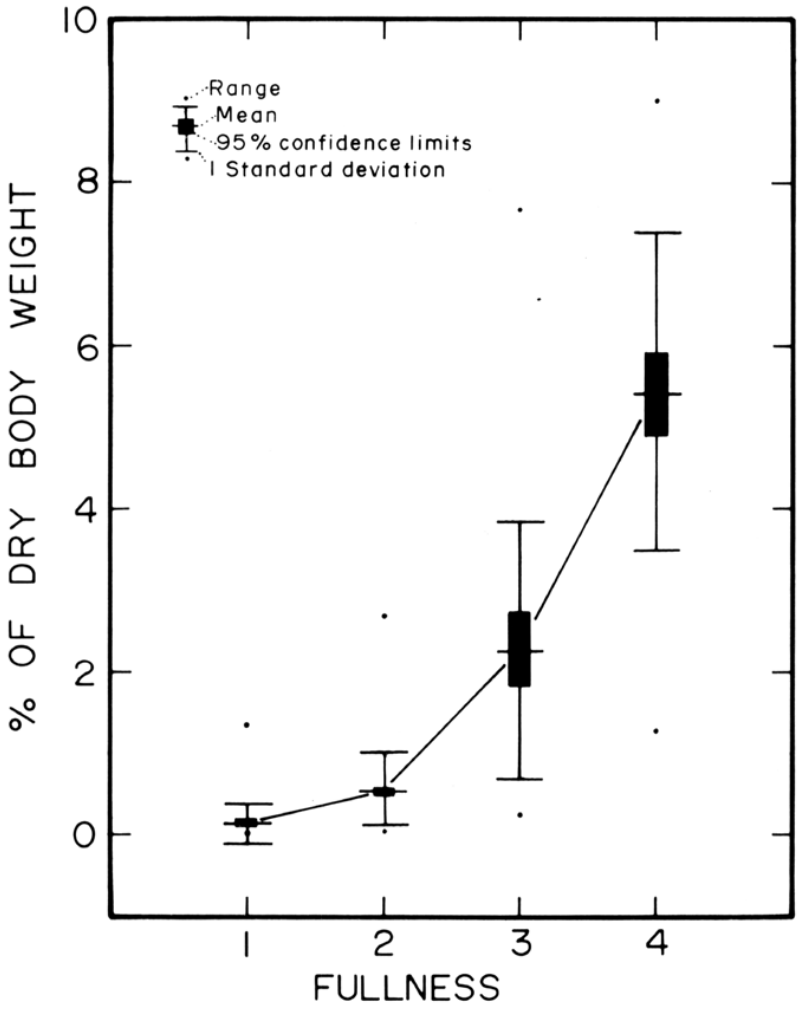

FIGURE 19. Comparison of fullness index to dry stomach content weight, as percent of dry body weight.

*FIGURE **19. Comparison of fullness index to dry stomach content weight, as percent of dry body weight.***

5.2.3. Field Digestion Experiment

Market squid used for field determination of stomach emptying time were taken by midwater trawl on August 24, 1976, aboard the PACIFIC RAIDER. This sample was taken at 1030 hours after a 30-minute trawl over a bottom depth of 140 to 225 fathoms several miles offshore between Santa Cruz and Davenport, California (36°50.0'N and 122°9.5'W). The depth of capture was estimated at between 105 to 125 fathoms. Initial examination of the captured squid revealed that most had full stomachs. Several hundred live animals were transferred by hand to a 50 gallon holding tank. In

all 105 animals survived to be sacrificed at: 0 time, 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 5 hours and 7 hours. All sacrificed samples were preserved in 10% seawater formalin. Running seawater maintained a holding tank temperature of 18.1 to 18.9 C throughout the duration of the experiment. An XBT cast indicated a surface temperature of 15.8 C, while the temperature at depth of capture was 8.4 C. For each squid, sex, state of maturity, and dorsal mantle length were ascertained and the stomach rated for state of fullness. Detailed analysis of prey composition was carried out on 35 of the 105 squid used in this experiment.

The percentage of dry body weight comprised of dry stomach contents was calculated and plotted against time of sacrifice. Model 1 linear regression (Sokal and Rohlf, 1973) was applied to these data and used to estimate the stomach emptying time (time intercepted by the regression line) and the average digestion rate (slope).

5.3. RESULTS

5.3.1. Prey Determination—Large vs. Small Squid

In general, squid fed mostly on crustacea and to a much lesser degree on fish, cephalopods, gastropods, and polychaetes (Table 5). In most categories, euphausiids and copepods dominated the diet, but other crustaceans such as mysids, megalops larvae, cumaceans, and amphipods were important food items.

TABLE 5
Index of Relative Importance in Prey Composition

	Squid size		*Sample location*		
Number of Squid:	*Small 123*	*Large 103*	*Deep water 134*	*Shallow water 94*	*Spawning grounds 52*
Crustacea unknown	73.3	17.5	23.8	135.2	95.4
Euphausiacea	3988.0	5400.0	6552.2	1553.4	0.0
Copepoda	97.5	37.5	103.8	1.0	0.0
Mysidacea	7.4	10.6	6.3	28.2	54.7
Megalops	4.9	0.0	0.1	23.3	1088.0
Natantia	0.2	0.6	0.0	1.9	0.0
Cumacea	12.2	0.0	2.6	1.0	0.0
Amphipoda	0.0	0.1	0.1	0.0	11.3
Ostracoda	0.0	0.1	0.1	0.0	0.0
Cephalopoda (whole)	16.2	9.7	2.6	187.6	0.0
Gastropoda	5.0	1.2	1.9	5.7	295.8
Radiolaria	0.0	0.4	0.1	1.0	0.0
Polychaeta	0.0	0.0	0.0	0.0	24.9
Fish	2.0	8.8	1.3	44.8	11.3
Miscellaneous	–	–	–	–	197.2

*TABLE **5***
Index of Relative Importance in Prey Composition

Using indices of relative importance, the comparison of prey composition of large (101–180 mm mantle length) versus small (21–100 mm mantle length) L. opalescens from off the spawning grounds revealed few major differences (Table 5). Both size categories fed mostly on crustaceans, primarily the euphausiids, Euphausia pacifica and Thysanoessa spinifera.

Other crustaceans taken included calanoid copepods, cumaceans, mysids, and the decapod shrimp, Sergestes sp. In both size categories, cephalopods and other non-crustaceans played a small role in the diet. Whole cephalopods eaten included Gonatus sp. and other L. opalescens individuals (cannibalism). Fragments of L. opalescens were also ingested and were most often identifiable as tentacle tips. Fish eaten were either unidentifiable species or Engraulis mordax. Gastropods and bottom debris were also ingested.

Percent frequency and percent by number comparisons of prey species indicated that large squid fed more frequently on euphausiids, cephalopods (whole and fragments), and fish. Rare taxa encountered only in the large squid feeding were the amphipod, Jassa sp., ostracods, and radiolarians. Small squid fed more frequently on other crustaceans such as megalops larvae and cumaceans. Few inferences can be drawn from percent by number of prey species since both size classes were overwhelmed by the number of euphausiids eaten. The percent similarity index between the two size groups was high (84.9%) and the Spearman rank correlation test showed these two groups to have similar proportions of food items in percent frequency of occurrence (P 0.025), but not in percent by number (Table 6).

5.3.2. Prey Determination—Deep vs. Shallow Water

Comparison of prey composition by depth of capture revealed major differences (Table 5). Squid captured in deeper water fed more on euphausiids and copepods. Squid taken nearer the surface fed far less, although still predominantly, on euphausiids, while fish, whole cephalopods, mysids, and megalops larvae were more important to these squid. Despite a relatively high similarity index (71.8%), no significant correlation of prey item ranks was found in either percent frequency of occurrence or percent by number of prey species between deep and shallow water (Table 6).

TABLE 6
Comparison Between Squid Sex, Sizes, and Location of Capture Using Percent Similarity Index and Spearman Rank Correlation Coefficient (r_s).

		Percent by Number	
H_o: $r_s=0$	*Percent Frequency of Occurrence* r_s	*Percent Similarity Index*	r_s
Large squid vs. small squid (101–180 mm DML vs. 20–100 mm DML †)	0.699 *	84.9	0.506 n.s.
Deep samples vs. shallow (0–40 fathoms = shallow)	0.549 n.s.	71.8	–0.230 n.s.
Shallow samples vs. spawning ground samples	–0.272 n.s.	16.8	–0.350 n.s.
Spawning ground samples male vs. female	0.852 *	60.8	0.697 n.s.

* Significant at P = 0.025
† Dorsal mantle length

*TABLE **6***
Comparison Between Squid Sex, Sizes, and Location of Capture Using Percent Similarity Index and Spearman Rank Correlation Coefficient (r_s).

5.3.3. Prey Determination—Spawning Ground vs. Shallow Water

A marked contrast was found in food items eated by squid taken from spawning grounds compared to squid taken in near surface waters (Table 5). On the spawning grounds, crustacean feeding still dominated, although of a different kind, with megalops larvae replacing euphausiids. Polychaetes, juvenile gastropods, and egg-like spheres also became common, replacing fish and whole cephalopods. Cephalopod fragments played a much larger role on the spawning grounds. A very low similarity index (16.8%) agreed well with the finding that no significant association occurred between prey ranks of spawning ground and shallow water squid in either percent frequency of occurrence or percent by number of prey species (Table 6)

5.3.4. Prey Determination—spawning Ground Males vs. Females

Little difference was found between the feeding habits of male and female L. opalescens from the spawning grounds. In both sexes crustacean feeding dominated, with mysids and megalops larvae being the primary foods. Juvenile gastropods also were important, with nereid polychaetes, and fish (juvenile Sebastes sp. and pleuronectiforms) playing lesser roles. No whole cephalopod feeding was found, although cephalopod fragments were ingested more often than off the spawning grounds. The miscellaneous category was dominated by egg-like spheres, but sand particles were also found.

The only major differences between sexes were in megalops larvae and cephalopod fragments. Male squid took cephalopod fragments more frequently and ate more megalops per meal than females. Females fed more on polychaetes, egg-like spheres, and cumaceans. A significant association between prey ranks was found in percent frequency of occurrence, (Table 6), but not in percent by number of prey species while the similarity index was relatively high (60.8%).

5.3.5. Feeding Chronology Determinations

The frequency histogram of the major feeding states revealed squid feeding to be most pronounced during daylight hours and least during night hours (Figure 20). Night samples (2201 to 0600) showed no significant feeding activity, with most of the stomach contents empty, not full or recent (Category A). Most of the night samples did show some feeding activity with small percentages of recent but not full stomachs (Category B). One interval (2201 to 2400) had 1.5% stomachs in the full and recent category (C). Daylight samples showed the only significant frequency of full stomachs. The late morning interval (1001 to 1200) had a large frequency, 25%, of recent and full stomachs (Category C). During this same period, the recent but not full feeding state (Category B) increased to 11.4%. By 1201 to 1400 hours, most of the stomachs were full (82.1%), with half of these no longer recent (Category D). Early afternoon hours (1401 to 1600) were similar to night samples, with 86.4% of the stomachs empty,

not full or recent (Category A). These differed from night samples in having a higher percentage (9.9) of full but not recent stomachs (Category D). Late afternoon (1601 to 1800) showed an increase in the frequency of recent but not full stomachs (Category B).

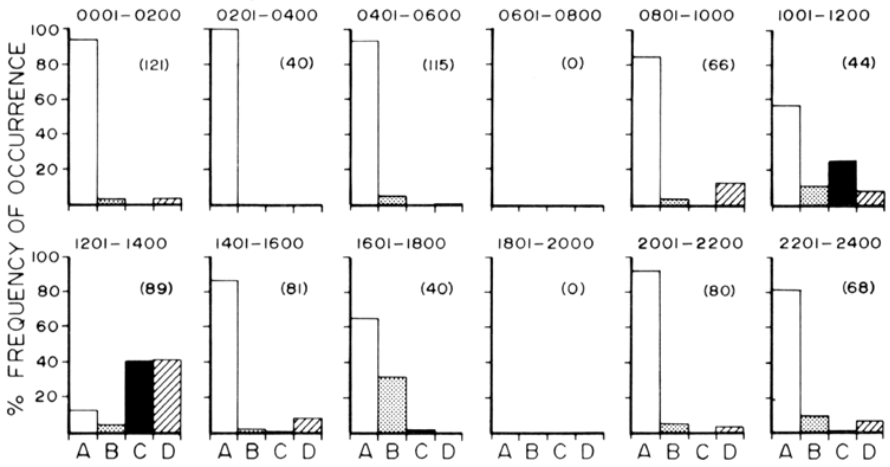

FIGURE 20. Diel feeding period determination using index of fullness and digestion combined. A = Empty, not full or recent; B = Recent but not full; C = Full and recent; D = Full but not recent.

*FIGURE **20. Diel feeding period determination using index of fullness and digestion combined. A = Empty, not full or recent; B = Recent but not full; C = Full and recent; D = Full but not recent.***

The results were examined qualitatively in greater detail by separating fullness from digestion; these were examined over 2 hour intervals for a 24-hour cycle (Figure 21). The fullness index alone showed night samples to be mostly empty or in State 1 (fragments to # full). Stomachs in State 4 (bulging) first occurred at 0801, peaked at 1201 to 1400, and decreased sharply at 1401 to 1600. During the noon peak, all but 1.1% of the squid had some stomach contents. The index of digestion revealed both night and day samples to have stomachs in State 3. Only during daylight was the most recent (State 4) ingested food encountered, with a maximum at 1001 to 1200.

Percentage of dry body weight also indicated daytime feeding, since almost all squid captured from 0945 to 1500 had some measurable stomach contents (Figure 22). From early evening to before dawn, stomach contents became progressively clustered near zero. By 0300 to 0600, all stomachs were essentially empty. The largest meal size occurred at 1255 when the sample averaged 6.3% dry body weight; this also was the time of the largest individual meal size of 9.0%.

5.3.6. Field Determination of Stomach Emptying Rates

All squid used in the field digestion experiments were sexually immature and of a relatively small size. The average dorsal mantle length was 87 mm (s = 1.5mm). Their average dry body weight was 2.8 gm (s = 1.0 gm).

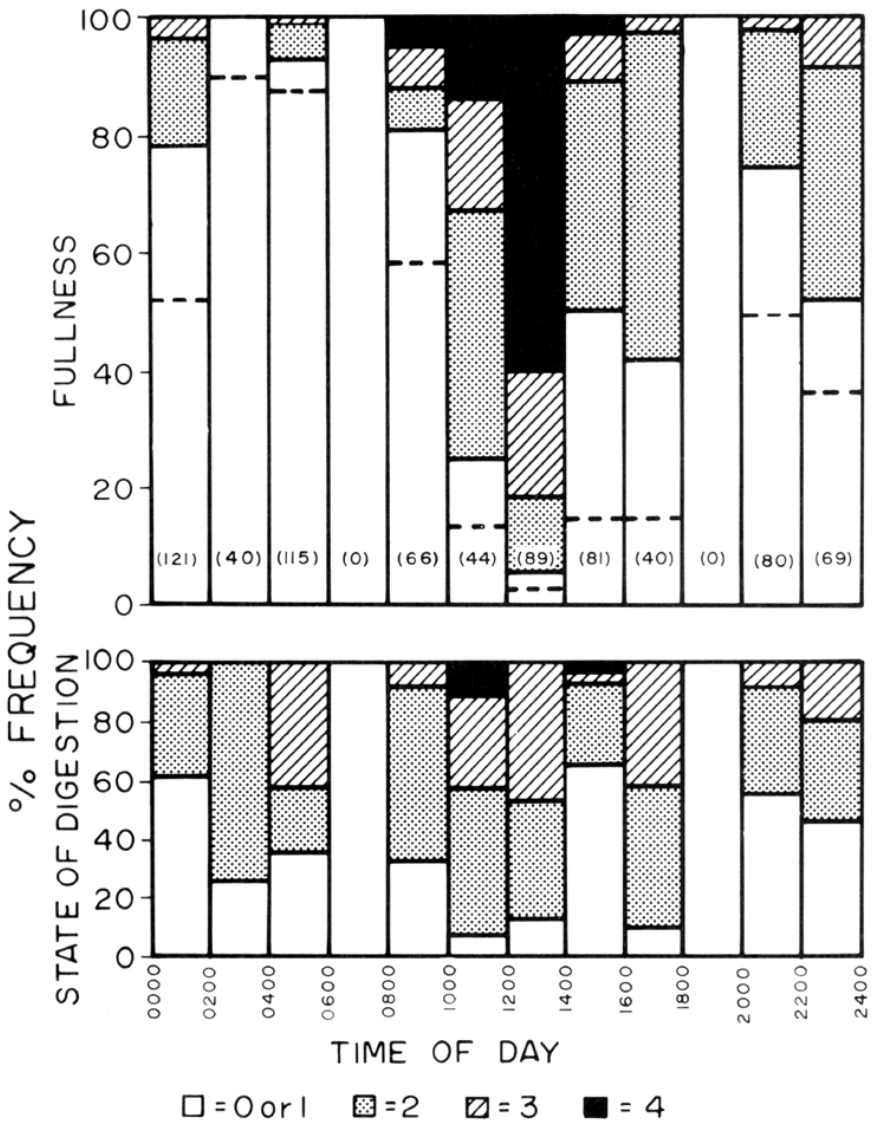

FIGURE 21. Diel feeding period determination using index of fullness and digestion separately.

*FIGURE **21. Diel feeding period determination using index of fullness and digestion separately.***

At the time of capture, the stomachs were generally full, although not largely distended. The average index of fullness was 3.2. The initial amount of stomach contents as a percent of dry body weight ranged from 0.18 to 4.07%. These corresponded to an actual dry weight of 0.004 to 1.140 gm, with an average meal of 0.041 gm.

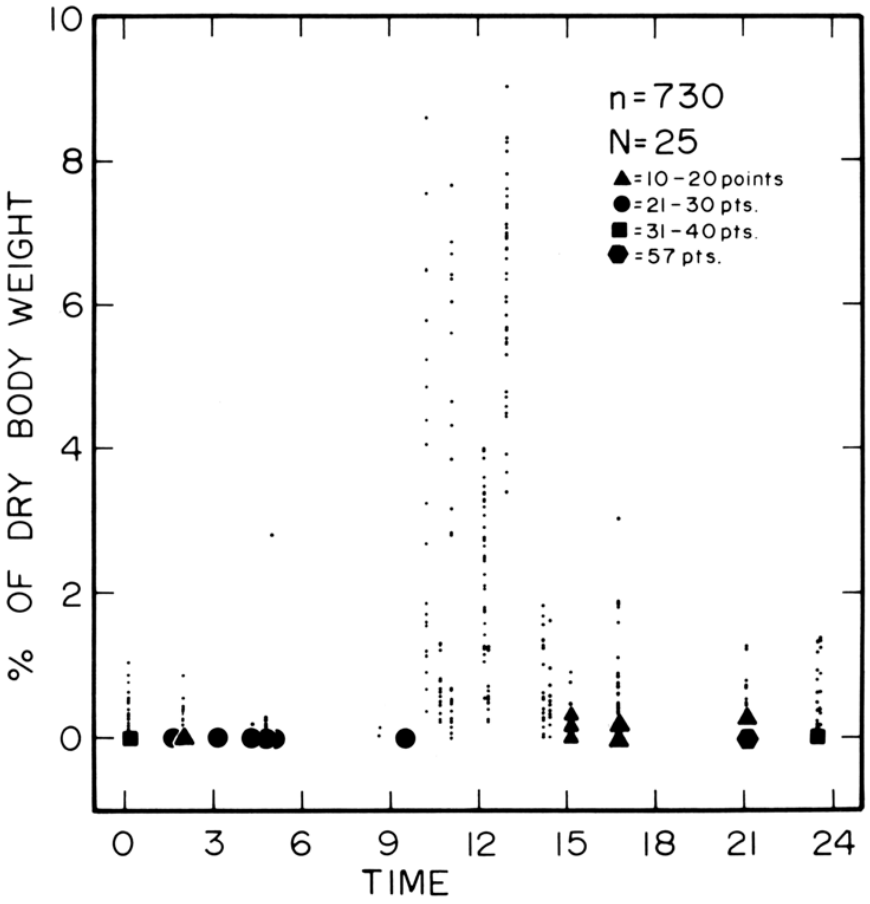

FIGURE 22. Diel variation of feeding intensity in terms of the dry weights of stomach contents as a percent of dry body weight.

*FIGURE **22. Diel variation of feeding intensity in terms of the dry weights of stomach contents as a percent of dry body weight.***

Detailed prey analysis revealed that euphausiids, Euphausia pacifica, dominated the diet, followed by small numbers of shrimp, Sergestes sp.; copepods; and fish, northern anchovy, Engraulis mordax.

Using the fullness and digestion state indices developed for the chronology study, most of the 0 to 60 minute samples were recently full (Category C). Within 2 to 3 hours, half of the stomachs were no longer full but still recent (Category B) and by 5 to 7 hours, all the stomachs were empty, not full or recent. (Category A).

Individual meal size as a percentage of dry body weight was compared to time of sacrifice (Figure 23), and no digestion occurred until after 2 hours. By 5 to 7 hours, the amount remaining clustered near 0. The slope of the regression line gave an average digestion rate of 0.29% dry body

weight per hour. The intercept gave a stomach emptying time of 6.6 hours. Using this emptying time and the average initial meal size, an average digestion rate of 0.006 gm per hour was calculated.

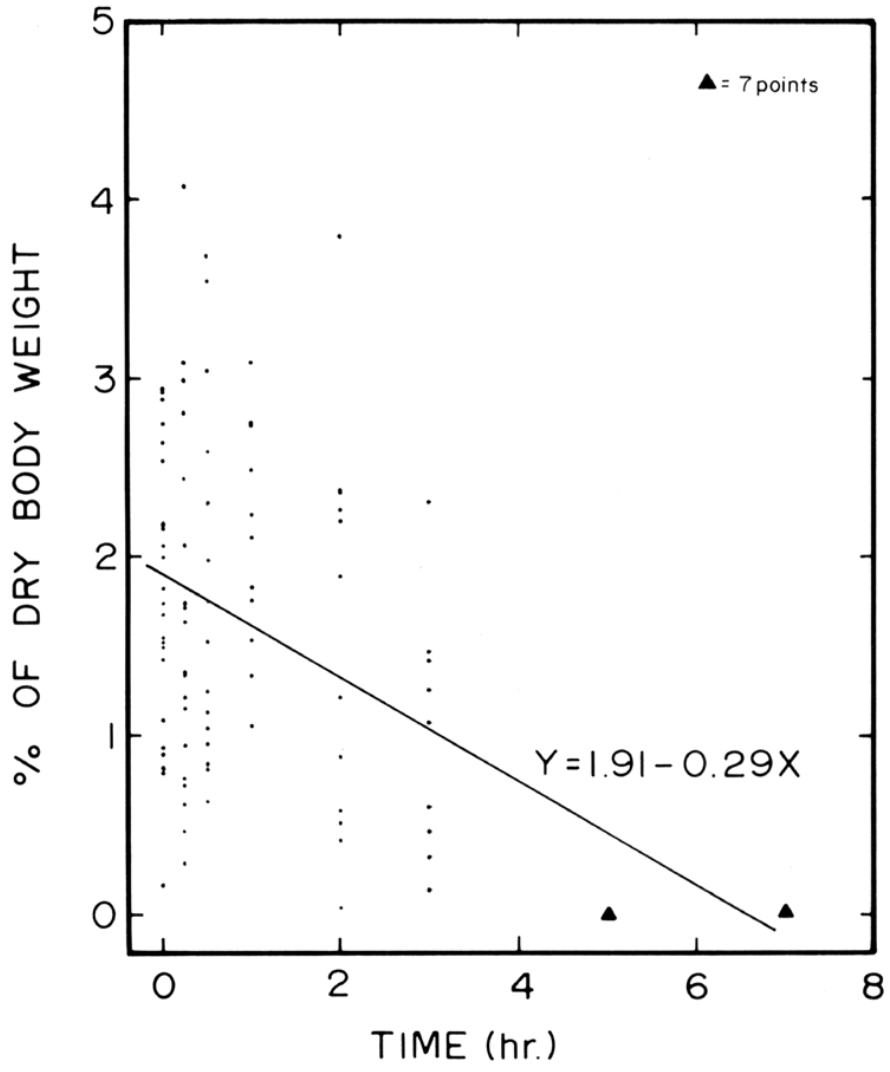

FIGURE 23. Field determination of digestion rate in terms of dry stomach weight, as percent of dry body weight.

*FIGURE **23. Field determination of digestion rate in terms of dry stomach weight, as percent of dry body weight.***

5.4. DISCUSSION

5.4.1. Prey Composition

Squid have been reported to change their feeding habits with growth in size. Squires (1967) described for the Newfoundland squid Illex illecebrosus a diet of mostly euphausiids in small animals (100–120 mm mantle length) with fish in only 12% of the food containing stomachs. In larger squid, the occurrence of crustacea declined to insignificance, with fish increasing in importance until they became the major component of the diet. Cannibalism increased among the largest animals (250–300 mm mantle length).

Vovk (1972b) reported a similar trend in the east coast squid L. pealei. Planktonic feeding was dominant in the smallest sized squid (75 mm mantle length). Euphausiid feeding became increasingly important to larger squid (125 mm mantle length). Cannibalism and fish feeding dominated in sizes larger than the 160 mm mantle length.

Kore and Joshi (1975), working with the Indian squid Loligo duvauceli, reported a similar increase in cannibalism and decrease in crustacean feeding for larger squid. These were the only authors that distiguished true cannibalism from the ingestion of cephalopod fragments such as skin and tentacle fragments.

Fields (1965) also reported a similar trend for L. opalescens captured in Monterey Bay. His study was based on a sample of 75 animals from commercial fish seiners, and 31 male squid from the spawning grounds. Only those squid whose stomachs appeared to have contents from external examination were used in his study. He reported a trend of crustacean to fish feeding of 3:1 in small squid, 1:1 for young squid, and 1:3 for adult squid (males from the spawning grounds). Fields stated that feeding on the spawning grounds was probably atypical because of the ground's localized nature and the increased crowding that the animals experienced. These spawning adults were reported to show 75% frequency of cannibalism in stomachs with contents. No distinction was made between true cannibalism and cephalopod fragments. In our study no spawning ground animals were included in the comparison between sizes in order to avoid the localized nature of feeding on these grounds.

We found a closer correlation in feeding habits between different sizes of L. opalescens than was reported by Fields (1965). Certainly there was a trend for larger animals to feed more on cephalopods and fish than the smaller sizes of squid, but significance of association by frequency of occurrence and a high percent similarity index does not support this trend. The possibility that other squid do show major differences in feeding habits between size categories can still be explained. Both L. pealei and I. illecebrosus are larger animals than L. opalescens. If feeding habits of sizes similar to large L. opalescens are examined for these two species, crustacean feeding dominates. Another possible explanation is that unlike our study, these authors did not separate location of capture from size of squid captured. Squire (1967) pointed out that Illex captured on the outer edge of the Grand Bank were also the smaller squid and were found to feed more on euphausiids than larger squid taken on the Grand Bank.

Our comparison by depth of capture could not clearly be separated from a comparison of location since the shallow water (less than 40 fathoms) also represented the more inshore areas. It is therefore not clear if the greater amount of euphausiid and copepod feeding in deep water samples resulted from increased availability in deeper waters, or offshore waters, or both. It does appear, however, that as was reported by Squires (1967) for Illex, L. opalescens taken inshore had a different diet from those taken offshore.

Fields (1965) suggested that female L. opalescens do not actively feed on the spawning grounds. Our results indicate that females do feed on the spawning grounds, although perhaps less intensively than males. Only percent frequency of occurrence of prey items between spawning ground males and females was significantly associated, while percent by number was not and had a low percent similarity index value. Males ate larger meals by number than females. Perhaps, as suggested by Fields (1965), female squid do have digestive tracts in less active condition than in males.

It became clear that spawning ground feeding was indeed atypical, as suggested by Fields (1965), when these samples were compared to other areas at similar depths. The percent similarity index was lowest in this comparison, and no correlation was found in either percent by number or frequency of occurrence.

Demersal feeding was more important on the spawning grounds, with bottom-associated organisms such as megalops larvae, polychaetes, gastropods, and eggs being more common in the diet. Crustacean feeding still dominated, with euphausiids being replaced by the more seasonal, and perhaps more localized, megalops larvae. Cephalopod fragments occurred most frequently in spawning ground samples. True cannibalism, however, did not occur on these grounds. Cephalopod fragments probably do not reflect true feeding, but some form of behavior associated with crowding. This could explain the higher incidence of cephalopod fragments on the spawning grounds where animals tend to be more crowded.

Overall, it appears that Loligo opalescens is an important predator in the pelagic ecosystem of Monterey Bay, and presumably elsewhere in the California coastal waters. It feeds primarily on smaller crustaceans such as euphausiids, copepods, megalops larvae, mysids, and amphipods, but also utilizes larger prey items such as fish and other cephalopods. The diet of L. opalescens changes markedly with depth of water and location, but does not differ much between size categories or sexes. This appears to indicate that market squid tend to utilize similar prey items regardless of sex or size, but that differences in prey utilization may result from changes in patches of available prey or different behavior of this predator at different locations.

5.4.2. Feeding Chronology

Several approaches have been used by others to assess diel feeding periodicity in both laboratory and field experiments. Most of these have dealt with studies on fish and only one has dealt with squid; however, some of the assumptions made for these field studies are applicable to our study.

Magnuson (1969) fed skipjack tuna in the laboratory at fixed intervals, removing rejected portions of the meal. Using this method, he was able to estimate both the peak feeding period and the size of the daily meal. Darnell and Meierotto (1962) suggested making field collections throughout the 24 hour period. They collected bullheads, Ictalurus meals, over a 24 hour period at fixed intervals. The durations of these intervals were determined in laboratory experiments by the time required for the most important natural prey item (their "standard food item") to pass through the first stage of digestion (their Phase 1). By staggering the actual amount of stomach contents and the phase of digestion, the actual peak feeding period was determined. Vovk (1972b) in his work with L. pealei, used a subjective state of fullness assigned to catches grouped into 4-hour intervals over a 24 hour cycle. These collections were made over a 10 day period. However, he erroneously interpreted peak fullness as entirely representing the peak period of feeding intensity without regard for state of stomach recency.

In this study, we attempted to use a method similar to the one developed by Darnell and Meierotto (1962) with several inherent limitations: 1. Animals could not be taken over a 24 hour period and, in fact, were taken over a period of several months. The assumption made here is that these animals represented a single feeding population—a necessarily false assumption. 2. Samples could not be taken at fixed intervals and instead were taken whenever trawls captured squid throughout the 24 hour period. 3. The phases of digestion were determined using artificial food types and not the "standard food item" (euphausiids) for squid.

With these limitations in mind, peak feeding intensity for Loligo opalescens occurred during the summer months between 0900 and 1200, when most of the stomach contents were still recent in terms of state of digestion. Although by 1200 to 1400, more stomachs had contents, half of these were no longer recent. Intensive feeding may resume for a short burst at 1500 to 1600 hours.

An examination of the actual amount of stomach contents as a percentage of the squid's dry body weight throughout the 24 hour period revealed a similar picture. None of the assumptions made earlier were necessary to conclude that squid feed most intensively during daylight hours. The gradual tapering off of stomach contents toward the early morning hours can be interpreted in two ways: 1. Squid may slow their digestion during night hours, thus having small amounts of food in their stomachs throughout the night hours. 2. A small amount of feeding may occur in the early evening during chance encounters with prey. The first possibility seems most important in light of the fact that only during daylight hours was Category 4 (the most recent digestion) encountered.

Vovk (1972b) reported that L. pealei also fed less on cloud-covered days during daylight hours. He based this conclusion on only two samples taken at the same time on different days. We found a similar trend for L. opalescens, again based on only a few samples. While using night lights to jig for squid, we have observed L. opalescens feeding on prey attracted to the lights. This and the daylight feeding indicate that this squid is a visual predator, feeding optimally when the most light is available. It is certainly

probable that other factors influenced this feeding period. Ivlev (1961) suggested that factors such as prey concentration and patchiness could affect prey availability. Investigating these possibilities was beyond the scope of this study.

5.4.3. Digestion Rates

Caution was exercised in interpreting the rates of digestion obtained in the field experiments. A number of factors known to affect digestion processes in animals could not be controlled. These squid were small, of uniform size, and sexually immature. They had been feeding on natural prey at the time of capture and had not had a chance to complete their meal. Disadvantages of the field experiment included inability to gauge precisely when their meal was initiated, an experimental temperature that was higher than surface water temperature, and the rough handling experienced in the trawling operation. Bidder (1950) reported that digestion stopped for several hours in trawled squid. This probably explains the lack of significant change in stomach contents during the first few hours.

Our subjective index of digestion (Tables 3 and 4; Karpov, 1977) provided a means whereby recency of feeding could be estimated. Even though most of the squid stomachs taken in the field represented Category (4), it should be remembered that this index value has a range of sensitivity of several hours and thus possibly reflects a meal several hours old. Several authors have shown that in fish acclimated to higher temperatures, digestion rates are higher (Hathaway, 1927; Molnar and Tolg, 1962; and Brett and Higgs, 1970). The temperature of the experiment was elevated 2 to 3 C above the surface water temperature (possibly 10 to 11 C above the temperature at the depth of capture). These animals had not had a chance to acclimate to these higher temperatures.

A digestion rate value of 0.29% (0.34% if time 0 is assumed to start at 1 hour) of dry body weight per hour was obtained. This result is comparable to that found in a laboratory study of Loligo opalescens' digestion rate for mosquito fish, Gambusia sp., and grass shrimp, Crangon sp., where a range of 0.41% to 0.9% was calculated (Karpov, 1977). Magnuson (1969) found that skipjack tuna, Katsuwonus pelamis, one of the most rapidly digesting fishes, has a similar rate of 0.72% of dry body weight per hour (meal weight adjusted for water loss).

An estimate of the population daily meal size can be made for L. opalescens by combining the duration of actual intense feeding, determined in the field feeding chronology study, with an estimate of the instantaneous depletion rate determined in the laboratory. Using this depletion rate and the initial meal size the hourly meal can be calculated. In Karpov's (1977) study, the depletion rate was 0.39 for the combined sizes of crustacean meal, and the initial meal size was 0.69 gm dry weight (6.48% dry body weight). The hourly meal is then calculated to be 0.27 gm dry weight (2.54% dry body weight).

Our field studies showed two periods of intense feeding: 3 hours during the morning and 1 hour before sunset. The calculated morning consumption is 7.61% dry body weight. Depletion rates cannot be applied to the

sunset meal because of the small average meal size (0.47% dry body weight). Instead, the average value itself will be assumed to represent the total amount consumed. The average meal size at midday must also be included, with a peak value of 6.26% dry body weight. This gives a total daily consumption of 14.4% dry body weight per day.

LaRoe (1971) estimated that in the laboratory 10 week old squid, Sepioteuthis sepiodea, consumed 17 to 25% of their body weight (wet) per day. He also noted a gradual decrease with age. Hurley (1976) reported a daily meal of 35 to 80% of wet body weight for L. opalescens young.

Our studies indicate that Loligo opalescens is an active, visual predator, feeding mostly during midday on euphausiids and other crustacea, has a rapid digestion rate, and consumes a rather large daily meal. Since they consume at least 14% of their total biomass daily, are very abundant in California Current waters, and act as a major food source for many other marine predators, market squid must indeed play a large role as a vital link between zooplankton and higher trophic levels in this pelagic environment.

5.5. ACKNOWLEDGEMENTS

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* **Present Address: California Department of Fish and Game Long Beach, California**

6. THE IMPORTANCE OF LOLIGO OPALESCENS IN THE FOOD WEB OF MARINE VERTEBRATES IN MONTEREY BAY, CALIFORNIA

by
G. VICTOR MOREJOHN
JAMES T. HARVEY
LYNNE T. KRASNOW
Moss Landing Marine Laboratories
Moss Landing, Californiaq

6.1. INTRODUCTION

Cephalopods have long been known to be important prey of many marine vertebrates. Clarke (1966) lists the known cephalopod species and the fish, bird, and mammal predators recognized at that time. The importance of cephalopod species in the diet of commercially important food fishes such as yellowfin tuna and albacore has been noted by Perrin, *et al.* (1973). However, Loligo opalescens, among the cephalopod species, has been found in the diet of several important commercial and recreational fishes (Fields, 1965), in bonito, bluefin tuna, and albacore (Pinkas, Oliphant, and Iverson, 1971), and in king salmon (Merkel, 1957) and silver salmon (Silliman, 1941).

The intent of the present investigation was to provide information on the extent of predation by marine fishes, seabirds, and marine mammals on L. opalescens in Monterey Bay. Beyond this major purpose, this study provided valuable information on other forage (prey) species consumed by these predators. Additionally, we now have baseline knowledge of the importance of L. opalescens, as food, relative to other species of cephalopods, several species of crustaceans, and many species of fishes that are important in life cycles of abundant or economically important marine vertebrates.

6.2. MATERIALS AND METHODS

Gastrointestinal or stomach contents from 1,928 marine fish of 86 species in 33 families; 513 seabirds for 28 species in 8 families, and 143 marine mammals of 15 species in 8 families (Table 7) were obtained from specimens collected in various ways. Fishes were collected with hook and line, gill net, beach seine, purse seine, midwater trawls, and otter trawls. Birds were collected by hand, shotgun, hoop net, and as beach-cast dead carcasses. Mammals were collected only as beach-cast dead carcasses, however, oral rejecta (vomitus) from seals on hauling grounds and scats were also utilized.

TABLE 7
Marine Fishes, Seabirds, and Marine Mammals Collected for Study of Feeding Habits in Monterey Bay, California

Class	*Family*	*Species*
Chondrichthyes	Hexanchidae	*Hexanchus griseus*
	Squalidae	*Squalus acanthias*
		Somniosus pacificus
		Echinorhinus cookei
	Alopiidae	*Alopias vulpinus*
	Scyliorhynidae	*Parmaturus xaniurus*
	Lamnidae	*Carcharodon carcharias*
	Carcharhinidae	*Triakis semifasciata*
		Mustelus californicus
		Mustelus henlei
		Prionace glauca
	Rhinobatidae	*Rhinobatus productus*
	Rajidae	*Raja binoculata*
	Chimaeridae	*Hydrolagus collei*
Osteichthyes	Clupeidae	*Alosa sapidissima*
		Clupea harengus
	Engraulidae	*Engraulis mordax*
	Salmonidae	*Oncorhynchus kisutch*
		Oncorhynchus tshawytscha
	Alepisauridae	*Alepisaurus ferox*
	Batrachoididae	*Porichthys notatus*
	Ophidiidae	*Chilara taylori*
	Macrouridae	*Coryphaenoides acrolepis*
	Merlucciidae	*Merluccius productus*
	Zoarcidae	*Lycodes diapterus*
		Lycodapus mandibularis
	Trachipteridae	*Trachipterus altivelis*
	Scorpaenidae	*Sebastolobus alascanus*
		Sebastes cauriuus
		Sebastes rexillaris
		Sebastes nebulosus
		Sebastes chrysomelas
		Sebastes carnatus
		Sebastes rastrelliger
		Sebastes atrovirens
		Sebastes melanops
		Sebastes mystinus
		Sebastes flavidus
		Sebastes serranoides
		Sebastes constellatus
		Sebastes chlorostictus
		Sebastes jordani
		Sebastes paucispinis
		Sebastes goodei
		Sebastes diploproa
		Sebastes saxicola
	Zaniolepididae	*Zaniolepis latipinnis*
	Hexigrammidae	*Oxylebius pictus*
		Ophiodon elongatus
	Cottidae	*Scorpaenichthys marmoratus*
		Leptocottus armatus
		Chitonotus pugetensis
		Artedius notospilotus
	Carangidae	*Trachurus symmetricus*
	Sciaenidae	*Genyonemus lineatus*
	Scorpididae	*Medialuna californiensis*
	Embiotocidae	*Embiotoca jacksoni*
		Amphistichus argenteus
		Hyperprosopon anale

TABLE 7
Marine Fishes, Seabirds, and Marine Mammals Collected for Study of Feeding Habits in Monterey Bay, California

TABLE 7—Continued
Marine Fishes, Seabirds, and Marine Mammals Collected for Study of Feeding Habits in Monterey Bay, California

Class	*Family*	*Species*
		Hyperprosopon argentium
		Cymatogaster aggregata
		Zalembius rosaceus
		Micrometrus minimus
		Damalichthys vacca
		Phanerodon furcatus
	Anarhichadidae	*Anarrhichthys ocellatus*
	Clinidae	*Neoclinus uninotatus*
	Scombridae	*Sarda chiliensis*
		Thunnus alalunga
	Centrolophidae	*Icichthys lockingtoni*
	Cynoglossidae	*Symphurus atricauda*
	Bothidae	*Paralichthys californicus*
		Citharichthys sordidus
		Citharichthys stigmaeus
	Pleuronectidae	*Hippoglossus stenolepis*
		Pleuronichthys decurrens
		Pleuronichthys verticalis
		Psettichthys melanostictus
		Hypsopsetta guttulata
		Parophrys vetulus
		Platichthys stellatus
		Glytocephalus zachirus
		Atheresthes stomias
		Microstomus pacificus
		Lyopsetta exilis
		Eopsetta jordani
Aves	Gaviidae	*Gavia arctica*
	Phalacrocoracidae	*Phalacrocorax penicillatus*
	Diomedeidae	*Diomedea nigripes*
	Procellariidae	*Fulmarus glacialis*
		Puffinus griseus
		Puffinus tenuirostris
		Puffinus creatopus
		Puffinus bulleri
		Puffinus (calonectris) leucomelas
	Stercorariidae	*Stercorarius pomerinus*
		Stercorarius parasiticus
	Laridae	*Larus occidentalis*
		Larus glaucescens
		Larus californicus
		Larus argentatus
		Larus heermanni
		Larus canus
		Larus philadelphia
		Rissa tridactyla
	Stemidae	*Sterna forsteri*
		Sterna caspia
		Sterna elegans
	Alcidae	*Uria aalge*
		Cerorhinca monocerata
		Ptychoramphus aleutica
		Synthliboramphus antiguum
		Brachyramphus marmoratum
		Endomychura hypoleuca
Mammalia	Mustelidae	*Enhydra lutris*
	Phocidae	*Phoca vitulina*
		Mirounga angustirostris
	Otariidae	*Callorhinus ursinus*
		Zalophus californianus
		Eumetopias jubata

TABLE 7
Marine Fishes, Seabirds, and Marine Mammals Collected for Study of Feeding Habits in Monterey Bay, California

TABLE 7—Continued
Marine Fishes, Seabirds, and Marine Mammals Collected for Study of Feeding Habits in Monterey Bay, California

Class	*Family*	*Species*
	Ziphiidae	*Mesoplodon stejnegeri*
	Kogiidae	*Kogia breviceps*
	Delphinidae	*Pseudorca crassidens*
		Lagenorhynchus obliquidens
		Lissodelphis borealis
	Phocoenidae	*Phocoena phocoena*
		Phocoenoides dalli
	Eschrichtidae	*Eschrichtius robustus*
		Balaenoptera acutorostrata

TABLE 7
Marine Fishes, Seabirds, and Marine Mammals Collected for Study of Feeding Habits in Monterey Bay, California

The gastrointestinal, stomach, vomitus, or scat contents were rinsed in water and sorted into their taxonomic categories. They were then identified to family, genus, and species, when possible. Animals with sufficiently undigested materials in their stomachs had the contents removed and placed in 10% formalin for about 2 days, then transferred to 40% or 50% isopropyl alcohol until the items could be studied.

All prey from these stomachs were weighed, enumerated, and subjectively evaluated on the basis of percent volume of the total stomach contents. Some recently ingested prey or partly digested prey items were readily identifiable. In most cases, however, species identifications were largely made using fish otoliths (sagittae) and cephalopod beaks using our reference collections and keys by Iverson and Pinkas (1971). John Fitch (California Department of Fish and Game, Long Beach) either confirmed our tentative otolith identifications or initially identified the otoliths. Gilbert L. Voss (University of Miami) confirmed most of our cephalopod beak identifications.

Most fish, some of the birds, and a few of the mammals had stomach contents evaluated on the basis of the Index of Relative Importance (IRI) developed by Pinkas, Oliphant, and Iverson (1971). Prey items identified from stomachs of dead, beach-cast, or stranded marine mammals restricted conventional methods of analysis. In most instances stomach contents of mammals were completely digested leaving only fish bones, lenses, otoliths, cephalopod beaks, and pens. For this reason it was not often possible to determine volume of food items. Thus we used both numerical percentage and frequency of occurrence percentage as well as the IRI method of Pinkas, Oliphant, and Iverson (1971).

In order to arrive at an estimation of dorsal mantle length (DML) of L. opalescens, the upper and lower beaks were measured according of Kashiwada, Recksiek, and Karpov (In press, Calif. Coop. Ocean. Fish. Invest. Rept. 20).

Several research vessels were utilized: ORCA, ARTEMIA, OCONOSTOTA from Moss Landing Marine Laboratories; National Marine Fisheries Service (NMFS) vessel JOHN N. COBB, and the NMFS charter vessel PACIFIC RAIDER.

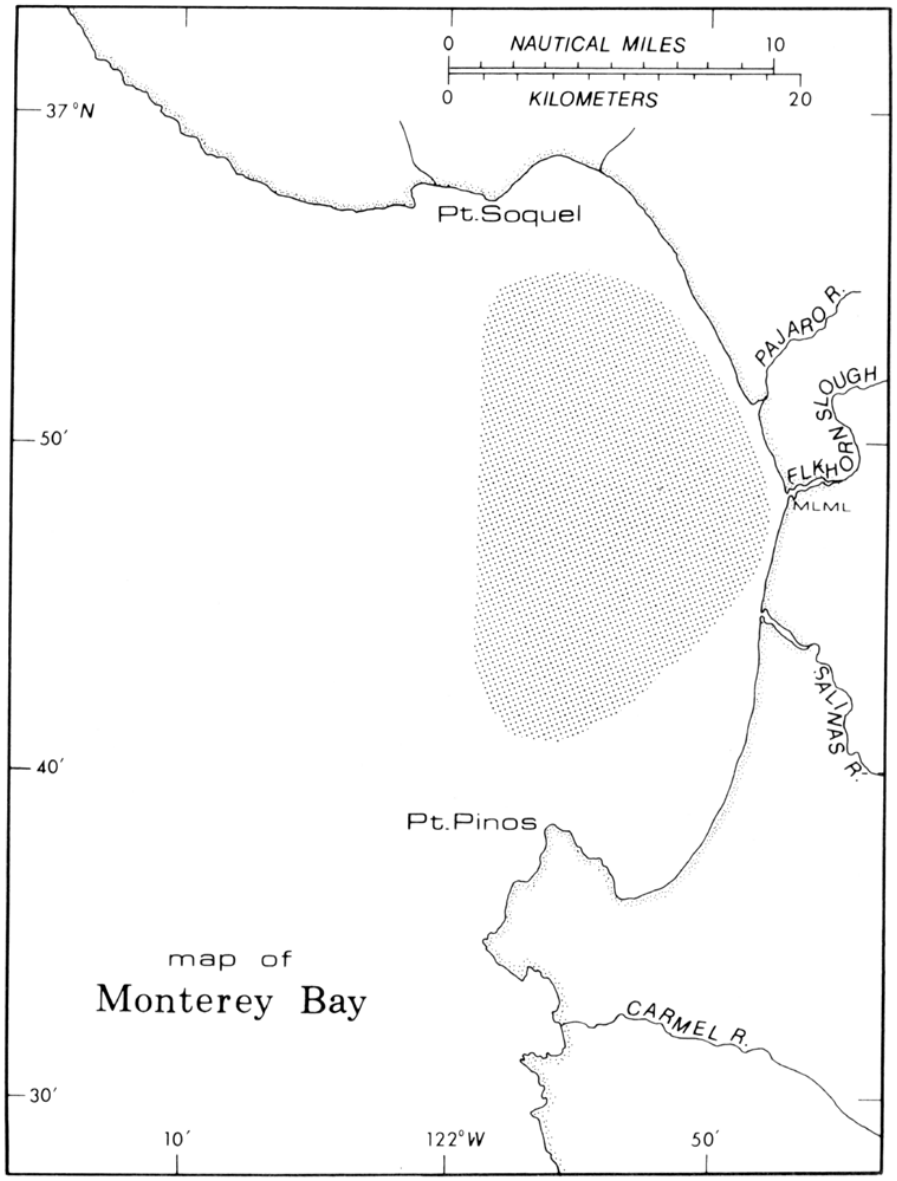


FIGURE 24. Principal collecting areas for fish and birds in Monterey Bay.

*FIGURE **24. Principal collecting areas for fish and birds in Monterey Bay.***

Our principal collection stations for fish and birds (Figure 24) were at:

1. West of Pt. Piños near the squid spawning grounds off Cannery Row in waters of 8 to 10 fathom depth;
2. Petrale Hill and vicinity approximately due west of Moss Landing, 1.5 miles, in waters of 20 to 40 fathoms depth; and
3. Soquel—an area due south of Santa Cruz, 5 miles, in waters of 30 to 40 fathoms depth.

Collections of fish by trolling, set lines, and other trawls were made in oceanic areas in between these stations. The NMFS cruises were largely on the continental shelf north and south of Monterey Bay.

An energetics study of sooty shearwaters, Puffinus griseus, was undertaken to determine the demand by these birds on L. opalescens as prey. An equally important resource species, the northern anchovy, Engraulis mordax, was used in the experiments for comparison with Loligo. For these metabolic studies, sooty shearwaters were captured with a hoop net thrown from the bow of a boat as they sat on the water or took flight from the water. The net was made of 10 cm stretch-mesh gill net bound onto a 2.5 m diameter black plastic hose connected at each end by a piece of dowel fastened with metal clamps as modified from Gill, Sladen, and Huntington (1970). Shearwaters captured in this manner were held in captivity in individual cages made of 1 inch square welded wire fabric 24 by 24 by 20 inches tall. Each cage was placed in a plywood box which extended 6 inches up each side as a splash guard. A plastic garbage bag, slit and unfolded, was placed between each box and cage to collect excreta.

Existence energy requirements (basal metabolic rate) and assimilation efficiency (amount of nutrients in food utilized) of Loligo and Engraulis fed to captured birds were determined. Details of the experimental procedure are provided in Krasnow (1978). Calorimetric analyses of intact specimens of male and female Loligo and Engraulis, as well as the excreta from shearwaters following consumption of these prey items, were made after combustion in a Parr nonadiabatic semi-microbomb calorimeter. The digestibility of the two prey species and their mean caloric densities were used to determine the approximate quantity of each that would be required to supply an individual sooty shearwater's daily metabolic needs. Abundance estimates (Ainley, 1976; Elliott, Gill, and Morejohn, unpublished manuscript) of sooty shearwaters in Monterey Bay were used to calculate the maximum and minimum annual demand by this species on Loligo and Engraulis in Monterey Bay.

6.3. RESULTS

6.3.1. Species Accounts

6.3.1.1. Fish

Nineteen species of fish in Monterey Bay were found to consume Loligo. Most species are associated with the bottom, but several species range widely in the water column such as salmon, oncorhynchus spp.; midshipman, Porichthys spp.; sanddabs, Citharichthys spp. (Fitch and Lavenberg, 1968). The blue shark, Prionace glauca, occurs principally in the epipelagic zone in Monterey Bay (Harvey, unpublished manuscript). Feeding habits of some fishes of economic importance in Monterey Bay are reviewed by Frey (1971).

Only three species of fish studied were seasonal in occurrence: king and silver salmon, and the blue shark. All the others were resident in Monterey Bay and vicinity. King and silver salmon are important economically as game and food fish. The blue shark, abundant in Monterey Bay, plays a minor role as a game fish, however, because of its abundance it plays a major role in the food web in Monterey Bay. Anchovies, rockfish, hake, croakers, midshipman, and several species of flatfish, as well as the two species of salmon and the blue shark, are of major importance in the complex food web which involves L. opalescens as a prey species (with the exception of anchovies of which Loligo is the predator).

King salmon, Oncorhynchus tshawytscha—Most king salmon were taken between the Petrale Hill and Soquel Stations. Some gastrointestinal tracts were provided by local fishermen, but the area of capture in Monterey Bay could not be related to our collecting station localities. The most important prey eaten in Monterey Bay was the northern anchovy (Table 8, Figure 25 A). Other important food items were euphausiids, Thysanoessa spinifera; juvenile rockfish, Sebastes sp.; crab, Cancer sp.; megalopa; and squid, Loligo. Anchovies ranked highest in frequency of occurrence and volume (IRI = 3834), juvenile rockfish, Sebastes sp., ranked third (IRI = 242) after fish parts (IRI = 1,749); and Loligo ranked fourth (IRI = 190). The range in size of Loligo consumed ranged between 30 to 130 mm DML.

TABLE 8
A List of Marine Vertebrate Species Studied Showing Selected Prey Items Ranked According to Their Highest Index of Relative Importance (IRI).

Predators	*Prey categories* * Fish parts	Digested tissue	Euphausiids	Crustacea	*Engraulis mordax*
Fish:					
Oncorhynchus tshawytscha (n=99)	1749	14	9	—	3834
Oncorhynchus kisutch (n=34)	251	260	5	—	—
Porichthys notatus					
(n=25) Petrale Hill Station:	167	318	2957	376	16
(n=35) Soquel Station:	386	1219	47	2837	—
Genyonemus lineatus (n=12)	1210	5233	—	97 (11)	139 (8)
Sebastes goodei (n=5)	1472	—	392	—	—
Citharichthys stigmaeus (n=6)	4100	794	—	983	—
Citharichthys sordidus					
(n=98) Monterey Station:	532	1095	—	30	—
(n=75) Petrale Hill Station:	515	1895	—	570	34
(n=56) Soquel Station:	868	757	304	50	—
Pleuronichthys decurrens					
(n=37) Monterey Station:	—	1657	—	7	—
Opiodon elongatus					
(n=7) Monterey Station:	2314	—	—	—	284
(n=3) Petrale Hill Station:	1250	1688	1000	—	—
(n=4) Soquel Station:	10219	—	—	—	—
Eopsetta jordani					
(n=10) Petrale Hill Station:	200	23	—	1041	200
(n=4) Soquel Station:	5000	—	1250	213	388
Hippoglossus stenolepis					
(n=7) Monterey Station:	543 (3)	—	—	147 (8)	—
Anaplopoma fimbria					
(n=21) Soquel Station:	5987	158 (4)	—	93 (5)	—
Merluccius productus					
(n=13) Monterey Station:	135	1169	118	246	—
(n=4) Soquel Station:	769	—	163 (5)	10688	—
Prionace glauca (n=121)	1985	384 (3)	198 (5)	—	301 (4)
Birds:					
Gavia arctica (n=7)	90 (5)	—	—	320 (3)	35 (6)
Phalacrocorax penicillatus (n=14)	908	912	—	—	1301
Puffinus griseus (n=95)	571 (4)	130 (8)	82 (9)	10 (12)	665 (3)
Puffinus tenuirostris (n=20)	414 (5)	60 (9)	—	—	70 (8)
Puffinus creatopus (n=13)	4740	57 (7)	—	—	1818
Fulmaris glacialis (n=14)	33 (9)	1425 (2)	—	—	13 (7)
Larus heermanni (n=24)	2310	14 (11)	—	—	826
Larus canus (n=3)	1667	—	—	56 (9)	11900
Larus glaucescens (n=10)	630	520	—	200 (6)	120 (9)
Larus californicus (n=11)	631 (3)	466 (4)	—	105 (9)	180 (7)
Rissa tridactyla (n=21)	241 (4)	100 (7)	—	19 (8)	630
Cerorhinca monocerata (n=37)	597	42 (6)	—	—	593
Uria aalge (n=73)	4551	86 (6)	—	—	1188
Mammals:					
Mirounga angustirostris (n=2)	2800 (2)	—	—	—	—
Callorhinus ursinus (n=3)	8267	—	—	—	—
Zalophus californianus (n=21)	5025	—	—	—	513
Phocoena phocoena (n=10)	1668	—	—	—	450 (6)
Lagenorhynchus obliquidens (n=5)	2640	—	—	—	1212 (3)

* Numbers in parentheses refer to ranks of prey items relative to other prey categories not listed above.

*TABLE **8***
A List of Marine Vertebrate Species Studied Showing Selected Prey Items Ranked According to Their Highest Index of Relative Importance (IRI).

Prey categories *							
Sebastes sp.	*Loligo opalescens*	*Thysanoessa spinifera*	Megalopa	*Clupea harengus*	*Euphausia pacifica*	*Pagrurus* sp.	*Merluccius productus*
242	190	151	31	4	—	—	—
1	581	10060	239	30	—	—	—
—	100	68	—	—	800	—	—
—	296	342	—	—	70	—	—
—	286 (5)	—	—	—	—	1396	—
11740	520	—	—	—	—	—	—
—	2000	—	—	—	—	—	—
62	866	—	96	—	107	—	—
—	25	23	—	—	—	—	—
—	—	106	22	—	831	—	—
—	3745	—	—	—	—	—	—
31	—	—	—	—	—	—	—
—	656	—	—	—	—	—	—
—	3188	—	—	—	—	—	—
460	43	—	—	—	—	—	—
—	644	—	—	—	—	—	—
190	33 (17)	—	—	—	—	—	—
—	12 (9)	—	—	—	1094	—	—
—	—	9876	—	118	—	—	—
—	—	188 (4)	—	—	—	—	—
6 (23)	41 (12)	174	—	11 (16)	1 (30)	—	169 (7)
—	890 (2)	—	—	—	—	—	—
167 (7)	316	—	—	—	—	—	—
60 (10)	1478 (2)	335 (6)	—	—	—	—	1 (15)
—	1360 (4)	—	—	—	—	—	—
3 (12)	1657	—	—	—	—	—	—
—	120 (7)	—	—	—	—	—	—
35 (8)	276 (6)	310 (5)	—	—	—	—	—
—	389 (4)	—	—	—	—	—	—
—	506	—	—	—	—	—	—
—	2192	—	—	—	—	—	—
—	6149	—	—	—	—	—	—
25 (9)	12810	—	—	2 (13)	—	—	—
225	1286	12 (11)	—	10 (14)	—	—	3 (14)
200 (6)	225 (5)	—	—	75 (9)	—	—	—
—	11400	—	—	—	—	—	—
578	967	—	—	—	—	—	316 (6)
849	3654	—	1 (15)	—	—	—	471 (4)
828 (5)	1068 (4)	—	—	—	—	—	708 (6)

TABLE 8—Cont'd.

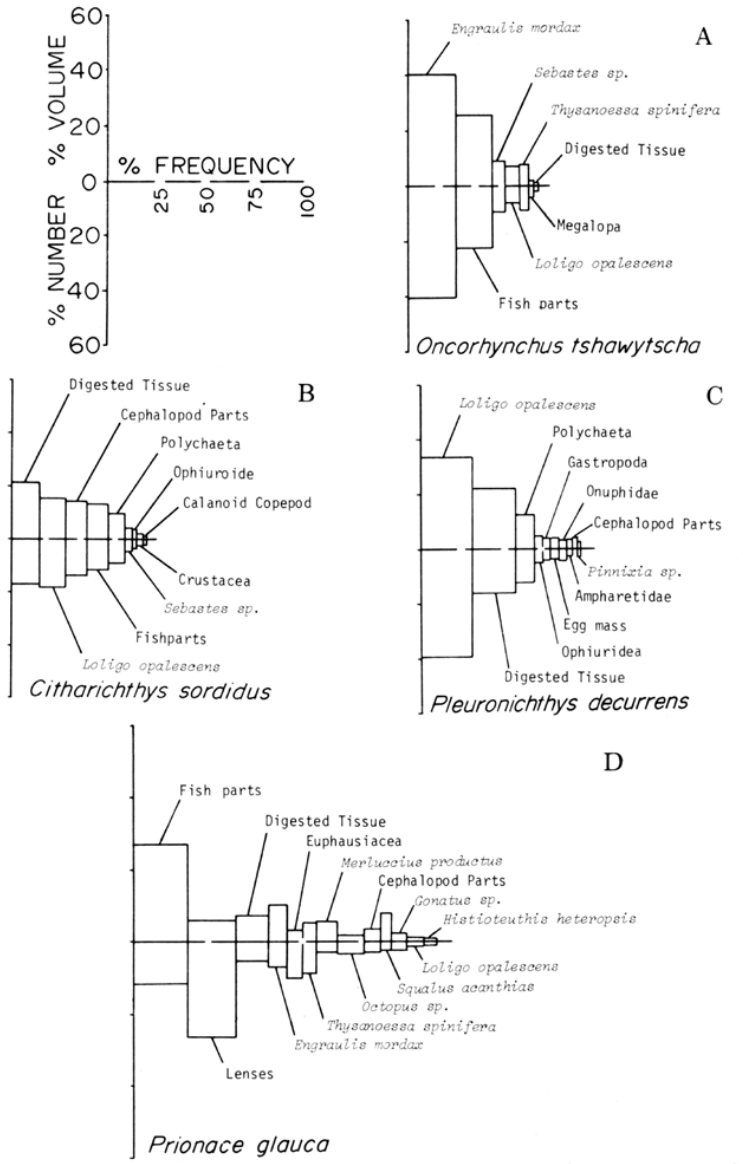


FIGURE 25. **Percent composition of major food categories in number, volume, and frequency of occurrence in Monterey Bay for: *A.* king salmon, *B.* Pacific sanddab, *C.* curlfin turbot, *D.* blue shark.**

FIGURE ***25. Percent composition of major food categories in number, volume, and frequency of occurrence in Monterey Bay for:*** *A.* ***king salmon,*** *B.* ***Pacific sanddab,*** *C.* ***curlfin turbot,*** *D.* ***blue shark.***

Silver salmon, Oncorhynchus kisutch. This species was collected between Petrale Hill and Soquel Stations. The highest ranking prey species was the euphausiid Thysanoessa spinifera (IRI = 10,060) followed by Loligo (IRI = 581). Crab, Cancer sp.; megalopa; and two fish species were the other prey items (Table 8). The DML of Loligo consumed ranged between 30 and 130 mm.

Plainfin midshipman, Porichthys notatus. This species was collected at two stations in the bay. At both they fed heavily on crustaceans consisting mostly of the two species Euphausia pacifica and Thysanoessa spinifera, and Loligo (Table 8). At Soquel Station crustacean parts had the highest rank (IRI = 2,837). As identifiable species consumed, Thysanoessa and Loligo were near equal in rank (IRI = 342 and IRI = 296). At Petrale Hill Station (Table 8), E. pacifica, as an identifiable species, had the highest rank (IRI = 800) after euphausiid parts (IRI = 2,957). Range in size of Loligo consumed at both stations was between 34 to 115 mm DML.

White croaker, Genyonemus lineatus. This species was collected only at Petrale Hill Station. Among identifiable prey items (Table 8), hermit crabs, Pagurus sp., had the highest rank (IRI = 1,396) followed by polychaetes (IRI = 371) and then Loligo (IRI = 286). Unidentifiable fish parts, however, ranked third (IRI = 1,210). No squid beak measurements were available.

Chilipepper rockfish, Sebastes goodei. Chilipepper rockfish were taken only at Soquel Station and showed an extremely low prey diversity (Table 8). Parts of Sebastes sp. ranked highest (IRI = 11,740) followed by fish parts (IRI = 1,472); Loligo ranked third (IRI = 520) then euphausiids (IRI = 392). No squid beak measurements were available.

Speckled sanddab, Citharichthys stigmaeus. This species was collected only in the shallowest station, at Monterey. It fed largely on fish (IRI = 4,100), Loligo (IRI = 2,000), and calanoid copepods (IRI = 1,725). No squid beak measurements were available.

Pacific sanddab, Citharichthys sordidus. All stations provided samples of this species since it normally occurs in deeper waters (Fitch and Lavenberg, 1968; Miller and Lea, 1972). At all stations there was a high prey species diversity. At Monterey Station, among 39 prey items (Table 8, Figure 25B) Loligo (IRI = 866) ranked second after the prey item "digested tissue" (IRI = 1,095); cephalopod parts ranked third (IRI = 567). Several species of fishes, numerous species of crustaceans, and other invertebrates comprised the prey eaten. At Petrale Hill Station there were only 26 prey items (Table 8) with crustaceans and fish parts nearly equal (IRI = 570 and IRI = 515). Loligo ranked 11th on the list (IRI = 25). Numerous crustacea and only one identifiable fish, E. mordax, were also consumed. A lower prey diversity was found at Soquel Station with only 22 prey items recorded (Table 8). Again a mixed diet of mainly fish and crustaceans, with other invertebrates, typified the food eaten by this species. No Loligo could be confirmed as prey eaten at Soquel Station. Loligo beak measurements from the two stations containing them provided a DML range of 30 to 160 mm for squid consumed.

Curlfin turbot, Pleuronichthys decurrens. This species of turbot is an obvious bottom feeder based on the large variety of burrowing invertebrates

it consumed at Petrale Hill and Monterey Stations (Table 8; Figure 25 C). At the Monterey Station (on Loligo spawning grounds) post-hatch Loligo (2 to 3 mm DML) had the highest rank (IRI = 3,745) among the 37 prey items consumed. Some individual specimens of curlfin turbot had eaten in excess of 600 post-hatch squid. A small proportion of squid eaten (less than 5%) had a size range of 100 to 110 mm DML. No Loligo were found in curlfin stomachs from Petrale Hill Station.

Lingcod, Ophidon elongatus. This species was collected in small numbers at all stations. Depending upon the station at which they were collected, prey items varied from fish and crustaceans to fish, Loligo, and crustaceans. No Loligo were consumed at the Monterey Station (Table 8). At Petrale Hill (Table 8) Loligo ranked fourth (IRI = 656) compared to the Soquel Station (Table 8) where unidentifiable fish parts ranked first (IRI = 10,219) and Loligo ranked second (IRI = 3,188). No squid beak measurements were available.

Petrale sole, Eopsetta jordani. This species was collected only at Petrale Hill and Soquel Stations. Stomach contents indicated epibenthic prey consumption. At Petrale Hill (Table 8) Octopus sp. had the highest rank (IRI = 1,710). Crustacea and other fish were also represented. Loligo had an IRI = 43. At Soquel Station (Table 8) similar food was eaten. Fish parts had the highest rank (IRI = 5,000) and Loligo had an IRI = 43. No squid beak measurements were available.

Pacific halibut, Hippoglossus stenolepis. In comparison to the other species of flatfish, predation of Loligo by this species was exceedingly low. Fish, echinoderms, molluscs, polychaetes, crustaceans, and other assorted invertebrates were consumed (Table 8). Among 17 prey items, the spotted cusk eel, Chilara taylori, had the highest IRI rank of 2,375. Loligo the lowest IRI of 33. This species was taken only at the Monterey Station. No squid beak measurements were available.

Sablefish, Anoplopoma fimbria. Sablefish were collected only at Soquel Station (Table 8). They ate primarily fish (IRI = 5,987) and Euphausiia pacifica (IRI = 1,094). Among nine prey items categorized, Loligo ranked ninth with an IRI of 12. No squid beak measurements were available.

Pacific hake, Merluccius productus. Hake were collected only at the Monterey and Soquel Stations. At the Monterey Station they fed primarily on Thysanoessa spinifera (IRI = 9,876). The remaining five prey items consisted mostly of crustaceans, fish parts, and herring, Clupea harengus (Table 8). At Soquel Station (Table 8) crustaceans were largely eaten (IRI = 10,688), followed by fish (IRI = 769), and then cephalopod parts (IRI = 319). No squid beak measurements were available.

Blue shark, Prionace glauca. This migratory elasmobranch was largely collected during summer. Sometimes they arrived in Monterey Bay during the month of May; other years they did not appear until mid June. They stayed in the area 3 to 5 months. Apparently water temperatures are the controlling factors (Harvey, unpublished manuscript). The specimens collected ranged in total length from 958 mm to 2,045 mm with a mean of 1,623.2 mm. Their weights ranged from 3.6 to 38.1 kg with a mean of 15.6 ± 1.25 kg. Females predominated in the catch with a ratio of 5.1

females to 1 male (124:24). Blue sharks had the highest prey diversity (48 prey items) among all fishes collected. Most of the sharks were hooked with set lines between Soquel and Petrale Hill Stations. The most important prey item in the diet was the northern anchovy (IRI = 384). Fish parts, eye lenses, and partly digested tissue ranked high mainly due to a high frequency of occurrence in the stomachs (Table 8, Figure 25D). The remaining important prey items included unidentifiable euphausiid fragments and the euphausiid, Thysanoessa spinifera (IRI = 174), along with hake, Merluccius sp.; dogfish, Squalus sp.; and the cephalopods, Octopus sp., Gonatus sp., L. opalescens, histioteuthis heteropsis, and Octopoteuthis deletron. Based on beak measurements the Loligo consumed ranged between 28 and 134 mm DLM.

Five other species of fish were found to feed on Loligo: filetail cat shark, Parmaturus xaniurus; common thresher shark, Alopias vulpinus; long nose lancetfish, Alepsisaurus ferox; albacore, Thunnus alalunga; and the splitnose rockfish, Sebastes diploproa (Table 8). Some of these were brought in by fishermen; all contained only traces of food in their digestive tracts which contained Loligo beaks.

6.3.1.2. Birds

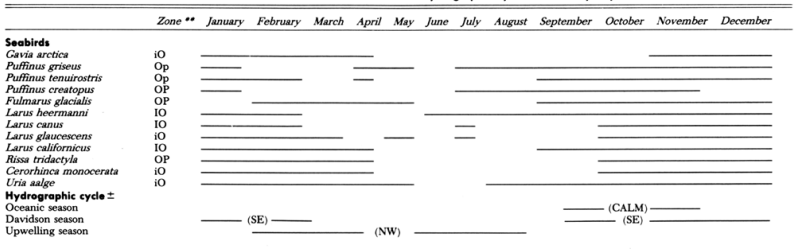
Several species of sea birds occurring off California feed extensively on cephalopods and many particularly on Loligo (see Baltz and Morejohn, 1977, for most recent review). Most are migratory and are absent from the Monterey Bay area during their breeding seasons, with the exception of some juvenile and vagrant individuals. The seasonal occurrence of selected seabirds in relation to the hydrographic cycle in central California was considered (Table 9). Following the classification of marine zones by Wynne-Edwards (1930) the usual areas of occurrence of birds in this study were: 1. inshore zone—that area of the sea within sight of shore; 2. offshore zone—waters over the continental shelf out of sight of shore; and 3. pelagic zone—waters beyond the continental shelf, delimited arbitrarily from the offshore zone by the 100 fathom contour.

Arctic loon, Gavia arctica. These loons arrived in the area in late October or early November and usually remained in the offshore zone into April (Table 9). Unidentifiable detritus made up the largest portion of stomach contents (IRI = 12,886). Loligo ranked second (IRI = 890); crabs and fish made up the remainder of the diet (Table 8). Loligo consumed ranged in size from 40 to 130 mm DML.

Brandt's cormorant, Phalacrocorax penicillatus. This species is a year-round resident in Monterey Bay found in association with kelp beds, especially during the breeding season. To a lesser extent they may be found singly or in small groups in the offshore zone throughout the year. Specimens studied were collected in the offshore zone and had fed primarily on several species of fishes. Anchovies had the highest rank (IRI = 1,301). Loligo ranked fourth (IRI = 316) among 19 prey items (Table 8). The size range of Loligo was between 40 to 130 mm DML.

Sooty shearwater, Puffinus griseus. This species is the most common shearwater on Monterey Bay seasonally during April and May; and on an annual basis it is the most common seabird species encountered (Ainley,

TABLE 9
Seasonal Seabird Occurrence in Relation to the Hydrographic Cycle in Monterey Bay *



* Only selected migratory species used in this study are shown.
** Habitat indicated by zone: inshore (I), offshore (O), and pelagic (P). Lower case indicates secondary zone of occurrence.
± For comparison with avian occurrence, the generalized hydrographic cycle proposed by Bolin and Abbott (1962) is included with direction of prevailing wind.

*TABLE **9***
Seasonal Seabird Occurrence in Relation to the Hydrographic Cycle in Monterey Bay

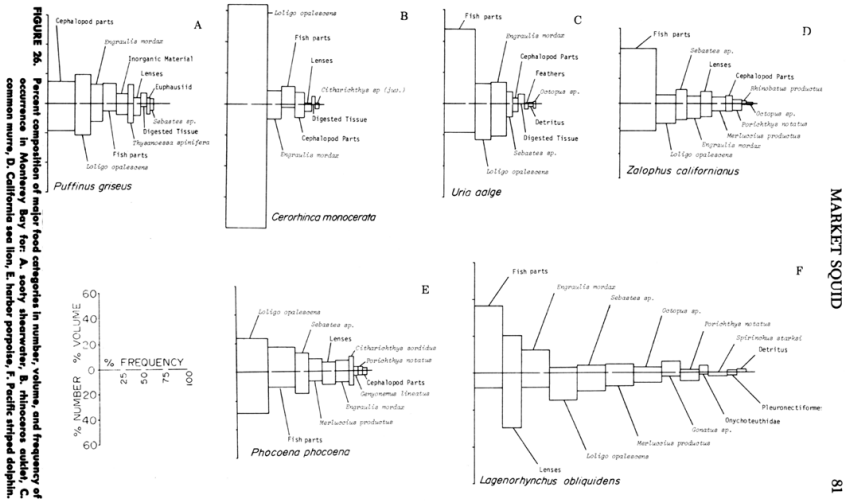


FIGURE ***26. Percent composition of major food categories in number, volume, and frequency of occurrence in Monterey Bay for: A. sooty shearwater, B. rhinoceros auklet, C. common murre, D. California sea lion, E. harbor porpoise, F. Pacific striped dolphin.***

1976). It was collected most frequently in the offshore and pelagic zones. The highest ranking prey item of 19 (Table 8, Figure 26A) was cephalopod parts (IRI = 2,000), followed by Loligo (IRI = 1,478). The northern anchovy was the second most important species (IRI = 665). of lesser importance was the euphausiid, Thysanoessa spinifera (IRI = 335). Other prey consumed in lesser quantities were the pacific herring, Clupea harengus; jack smelt, Atherinopsis californiensis; juvenile rockfish, Sebastes sp; and hake, Merluccius productus. Other cephalopods consumed were Gonatus sp. and Onychoteuthis boreali-japonicus. The range in size of Loligo bases on beak measurements was 30 to 180 mm DML.

Short tailed shearwater, Puffinus tenuirostris. This shearwater is second to the sooty shearwater in abundance and has its peak occurrence during January and February (Baltz and Morejohn, 1977). It was collected mainly in the offshore zone. As an identifiable prey species, Loligo (IRI 1,360) was the most abundant prey item of 15 taken by this shearwater. Anchovies (IRI 70) and other species of cephalopods (Onychoteuthis boreali-japonicus, Gonatus, and Argonauta octopoteuthis) were less abundant (Table 8). Loligo beak measurements provided a size range of 40 to 150 mm DML.

Pink-footed shearwater, Puffinus creatopus. This species occurred less frequently on Monterey Bay than other shearwaters, and was encountered mostly in the offshore and pelagic zones. Fish parts had the highest ranking IRI (4,740) followed by anchovies (IRI 1,818), then Loligo (IRI 1,657). Other cephalopods included Onychoteuthis boreali-japonicus (Table 8).

Northern fulmar, Fulmaris glacialis. The northern fulmar occurred sporadically on Monterey Bay during this study. In the winter and spring of 1976–1977 there was a mass die-off of fulmars along the pacific coast from Washington to southern California. Prey items consumed by these die-off birds were reported by Morejohn and Cross (1978). Specimens collected for the present study were collected in the pelagic inshore and offshore zones on Monterey Bay; however, they were encountered in large rafts in the pelagic and offshore zones. They are primarily cephalopod feeders, and Gonatus ranked first (IRI = 2,018). Loligo ranked seventh (IRI = 120). They fed on four other cephalopod genera (Table 8); however, ten cephalopod genera were identified from a larger sample (Table 10) of the 1976–1977 die-off specimens (Morejohn and Cross, 1978). No Loligo beak measurements were made.

Heermann's gull, Larus heermanni. This species occurs on Monterey Bay from mid-June to February. It is distributed inshore and offshore. Fishes, mollusks, crustaceans, and cephalopods were the predominant prey consumed (Table 8). Fish parts ranked first (IRI = 2,310), anchovies second (IRI = 826), Thysauoessa spinifera fifth (IRI = 310) and Loligo ranked sixth (IRI = 276). All Loligo beak measurements were pooled for gulls of the genus Larus and ranged from 20 to 140 mm DML.

Mew gull, Larus canus. Mew gulls occur on Monterey Bay from October to March in the inshore and offshore zones. Anchovies ranked first (IRI = 11,900); Loligo ranked fourth (IRI = 389). Another cephalopod, Onychoteuthis boreali-japonicus, had equal rank with Loligo (Table 8).

TABLE 10
Prey Items Eaten by the Northern Fulmar, *Fulmarus glacialis*, During the Die-off Period of 1976–1977 *

	Prey items	Birds examined February (n=55)	March (n=135)	April (n=13)
Fish:	*Engraulis mordax*	–	x	–
	Chilara taylori	x	–	–
	Fish parts	–	x	–
Cephalopods:	*Abraliopsis* sp.			
	Dosidicus gigas	–	x	–
	Octopoteuthis deletron	x	x	–
	Gonatus sp.	x	x	–
	Onychoteuthis boreali-japonicus	x	x	x
	Onychoteuthidae	–	x	–
	Chiroteuthidae	–	x	–
	Cranchiidae	–	x	–
	Vampyroteuthis infernalis	–	x	–
	Loligo opalescens	x	x	x
	Octopoda sp.	x	x	–
	Unidentified caphalopod beaks	x	x	x
Crustaceans:	*Cancer gracilis* (?)	–	x	–
	Crab megalopa	–	x	–
Insects:	beetles	x	x	–
Polychaetes:	Nereidae	x	x	–

* Taken from Morejohn and Cross, 1978.

*TABLE **10***

Prey Items Eaten by the Northern Fulmar,** Fulmarus glacialis, **During the Die-off Period of 1976–1977

Glaucous-winged gull, Larus glaucescens. Adults of this species were seldom seen; immature (first year) birds were common but not abundant on Monterey Bay. They occurred on the bay from November to March (Table 9) and were found distributed over the three marine zones. Fish parts (IRI = 630) and Loligo (IRI = 506) were almost equal in occurrence in their diet (Table 8). Mole crabs; oceanic squid, Onychoteuthis sp.; mollusks; anchovies; and surfperch, Zalembius sp., were the remainder of prey consumed.

California gull, Larus californicus. This gull occurs on Monterey Bay from September to April (Table 9). It was found to be a major consumer of Loligo (IRI = 2,192). It fed on other assorted prey items including plant fragments, anchovies, oceanic squid, and crustaceans (Table 8).

Black-legged kittiwake, Rissa tridactyla. This species is normally found in largest numbers in the pelagic zone, but it occurs as well in the offshore and inshore zones. This species occurred on the bay from December to April (Table 9). Loligo had the highest rank (IRI = 6,149) followed by anchovies (IRI = 629). Other minor prey items occurred (Table 8). This species also suffered a high mortality during the winter of 1976–1977 together with the northern fulmar. Numerous carcasses were studied at that time, and it was determined that unavailability at the surface of both Loligo and anchovies was probably the major cause of mortality (Morejohn, Krasnow, Harvey, and Cross, 1978). Loligo beak measurements provided a range in size of 50 to 120 mm DML.

Rhinoceros auklet, Cerorhinca monocerata. These auklets are generally distributed in the offshore zone but may occasionally occur in the inshore zone. They are found on Monterey Bay from October to April (Table 9). Loligo ranked first (IRI = 12,810) with anchovies and fish parts

TABLE 11
Prey Items Eaten by the California Sea Otter, *Enhydra lutris* (n = 58)*

Mollusca	*Arthropoda*	*Echinodermata*	*Chordata*
Cryptochiton stelleri	Stalked barnacle	*Strongylocentrotus purpuratus*	Stalked tunicate
Ischnochiton sp.	*Pugetia producta*	*Strongylocentrotus franciscanus*	*Endistylia polymorpha*
Haliotis cracherodii	*Pugetia richii*	*Patiria miniata*	*Scorpaenichthys marmoratus*
Haliotis rufescens	*Cancer antennarius*	*Pisaster brevispinis*	*Phalacrocorax penicillatus*
Lottia gigantea	*Cancer magister*	*Pisaster ochraceous*	
Tegula brunnea	*Cryptolithodes sitchensis*	*Pycnopodia helianthoides*	
Tegula montereyi	*Haplogaster cavicauda*		
Polinices sp.	*Blepharipoda occidentalis*		
Astrea gibberosa	*Emerita analoga*		
Crepidula adunca			
Nassarius(cooperi ?)			
Mytilus californianus			
Mytilus edulis			
Hinnites multirugosus			
Pododesmus cepio			
Clinocardium nuttallii			
Protothaca sp.			
Saxidomus nuttallii			
Tivela stultorum			
Tresus nuttallii			
Loligo opalescens			
Octopus sp.			

* Taken from Morejohn and Hennessy (1978).

*TABLE **11***
Prey Items Eaten by the California Sea Otter,** Enhydra lutris **(n = 58)

second and third (IRI = 593 and IRI = 397 respectively). They fed on a large selection of fishes (Table 8, Figure 26B). Loligo beak measurements indicated that squid sizes ranged between 10 to 110 mm DML. The size between 50–60 mm was the most frequently taken.

Common murre, Uria aalge. Murres occur on Monterey Bay all months of the year, but they are abundant between August and May (Table 9). Fish parts (IRI = 4,550) made up the major portion of their prey; Loligo ranked second (IRI = 1,286), and anchovies ranked third (IRI = 1,188). Juvenile rockfish, Sebastes spp. ranked fourth (IRI = 1,225). Thirteen species of fishes were identified (Table 8, Figure 26C). The common murre's prey selection had the highest diversity among seabirds studied (23 prey items). The range in size of Loligo consumed was between 10 and 160 mm DML.

6.3.1.3. Marine Mammals

While many marine mammal species occur in Monterey Bay, we were only able to collect gastro-intestinal samples from nine species. Three species are residents throughout the year: sea otter, Enhydra lutris; harbor seal, Phoca vitulina; and harbor porpoise, Phocoena phocoena. All other species are migratory and seasonal in occurrence. In terms of numbers the California sea lion, Zalophus californianus, exceeds all others with greatest abundance from August to April. A recent pertinent literature review of distribution, feeding, and status of marine mammals off central and northern California is given by Morejohn (1977).

Sea otter, Enhydra lutris. In California waters the sea otter is generally found associated with kelp beds of the inshore zone. In its northern distribution (Alaska, Aleutians, etc.) there may be no kelp associated with the shoreline (Kenyon, 1969). The known prey items of sea otters from northern regions to California are reviewed by Calkins (1978). In gastrointestinal contents of sea otters from California, Morejohn and Hennessy (1978) added several species new to their diet (Table 11). Loligo was one of them.

Elephant seal, Mirounga angustirostris. This is the largest seal in the world. Seasonally, from December to March, they aggregate on Año Nuevo Island and vicinity for the breeding season. During this time adults do little or no feeding. Few individuals of other age groups haul out at this time. Where these animals feed is unknown presently. We have observed them at sea over the Monterey submarine canyon in the pelagic zone at other times of the year. Feeding habits of this seal have been reported by Huey (1930), and Morejohn and Baltz (1970). Fish and cephalopods (Table 8) had the highest rank (IRI = 9,850); fish parts ranked second (IRI = 2,800) and hake ranked third (IRI = 1,750). Loligo ranked fifth (IRI = 225). Other specimens with nearly empty stomachs increased the prey item list (Table 12).

Harbor seal, Phoca vitulina. In Monterey Bay we have no evidence that this seal feeds on Loligo. Most of our samples of prey items came from analyses of scat contents in the Elkhorn Slough area (Morejohn, Harvey, Helm and Cross, 1978). Thirty-five species of fishes were represented (Table 12). Among the oceanic fishes known not to enter the slough, five

TABLE 12
A Summary of Prey Items Fed Upon by Four Species of Pinnipeds *

Prey	*Phoca vitulina* (**)	*Mirounga angustirostris* (N=6)	*Callorhinus ursinus* (N=6)	*Zalophus californianus* (N=34)
Engraulis mordax	X	–	X	X
Clupea harengus	X	X	X	–
Allosmerus elongatus	X	–	–	–
Spirinchus starski	X	–	X	–
Porichthys notatus	X	X	X	X
Merluccius productus	X	X	–	X
Microgadus proximus	X	–	–	–
Chilara taylori	X	X	–	–
Lycodopsis pacifica	X	–	–	–
Atherinops affinis	X	–	–	–
Atherinopsis californiensis	X	–	X	–
Genyonemus lineatus	X	–	–	X
Ampistichus koelzi	X	–	–	–
Cymatogaster aggregata	X	–	–	–
Embiotoca jacksoni	X	–	–	–
Hyperprosopon argenteum	X	–	–	–
Phanerodon furcatus	X	–	–	–
Rhacochilus toxotes	X	–	–	–
Damalichthys vacca	X	–	–	–
Zalembius rosaceus	X	–	–	–
Sebastes paucispinus	X	X	–	–
Sebastes sp.	X	X	–	–
Anoplopoma fimbria	X	–	–	–
Ophiodon elongatus	–	–	X	–
Artedius notospilotus	X	–	–	–
Leptocottus armatus	X	–	–	–
Scorpaenichthys marmoratus	X (?)	–	–	–
Citharichthys sordidus	X (?)	–	X	–
Citharichthys stigmaeus	X	–	–	–
Eopsetta jordani	X	–	–	–
Glytocephalus zachirus	X	X (?)	–	–
Lepidopsetta bilineata	X	–	–	–
Lyopsetta exilis	–	X (?)	–	–
Microstomus pacificus	X	–	–	–
Parophrys vetulus	X	–	–	–
Platichthys stellatus	X	–	–	–
Pleuronichthys decurrens	X (?)	–	–	–
Pleuronichthys verticalis	X	–	–	–
Psettichthys melanostictus	X	–	–	–
Squalus acanthias	–	X	–	X
Apristurus brunneus (egg cases)	–	X	–	–
Molluscans:				
Clinocardium nuttallii	X (?)	–	–	–
Trachycardium quadragenarium	X (?)	–	–	–
Solen sp.	X	–	–	–
Loligo opalescens	–	X	X	X
Gonatus sp.	–	X	X	X
Onychoteuthis borealijaponicus	–	–	–	X
Octopoda	X	–	–	X
Crustaceans:				
Hemigrapsus oregonensis	X	–	–	–
Pachygrapsus crassipes	X	–	–	–
Brachyuran	X	–	–	–

* A question mark (?) after the indicated food item indicates some reservations on identity.
** Prey items fed upon by this species were identified from scat remains on hauling grounds (Morejohn, Harvey, Helm, and Cross, 1978).

*TABLE **12***
A Summary of Prey Items Fed Upon by Four Species of Pinnipeds

occurred most frequently in the seal's diet. Seals foraged over the sandy oceanic shelves north and south of Moss Landing. We concluded that they are benthic and epibenthic feeders and may descend to depths exceeding 7 to 8 fathoms.

Alaskan fur seal, Callorhinus ursinus. This seal away from its breeding grounds (Pribilof Islands, Bering Sea; San Miguel Island, California) spends the greater part of the year in its southern range of distribution in the pelagic zone, and apparently it need not haul out on land for many months. Since the establishment of the breeding colony on San Miguel Island (Peterson, LeBoeuf, and Delong, 1968) an increase in the number of specimens (pups and adult females) that wash in emaciated and near death or dead has increased in the Monterey Bay area. Feeding habits of this seal are well known (North Pacific Fur Seal Commission Reports, 1958–1972) in northern waters. Specimens found in the Monterey Bay area fed mainly on Loligo (IRI = 11,400), followed by digested fish parts (IRI = 8,267), and another cephalopod, Gonatus sp. (IRI = 111). For these the list of prey items is small; however, other specimens with nearly empty stomachs increased the list of prey items (Table 12).

California sea lion, Zalophus californianus. This species is perhaps the most common seal of coastal California. It breeds on the channel islands, and after the breeding season mostly males of all ages migrate northward (Peterson and Bartholomew, 1967). It frequently hauls out on land to rest

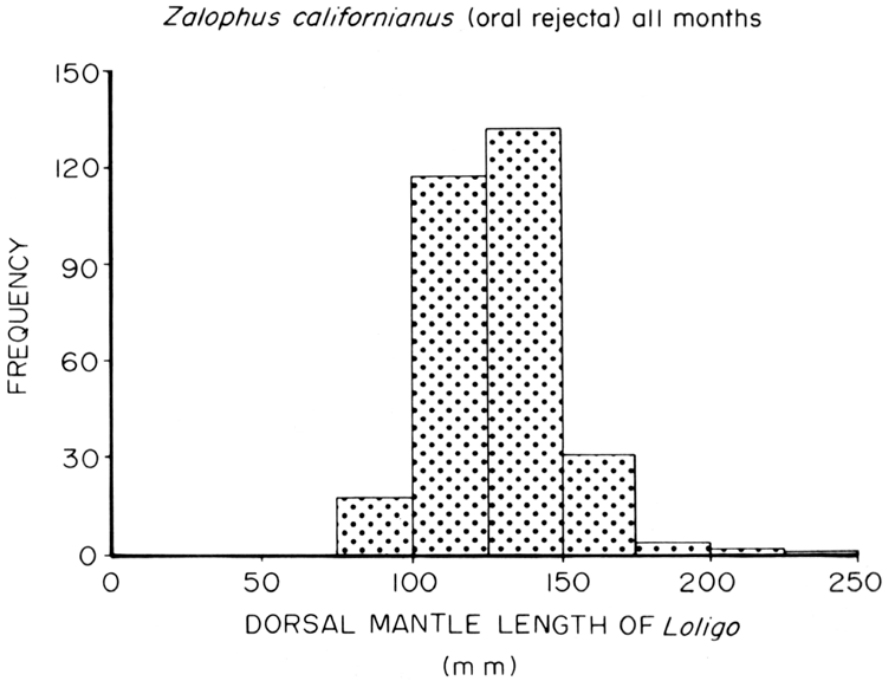


FIGURE 27. Beaks of *Loligo opalescens* recovered from oral rejecta of California sea lions on the Monterey breakwater jetty.

*FIGURE **27. Beaks of** Loligo opalescens **recovered from oral rejecta of California sea lions on the Monterey breakwater jetty.***

and may be encountered feeding in the inshore and offshore zones. On occasion an individual may be seen in the pelagic zone. Its feeding preference for anchovies, hake and the market squid, Loligo, is well established (Bonnot, 1928; Scheffer and Neff, 1948; and Fiscus and Barnes, 1966). Our samples support this view. Fish parts ranked first (IRI = 5,025), followed by Loligo (IRI = 967), juvenile rockfish (IRI = 578), anchovies (IRI = 513), and hake (IRI = 316). Many other fishes and two other cephalopods were on the list of prey items (Table 8, Table 12, and Figure 26D). DML of Loligo ranged from 95 to 175 mm.

On the Monterey breakwater, where several hundred Zalophus congregate to rest, we were given permission by the United States Coast Guard to walk out on the rocks to collect samples of fresh or dried vomitus of these seals. This material generally consists of mucus and the chitinous undigestible remains of cephalopods such as beaks and pens and fish otoliths and bones. From these samples, taken over a period of months when Zalophus is in abundance, thousands of Loligo beaks were recovered. We subsampled at random 50 beaks from each collection time and the DML of Loligo from which these beaks came provided a size range of 75 to 225 mm (Figure 27). Other cephalopod beaks were recovered and only a few otoliths.

Pigmy sperm whale, Kogia breviceps. This species is a rarely observed and ubiquitous species throughout most of its distribution. Stomachs were essentially empty with indigestible cephalopod beaks as the only prey items found in two specimens. Several hundred beaks of over 12 taxa were found (Table 13). The percent numerical of Loligo was 0.01.

Harbor porpoise, Phocoena phocoena. This inshore species is found along the entire coastline of Monterey Bay feeding in waters from 2 to 15 fathoms in depth. They generally are seen singly or in twos; sometimes they aggregate to feed in shallow water beyond the breakers in small groups of 12 to 15 animals. They shy away from boats and thus are difficult to observe.

Loligo was the most frequently consumed prey species (IRI = 3,654). Fish parts ranked second (IRI = 1,668). Juvenile rockfish, Sebastes sp., ranked third (IRI = 849). Hake and anchovies had nearly equal rank (Table 8, Figure 26E). DML of squid consumed ranged in size from 56 to 135 mm.

Dall's porpoise, Phocoenoides dalli. These porpoises occur in Monterey Bay every month of the year in the offshore and pelagic zones. The recorded feeding habits of this species were reviewed and expanded by Morejohn (1978). This porpoise feeds upon many of the same prey species as other porpoises (Table 13). Throughout the year the most extensive predation occurs on herring, hake, and juvenile rockfish (Table 8). Loligo and Gonatus are the most frequently eaten cephalopods. Loligo is preyed upon throughout the year. DML of Loligo ranged in size from 47 to 174 mm.

Pacific striped dolphin, Lagenorhynchus obliquidens. This species is generally encountered in the offshore and pelagic zones. Seasonally (late fall, winter, and early spring) they may be found feeding off headlands

(i.e. Point Pinos). Several large feeding aggregations (to 150 animals) have been observed in the deep water of the Monterey submarine canyon during late spring and early fall.

Fish parts ranked first in stomachs of this species (IRI = 2,640), and anchovies (IRI = 1,212) and Loligo (IRI = 1,068) were of near equal rank (Table 8, Figure 26F). Juvenile rockfish and hake also ranked relatively high (IRI = 828 and IRI = 708 respectively). Including Loligo, six species of cephalopods were consumed (Table 13). The range in DML of Loligo based on beak measurements was 47 to 176 mm.

TABLE 13
A Summary of Prey Items Fed Upon by Four Species of Small Toothed Whales

Prey	*Kogia breviceps* (N=2)	*Phocoena phocoena* (N=15)	*Phocoenoides dalli* (N=27)	*Lagenorhynchus obliquidens* (N=10)
Fishes:				
Engraulis mordax	–	X	X	X
Clupea harengus	–	–	X	X
Spirinchus starksi	–	–	X	X
Bathylagus pacificus	–	–	X	–
Myctophidae	–	–	X	–
Lampanyctus regalis	–	–	X	–
Chilara taylori	–	X	X	X
Porichthys notatus	X	X	–	X
Merlucccius productus	–	X	X	X
Atherinopsis californiensis	–	–	–	X
Embiotocidae	–	X	–	–
Zoarcidae	–	–	X	–
Macrouridae	–	–	X	–
Peprilus simillimus	–	–	X	–
Sebastes sp. (juv.)	–	X	X	X
Anoplopoma fimbria	–	–	X	–
Liparis sp.	–	–	X	–
Citharichthys sordidus	–	–	X	–
Pleuronectiform	–	X	–	X
Cephalopods:				
Loligo opalescens	X	X	X	X
Endoploteuthidae	X	–	X	X
Ommastrephidae	X	–	X	–
Histioteuthis heteropsis	X	–	–	–
Histioteuthidae	–	–	X	–
Octopoteuthis deletron	X	–	–	–
Octopoteuthis sicula	X	–	–	–
Octopoteuthidae	X	–	X	–
Gonatus sp.	X	–	X	X
Onychoteuthis borealijaponicus	X	–	–	X
Onychoteuthidae	X	–	X	X
Architeuthidae	X	–	–	–
Thysanoteuthidae	X	–	–	–
Cranchiidae	X	–	–	–
Sepiolidae	–	–	–	X
Argonauta	X	–	–	–
Octopus bimaculata	–	–	X	–
Octopus sp.	X	X	X	X
Crustaceans:				
Decapoda	–	–	X	–
Megalopa	–	X	–	–

*TABLE **13***
A Summary of Prey Items Fed Upon by Four Species of Small Toothed Whales

Feeding habits studies are best documented by complete gastrointestinal contents analysis. Reliance on contents of oral rejecta or fecal materials may have strong bias. Our studies of stomach contents of pinnipeds coupled with our studies of contents of pinniped vomitus and fecal material suggests that oral rejection (vomitus) consists largely of cephalopod beaks and pens with few otoliths. Fecal materials at sea lion rookeries contain hundreds of otoliths and little or no evidence of cephalopod beaks. The harbor seal fecal contents at Elkhorn Slough had little evidence of cephalopods (Morejohn, Harvey, Helm, and Cross, 1978). We do not know if harbor seals orally reject indigestible materials.

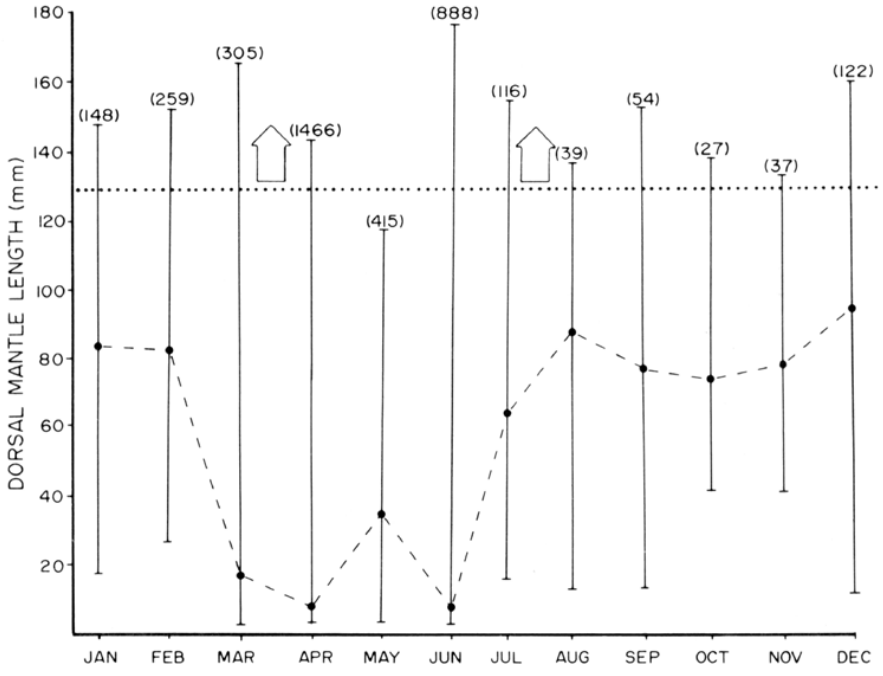


FIGURE 28. Dorsal mantle lengths of *Loligo opalescens* consumed by fish, birds, and mammals.

*FIGURE **28. Dorsal mantle lengths of** Loligo opalescens **consumed by fish, birds, and mammals.***

Market size squid were available in Monterey Bay away from the spawning grounds near Cannery Row 11 months of the year as demonstrated by squid beak measurements from all vertebrate predator gastrointestinal, stomach contents, or oral rejecta. The range and means of dorsal mantle lengths of these squid were plotted (Figure 28) for all months of the year. The mean DML of these squid was about 80 mm for the months of August through February. March through July were the months with the lowest frequency of occurrence of large squid from predators. Various sizes (DML) of Loligo were consumed by all fish, birds, and mammals (Figure 29).

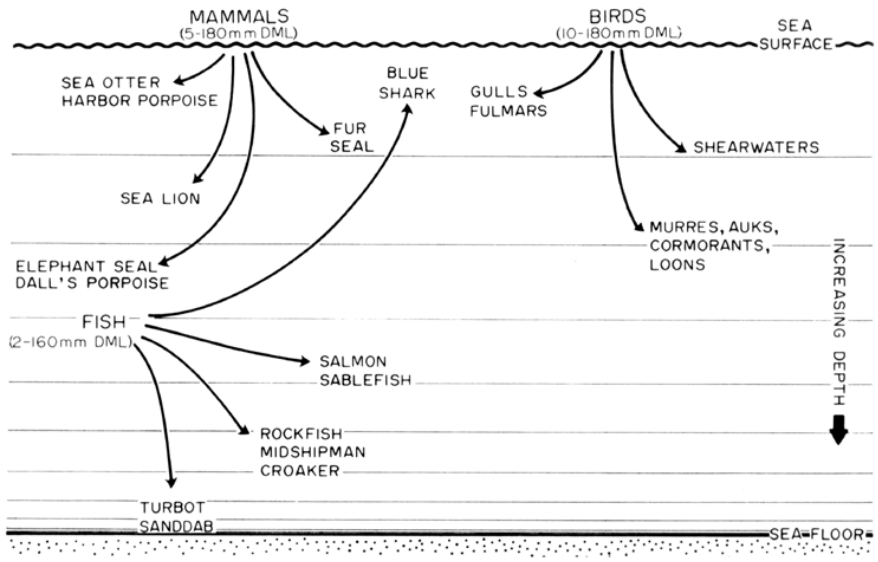


FIGURE 29. Dorsal mantle lengths of all squid measured from marine vertebrates throughout the year. Arrows indicate squid exceeding market size (horizontal dotted line).

*FIGURE **29. Dorsal mantle lengths of all squid measured from marine vertebrates throughout the year. Arrows indicate squid exceeding market size (horizontal dotted line).***

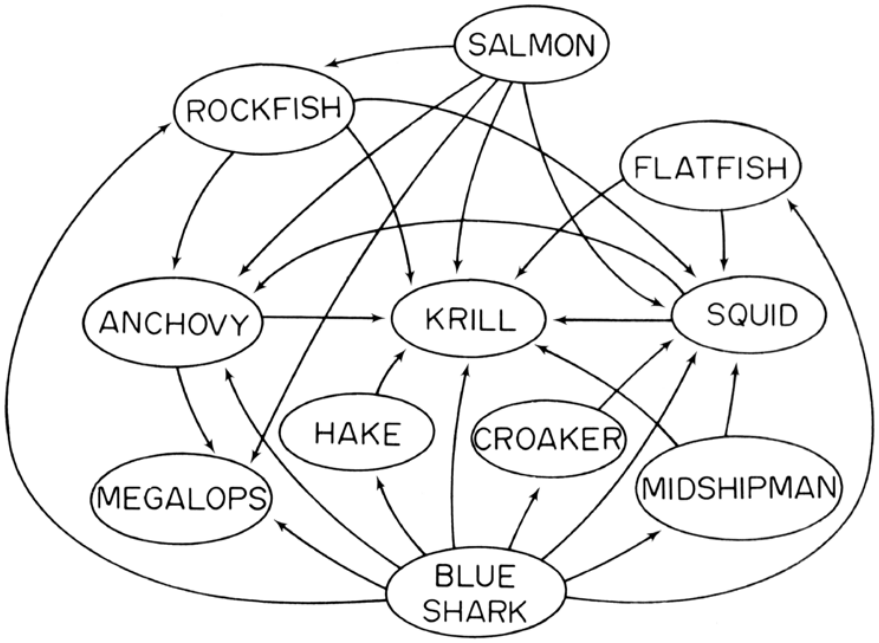


FIGURE 30. Food web involving commercially important or abundant fish and *Loligo opalescens.*

*FIGURE **30. Food web involving commercially important or abundant fish and** Loligo opalescens*

6.3.2. Food Webs

Among the fish species, several were found that not only fed upon the market squid and young of other species of commercial fish, but were, in turn, fed upon by seabirds and marine mammals. There were many examples involving different species at the different collecting stations in the bay. For each group of vertebrate predators (fish, bird, mammal) a food web was developed to show the inter-relationships between the species of each group. In each food web we have chosen only those species that either are important in most vertebrate food webs and/or that are important species (prey or predator) in commercial or recreational fisheries. In the fish group food web (Figure 30) the blue shark can be seen to be an obvious generalized predator. It overlaps considerably in prey preference with the two commercially important species of salmon. The three important prey species are Loligo, euphausiids, and anchovies. In the bird food web (Figure 31), recurring prey (squid, euphausiids, megalops) ranked high with seabirds and included important commercial and recreational fishes. Specialists among the birds are fulmars, kittiwakes, Bonaparte's gulls, and rhinoceros auklets. Generalized avian predators are the several shearwater species, murres, and Heermann's gulls. All species are dependent upon the important commercial and recreational species of squid and fish.

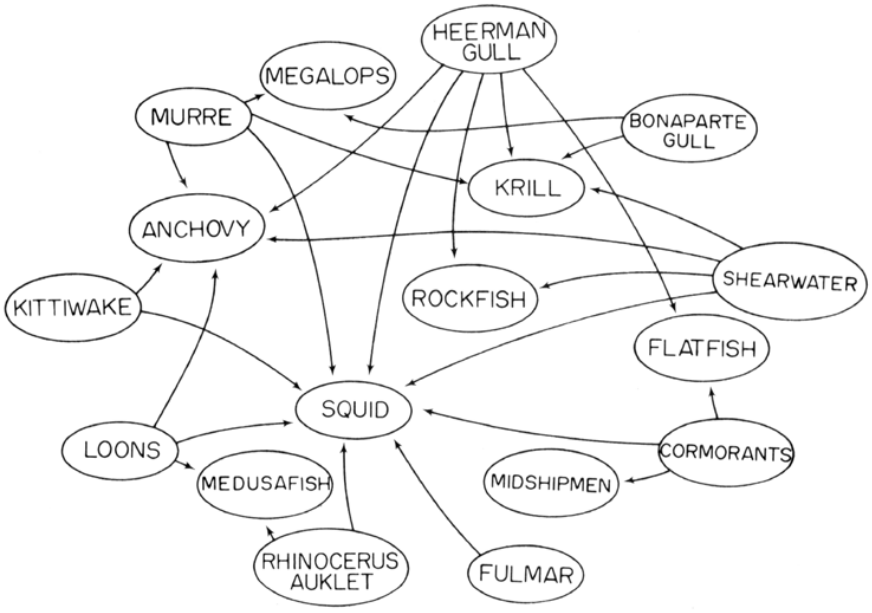


FIGURE 31. Food web involving commercially important or abundant fish, birds, and *Loligo opalescens*.

*FIGURE **31. Food web involving commercially important or abundant fish, birds, and** Loligo opalescens*

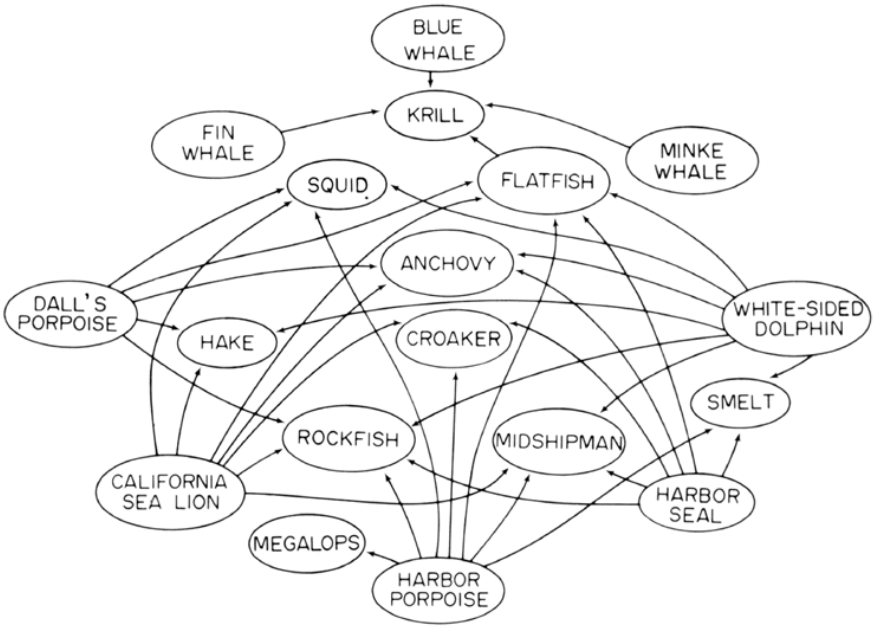


FIGURE 32. Food web involving commercially important or abundant fish, mammals, and *Loligo opalescens*.

*FIGURE **32. Food web involving commercially important or abundant fish, mammals, and** Loligo opalescens*

Among the marine mammals no stomach contents information from the large baleen whales in Monterey Bay was available, but we have observed them over several years periodically feeding in the bay. We have studied a number of important species (Figure 32). Three species of porpoises feed on much the same prey species but in different proportions. The sea lion also overlaps in dietary preference with these small cetaceans. The food web involving all the marine vertebrate predators of squid is complex (Figure 33).

6.3.3. Energetics

Because the sooty shearwater is the most commonly occurring seabird in Monterey Bay (Ainley, 1976) and has a high dependence on the market squid and anchovies in its diet, it was chosen for this feeding energetics study. Details of the experimental procedure were described by Krasnow (1978).

The total energy expenditure of the average 800 gm sooty shearwater was estimated following the least squares regression equation of King (1974) as 271 Kcals/bird/day. The mean assimilation efficiency of sooty shearwaters measured 75.9% and 80.2% for shearwaters fed Loligo, and 79.1% and 80.6% for those fed anchovies. The mean calorie density of anchovies, 5.649 ± .309 Kcals/gm, was higher than that of Lolego, 5.258 ± .111 Kcals/gm. This is probably due to the high oil content of anchovies, 5.8% to 17.4% of body weight (NOAA and Pacific Fishery Management Council, 1977) compared to 0.9% for squid (Watt and Merrill, 1970). Gravid

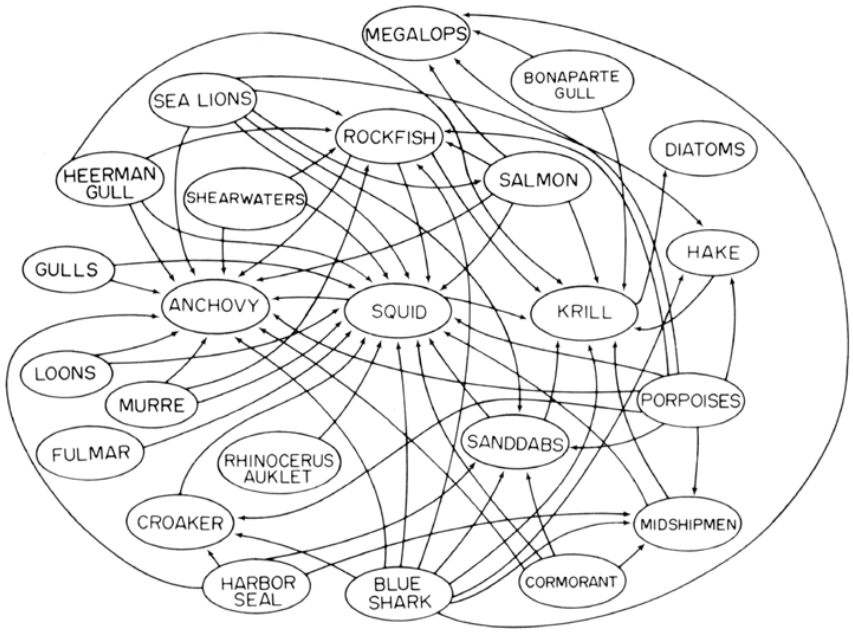


FIGURE 33. Food web involving commercially important or abundant fish, birds, mammals, and *Loligo opalescens*.

*FIGURE **33. Food web involving commercially important or abundant fish, birds, mammals, and** Loligo opalescens*

female and mature male squid were about equal in calorie content, likewise no differences were found between the sexes in anchovies.

On the assumption that sooty shearwaters require 271 Kcals/day and that 21.62% of its intact prey consists of Loligo, it would derive 58 Kcals/day from this food source. However, only 80% of the calories of Loligo are assimilable, thus a shearwater would need to consume 73 Kcals of Loligo per day to obtain the necessary net amount. An average Loligo provides 5.258 Kcals/gm dry weight, thus 14 gms (dry weight) of Loligo per day would be required to provide the shearwater with a gross energy intake of 73 Kcals. This is equivalent to 59 gms (fresh weight) of squid per day.

Employing the minimum and maximum abundance estimates for sooty sheerwaters in Monterey Bay (Ainley, 1976) and assuming that these birds are present on Monterey Bay from May to September (Elliott, Gill, and Morejohn, unpublished manuscript; and our personal observations) a range in demand of Loligo was estimated from 36 to 9,000 metric tons.

Anchovies represent 13.3% of the diet, but an individual sooty shearwater would need 36 Kcal/day from this source. Since only 81% of the calories from anchovies are assimilable, a total of 44 Kcal/day would be required to provide the net energy intake of 36 Kcal/day. An average anchovy supplies 5.65 Kcal/gm of dry weight. Eight gms (dry weight) of anchovies/day or 29 gms (fresh weight) would supply 44 Kcal/day. The demand by sooty shearwaters on anchovies in Monterey Bay is thus estimated to be from 18–4,400 metric tons.

6.4. DISCUSSION

During this study we have identified the major vertebrate predators of L. opalescens occurring in Monterey Bay and vicinity. Nineteen species of fishes feed on Loligo. Undoubtedly other species that we have not been able to sample due to limitations of our sampling gear also will be found to prey on this squid species.

Both the king and silver salmon fed on Loligo. In the former, Loligo ranked fourth (Figure 26A) among prey items eaten; in the latter, Loligo ranked second. The feeding habits of king salmon in the vicinity of San Francisco Bay were studied by Merkel (1957). The prey species eaten by kings in both areas were similar, however, by percent volume comparisons Monterey Bay kings consumed less squid, than those in the San Francisco Bay vicinity (7.4% compared to 9.3%). Consumption of euphausiids was also less for Monterey Bay (7.8%) than for San Francisco (14.9%). More anchovies were eaten by kings in Monterey Bay (40.3%) than in San Francisco (29.1%) and fewer rockfish were eaten in Monterey Bay (9.3%) than in San Francisco (22.5%). Feeding habits of silver salmon off Washington (Silliman, 1941) showed an extremely low predation on "squid." Squid, octopus, copepods and amphipods were grouped under "miscellaneous invertebrates" and represented less than 1.2% of the stomach contents. Our samples of king (n = 29) and silver salmon (n = 34) is inadequate to confirm temporal shifts in feeding habits from a fish eating phase to an invertebrate eating phase as Silliman (1941) demonstrated with troll caught kings and silvers off Washington.

The other fish species important in terms of numbers (biomass) that feed extensively on Loligo are the Pacific sanddab and the curlfin turbot. Only on the Loligo spawning grounds, off Cannery Row, Monterey Station, do they prey heavily on squid. Loligo ranked second after "digested tissue" on the prey species list (Table 8, Figure 26B) for C. sordidus. The species feeds on squid ranging is size from 30 to 160 mm DML. The latter size is well into the "market size" or adult squid. The curlfin turbot, on the other hand, selectively appears to feed on smaller squid preferring the post-hatch size of 2 to 3 mm DML. The high ranking (IRI = 3,744) for Loligo in the prey list for the curlfin (Table 8, Figure 26C) was largely brought about by the high frequency of occurrence (51.35%) of these small post-hatch squid in its diet.

Anchovies were the most important prey volumetrically and were the third most important in frequency of occurrence in blue shark stomachs (Table 8, Figure 26D). Only euphausiids outnumbered anchovies in the stomachs examined. In many instances anchovies were the only recognizable prey in stomachs. For this reason, predation upon anchovies probably occurred during the late night or early morning, since shark catches occurred in the mornings and other vertical migrants in shark stomachs were found to be well digested. The extensive predation on euphausiids has not been reported elsewhere in the Pacific (Strasburg, 1958; Bane, 1968; Tricas, 1977) and may be representative of a localized phenomenon in Monterey Bay.

The blue shark may be considered a biological sampler of certain prey

species as Gotshall, Smith, and Holbert (1965) demonstrated for the blue rockfish, Sebastes mystinus; Gotshall (1969) showed for the Pacific hake, Merluccius productus; and Prince and Gotshall (1976) suggested for the copper rockfish, Sebastes caurinus. These teleosts potentially can be used to estimate annual mortality rates and population sizes of species of economic importance such as Dungeness crabs, Cancer magister, and commercial shrimp, Pandalus jordani. The blue shark may serve as a sampler of anchovy; euphausiids, Thysanoessa spinifera; and hake among the three most important forage species that it feeds on which are interrelated in the food web (Figure 32) involving Loligo.

Several avian species that represent large biomasses in Monterey Bay consume Loligo as a high ranking prey species. The most important of these in decreasing order of number is the sooty shearwater, P. griseus; rhinocerus auklet, C. Monocerata; short-tailed shearwater, P. tenuirostris; California murre, U. aalge; and the kittiwake, R. tridactyla. All of these are non-resident birds that spend from 3 to 10 or more months in the Monterey Bay area (Table 9) during migration to or from the breeding grounds. In most of these species the anchovy follows Loligo in rank.

Wiens and Scott (1975) assessed the energy requirements of Oregon coastal seabird population by simulating their abundance patterns and prey consumption over time. Their model estimated that four seabird species could consume 28,000 metric tons of anchovies per year. Eighty-six percent of this tonnage was considered to be consumed by the sooty shearwater. Our study of the total daily expenditure of a typical sooty shearwater (800 gm) was calculated to be 271 Kcal/day. Since squid provide 22% of the shearwater's daily calorie intake, a gross consumption of 59 gms (fresh weight) of squid per day would be required. Anchovies provide only 13% of the shearwater's daily calorie intake or 36 Kcal/day. Twenty nine gms (fresh weight) of anchovies would meet this demand. Using Ainley's (1976) abundance estimates for sooty shearwaters in Monterey Bay (4,000 to 1,000,000 per year), the annual demand by sooty shearwaters for squid, Loligo, would be from 36 to 9,000 metric tons and for anchovies, 18 to 4,400 metric tons.

This calculated consumption of squid and anchovies by sooty shearwaters was compared with 10 year averages for commercial landings of squid and anchovies in Monterey Bay (Bell, 1971; Greenhood and Mackett, 1967; Heimann and Frey, 1968a, 1968b; McAllister, 1975, 1976; Oliphant, and statistics staff, 1973; Pinkas, 1970, 1974). The commercial squid catch for 1965 to 1974 averaged 5,000 metric tons per year compared to an estimated 36 to 9,000 metric tons consumed by shearwaters per year. The commercial anchovy catch for the same years averaged 3,400 metric tons per year compared to 18 to 4,400 metric tons consumed by shearwaters per year. A discussion of the possibilities of higher or lower consumptive rates on squid and anchovies is given in Krasnow (1978).

In consideration of the impact marine mammals play in predation on Loligo, the predator species with the highest biomass that feed heavily on this squid are the California sea lion, Z. californianus; the harbor porpoise, P. Phocoena; and Dall's porpoise, P. dalli. During the post breeding northward migration of Zalophus from the channel islands (Peterson and Bartholomew,

1967) over 14,000 individuals, largely males, were counted by Orr and Poulter (1965) hauled out on Año Neuvo Island north of Monterey Bay during the months of August and September. No data are available on the number that occur in Monterey Bay, however, monthly counts of individuals hauled out on the Monterey breakwater and adjacent to the U.S. Coast Guard Station reportedly (J. Vandevere, biologist, Friends of the Sea Otter, pers. comm.) average around 350 with lows of less than 50 and highs exceeding 500.

The prey of this species shows Loligo with second highest rank after fish parts. The Monterey breakwater jetty is conveniently located near Cannery Row and the squid spawning grounds. The numbers of sea lions hauled out have been correlated with high catches of anchovies and squid at the port of Monterey by R. Parrish (NMFS Monterey, pers. comm.). Oral rejecta gathered at the breakwater over the winter and spring months contained thousands of Loligo beaks for which dorsal mantle lengths were plotted by month (Figure 27). The squid size that most frequently occurred was over 125 DML or within the size of market squid.

Dall's porpoise may occur in groups of 6 to 20 or more in the bay throughout the year (Morejohn, 1978). It feeds on Loligo essentially every month of the year. The resident harbor porpoise probably numbers between 100 and 500 individuals in the bay throughout the year (Morejohn, Harvey, Moss Landing Marine Laboratories, personal observations, and John Hall, Univ. Calif. Santa Cruz, pers. comm.). Loligo has the highest ranking among the prey species eaten. The other mammals occur sporadically and in smaller numbers in the bay.

The dependence of these marine mammals on prey species that recurr in all vertebrate food webs is significant. The market squid, anchovy, and rockfish are important prey items for most marine mammals as well. The overall picture of predator—prey relationships (Figure 33) is somewhat unwieldy, but it is the best diagramatic manner of presenting this information. It is certain that latitudinally, north and south, changes in food habits (largely as species substitutions) will be encountered as faunal components change. What is clearly demonstrated is the central focal emphasis on market squid, anchovy, rockfish, and euphausiids. The dependence of fish, seabirds, and marine mammals on these major prey species cannot be over emphasized.

6.5. ACKNOWLEDGEMENTS

We are grateful to many students and faculty members at Moss Landing Marine Laboratories for assistance during this study. In particular, we appreciate the help of Donald M. Baltz, Janice L. Cross, Roger Helm, Bernadette Allen, Susan Stingle, and John Delorey for field and laboratory preparation of specimen materials. William Broenkow and Gregor Cailliet provided guidance with statistical problems. Many local fishermen provided entrails of salmon for analysis via Lillian Woodward of Moss Landing. We thank Donald Gunderson for providing us with ship time on the NMFS charter vessel PACIFIC RAIDER and the NMFS R/V JOHN COBB. John Fitch identified or confirmed our identifications of fish otoliths, and Gilbert Voss confirmed our identifications or identified unknown cephalopod beaks from our collections. To both of these scientists we offer a special thanks for untiring efforts to help us. Lynne McMaster expertly illustrated the figures.

7. POSSIBLE MORPHOLOGICAL INDICATORS OF POPULATION STRUCTURE IN THE MARKET SQUID, LOLIGO OPALESCENS

JERRY KASHIWADA
and
CONRAD W. RECKSIEK
Moss Landing Marine Laboratories
Moss Landing, California

7.1. INTRODUCTION

Most studies of morphological variation in loliginid cephalopods were undertaken to resolve systematic ambiguities. Haefner (1964) studied the morphology of Loligo pealei and Lolliguncula brevis to distinguish the two species in various size classes. LaRoe (1967) clarified the systematics of the loliginid squids of the tropical western Atlantic Ocean by developing a key based on his studies of morphological variation. Cohen (1976) continued the work on the systematics of these squids by investigating morphological variation with respect to geographical location and sex.

In contrast to the systematical complexities of the northwestern Atlantic Loliginidae, the loliginids of the northeastern Pacific Ocean are represented by three species in three genera. Loligo opalescens ranges from south Vancouver Island to Baja California (Fields, 1965). Lolliguncula panamensis occurs from Panama to Ecuador (Voss, 1971). The only loliginid close to L. opalescens geographically is Loliolopsis diomedeae; this species ranges from the Gulf of California to Peru (Voss, 1971) and apparently does not overlap with L. opalescens. Since these species appear to be distinct (Voss, 1971), the problems of systematics for these squids are largely resolved.

Even with a relatively fixed taxonomy, a study of morphometric variation can be of great value in researching the biology of a fisheries resource. Knowledge of whether or not a fishery depends on one or several stocks is mandatory for the regulation of the fishery (Rounsefell, 1975). Studies of variation in number of vertebral elements have been useful in indicating possible subpopulations in the Pacific sardine, Sardinops caerulea, (Clark, 1947) and in the northern anchovy, Engraulis mordax (McHugh, 1951). Some morphological studies of Loligo opalescens have been made in attempts to distinguish subpopulations. Evans (1976) found some statistical differences in morphology in comparing L. opalescens from Monterey Bay and southern California. Kashiwada, Recksiek, and Karpov (in press, CalCOFI Reports) did not find statistical differences in beak morphology of L. opalescens from the above two areas. Both studies are of questionable value in distinguishing subpopulations since only two locations were compared and these locations are close together when the

whole range of L. opalescens is considered.

Before a morphological comparison of squid from different areas can be made, there must be an investigation of other factors which are likely to cause differences in morphology. Fields (1965), in revising the description of L. opalescens given by Berry (1912), notes that sexual dimorphism is apparent in the size of arms and heads of mature squid. Since sexual dimorphism may be an influencing factor, state of sexual maturity may also have an effect.

This study investigates sources of morphological variation in Loligo opalescens to determine whether there are geopraphic subpopulations and if so, whether there are morpholgical characters that would be useful in the field to distinguish the subpopulations. The specimens examined cover a much wider geographical range than previous studies. The wide geographical range will become particularly important if the fishery expands beyond its current boundaries.

7.2. METHODS

Specimens of Loligo opalescens were collected between March 1976 and December 1977 at locations between Puget Sound and Rosario Bay, Baja California. A large majority was caught in midwater trawls; others were captured by jigging or dipnetting under a night-light or by shrimptrawl. For the remainder of this paper, the word "squid" refers to L. opalescens.

All specimens were placed in plastic bags and frozen as soon as possible in shipboard freezers, in ice chests with dry ice, or in freezers on shore. Care was taken to ensure the squid were frozen before muscels and skin could contract. This contraction begins to occur approximately 6 hours after death and may cause significant variation in some measurements. Specimens were transported in ice chests packed with dry ice to Moss Landing Marine Laboratories and were stored in freezers until the samples could be processed. Most samples were processed within 4 months after arrival, although some were kept in the freezers for up to 18 months.

Samples were thawed by placing them in seawater or leaving them at room temperature. Thawed squid were rinsed with sea water, then placed in trays with 10% freshwater formalin. As much as possible, the arms, tentacles and fins were kept extended away from the body. Squid were kept in formaline for a minimum of 2 weeks. Preliminary investigations showed most shrinkage due to formalin occurred within 24 hours and virtually no further shrinkage occurred after 1 week. After being fixed in formalin, the squid were rinsed with freshwater and stored in 70% ethanol for a minimum of 2 weeks before measurements and counts were taken.

For this study, the terms dorsal, ventral, anterior, and posterior refer to the functional orientation of the squid. The following list gives a description of the measurements used in this study. Most measurements are taken from Cohen (1976).

Dorsal mantle length (**DML**): length of mantle from anterior-most point on the dorsal side to the posterior body tip.

Fin length oblique (**FLO**): distance from insertion of left fin to posterior body tip, taken at an oblique angle to the main body axis. Fin length was measured in this manner because it seemed there would be less

bias caused by squid with flattened or narrow bodies.

Fin width (**FW**): distance between lateral-most points of fins.

Interorbital width (**IOW**): distance between dorsal margins of eyes.

Arm length: distance from proximal-most sucker to distal tip of arm. Arms are numbered starting from the dorsal side.

Arm length first arm (**AL I**): length of dorsal-most right arm. If right arm was damaged, the left arm was measured.

Arm length second arm (**AL II**): length of second right arm or second left arm if right arm was damaged.

Tentacle length (**TL**): distance along right tentacle from point of emergence from webbing to distal tip of tentacle.

Tentacle club length (**TCL**): distance from first proximal row containing four suckers (suckers may be staggered, but must at least overlap) to distal tip of right tentacle club.

Tentacle sucker width (**TSW**): diameter of widest sucker ring on right tentacle, measured from bases of sucker teeth.

Gill filament (**Gif**): number of filaments along the outside demibranch of right gill.

Funnel cartilage length (**FCL**): greatest length of right funnel cartilage.

Mantle circumference: measured by cutting open mantle along mid-ventral line and flattening mantle against ruler.

Anterior mantle circumference (**AMC**): circumference measured at mid-ventral point of anterior mantle margin.

Mid-mantle circumference (**MMC**): circumference measured at insertion point of fins.

DML was measured with a measuring board to the nearest millimeter. **FLO, FW, IOW,** and **FCL** were measured with vernier calipers to the nearest 0.1 mm. **AL I, AL II, TL, TCL, AMC,** and **MMC** were measured with a ruler to the nearest mm. **TSW** was measured with an occular micrometer on a dissection microscope to the nearest 0.1 mm.

TABLE 14
Criteria for Judging Sexual Maturity

	Males		*Females*	
Maturity Code	*Testis*	*Spermatophores*	*Nidamental glands*	*Eggs*
1 immature	short and thin to long and thick	none	small—less than 10% DML*	small—undeveloped
2 intermediate	long and thick	present—few in number and loosely packed	intermediate—between 10 and 20% DML*	large with distinct lobes
3 mature	long and either thick or thin	present—large numbers and densely packed	large—greater than 20% DML*	large and clear with no lobes
4 spent	long and thin	few in numbers and loosely packed or degenerating	large—greater than 20% DML*	large but few in number and opaque

* Estimated visually, not measured.

*TABLE **14***
Criteria for Judging Sexual Maturity

TABLE 15
Squid Sample Collection Information

Sample number	*Source*	*Method of capture*	*Date*	*Location*	*Number of Specimens*		
					Males	*Females*	*Total*
76A7-18	AL[1]	MWT[6]	19 September 1976	Rosario Bay, Baja CA.	10	3	13
76A7-19	AL	MWT	19 September 1976	Rosario Bay, Baja CA.	30	10	40
76A7-37	AL	MWT	23 September 1976	San Diego	22	10	32
77A15-1	AL	NL[7]	8 December 1977	San Diego	16	12	28
77A15-8	AL	NL	10 December 1977	Santa Catalina Island	14	10	24
76SR-1	KB[2]	NL	30 March 1976	Santa Rosa Island	9	6	15
76A4-44	AL	NL	8 June 1976	Santa Rosa Island	12	0	12
76A7-65	AL	MWT	27 September 1976	Santa Barbara	7	0	7
76A7-66	AL	MWT	27 September 1976	Santa Barbara	10	2	12
76A7-67	AL	MWT	27 September 1976	Santa Barbara	9	0	9
76A7-70	AL	MWT	28 September 1976	Santa Barbara	8	1	9
76A4-80	AL	MWT	17 June 1976	Monterey Bay	8	7	15
76A4-95	AL	MWT	20 June 1976	Monterey Bay	14	12	26
76PR-15	PR[3]	MWT	10 August 1976	Monterey Bay	15	3	18
77CR-1	CT[4]	ST[8]	July 1977	Crescent City	10	13	23
76W-1	CM[5]	MWT	20 December 1976	Puget Sound	2	6	8
76W-2	CM	MWT	20 December 1976	Puget Sound	7	4	11
77W-1	CM	MWT	11 February 1977	Puget Sound	8	8	16

[1] AL = California Department of Fish and Game ALASKA
[2] KB = California Department of Fish and Game KELP BASS
[3] PR = National Marine Fisheries Service PACIFIC RAIDER
[4] CT = Commercial shrimp trawler
[5] CM = Washington State Department of Fisheries COMMANDO
[6] MWT = Midwater trawl
[7] NL = Jigged or dipnetted under night-light
[8] ST = Shrimp trawl

*TABLE **15***
Squid Sample Collection Information

In addition to the above measurements, sex was recorded and a maturity code ranging from 1 to 4 (Table 14) was assigned using criteria modified from Vovk (1972), Holme (1974), and Evans (1976). The main criteria were presence and amount of spermatophores, size of testis, condition of eggs, and size of nidamental glands. Samples were grouped according to general geographical location (Table 15).

The "Scatterplot" routine in the Statistical Package for the Social Sciences (SPSS) Library Computer Program (Nie *et al,* 1975) was used to make scattergram plots of each measurement against **DML.** The program also allowed data to be segregated by different criteria (e.g., sex, maturity, location) and calculated linear regression statistics for the data. Plots of all measurements were made for all male squid, all females, immature males, mature males, Monterey Bay immature and mature males, Monterey Bay mature females, and mid-Baja California immature males and females.

Various plots were compared visually to investigate the effects of sex, maturity, and location. In general, we compared those groups in which the sample size was sufficiently large to make reasonable comparisons. Squid from Monterey Bay were used for comparisons of sex and maturity. Mid-Baja California squid were used to compare immature males with immature females. Geographical comparisons were made using all males from all areas when no morphological difference was noted for state of maturity. When maturity was a possible factor, only immature males were compared.

7.3. RESULTS

In comparing regressions of morphometric measurements plotted against **DML,** there were considerable differences in the amount of deviation from a straight line. The points for measurements such as **FLO** (Figure 34), **FW,** and **FC** appeared to cluster around a straight line very

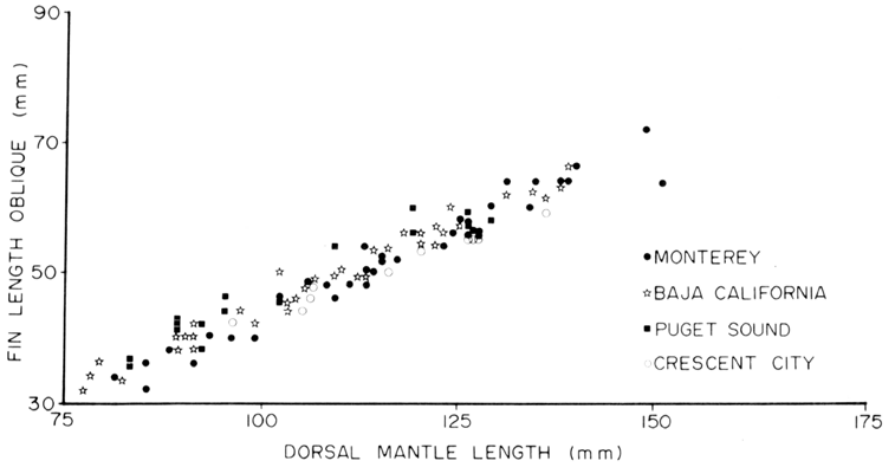


FIGURE 34. Scattergram of Fin Length Oblique (FLO) against Dorsal Mantle Length (DML) for Puget Sound, Monterey Bay, Baja California and Crescent City males.

*FIGURE **34. Scattergram of Fin Length Oblique (FLO) against Dorsal Mantle Length (DML) for Puget Sound, Monterey Bay, Baja California and Crescent City males.***

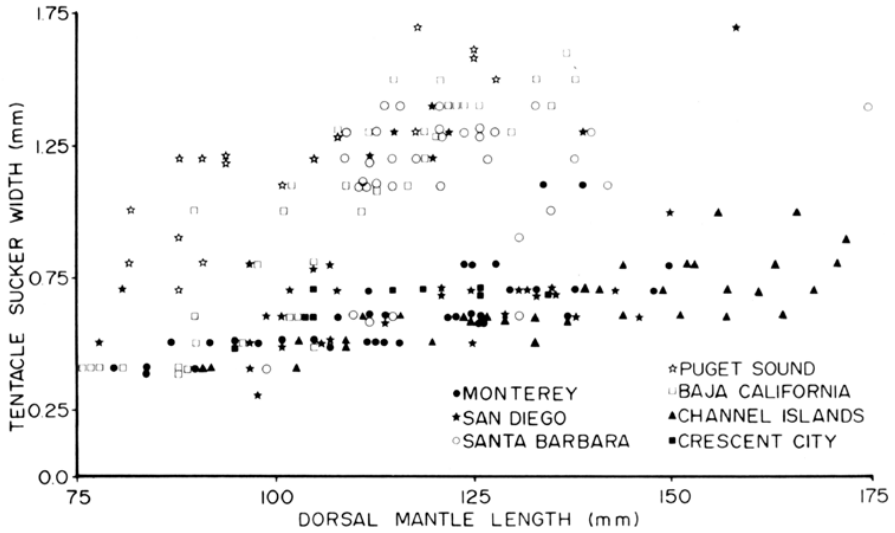


FIGURE 35. Scattergram of Tentacle Sucker Width (TSW) against Dorsal Mantle Length (DML) for all males.

*FIGURE **35. Scattergram of Tentacle Sucker Width (TSW) against Dorsal Mantle Length (DML) for all males.***

closely; regression statistics also reflect the close fit of points for these measurements (Table 16). In contrast **TSW** (Figure 35), **TL,** and **TCL** exhibited considerable scatter (Table 16). The only meristic character counted, **Gif,** demonstrated much variation for all geographical locations (Figure 36; Table 16).

TABLE 16
Linear Regression Statistics for Various Measurements Against Dorsal Mantle Length (DML)

Measurement code	*N*	*R^2*	*Intercept*	*Slope*
FLO	207	0.964	−6.07	0.511
FW	207	0.867	−2.32	0.472
IOW	207	0.607	5.85	0.083
AL I	207	0.773	−30.54	0.588
AL II	207	0.808	−28.80	0.605
TL	205	0.396	36.45	0.430
TCL	207	0.157	9.61	0.080
TSW	207	0.073	0.31	0.005
GIF	207	0.359	50.31	0.135
FCL	207	0.896	4.71	0.095
AMC	207	0.773	21.66	0.348
MMC	207	0.583	15.95	0.323

*TABLE **16***
Linear Regression Statistics for Various Measurements Against Dorsal Mantle Length (DML)

7.3.1. Sexual Dimorphism

The comparison of scattergrams of mature male and female L. opalescens from Monterey Bay indicated possible sexual dimorphism for several measurements. Arm length showed definite sexual dimorphism for both **AL I** and **AL II;** arms of males were longer than those of females and there was no overlap in the distribution of points (Figure 37). For large squid, **FLO** may be slightly longer in females; **IOW** and **TSW** tend to be smaller

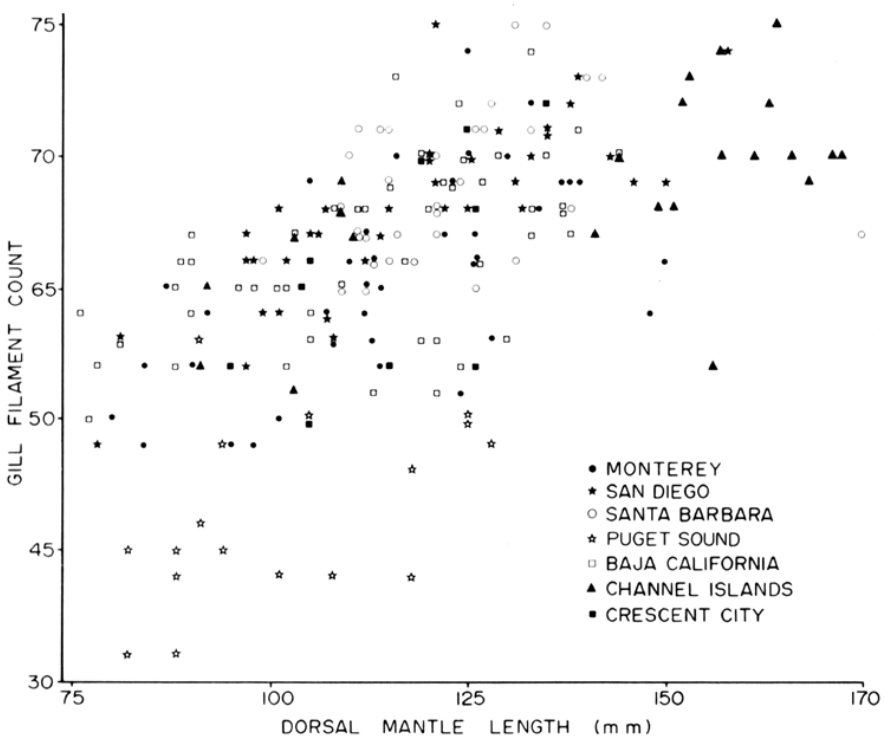


FIGURE 36. Scattergram of Gill Filament Count (Gif) against Dorsal Mantle Length (DML) for all males.

*FIGURE **36. Scattergram of Gill Filament Count (Gif) against Dorsal Mantle Length (DML) for all males.***

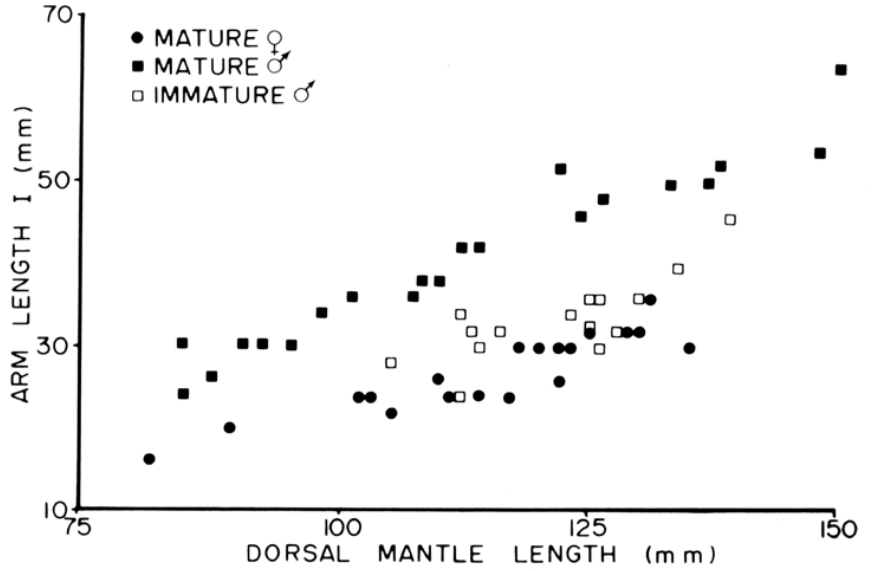


FIGURE 37. Scattergram of Arm Length I (AL I) against Dorsal Mantle Length (DML) for Monterey Bay gravid females, gravid males and immature males.

*FIGURE **37. Scattergram of Arm Length I (AL I) against Dorsal Mantle Length (DML) for Monterey Bay gravid females, gravid males and immature males.***

in females. In the plots of the three later measurements, there were considerable overlap and the differences may not have been significant (Statistical tests for significant differences between intercepts or slopes were not performed—see DISCUSSION.) Comparisons of immature male and female squid from mid-Baja California indicated little sexual dimorphism; only **AL I** (Figure 38) and **AL II** showed any possible differences. The dimorphism for arm length was much less pronounced in these immature squid than that in mature squid from Monterey Bay.

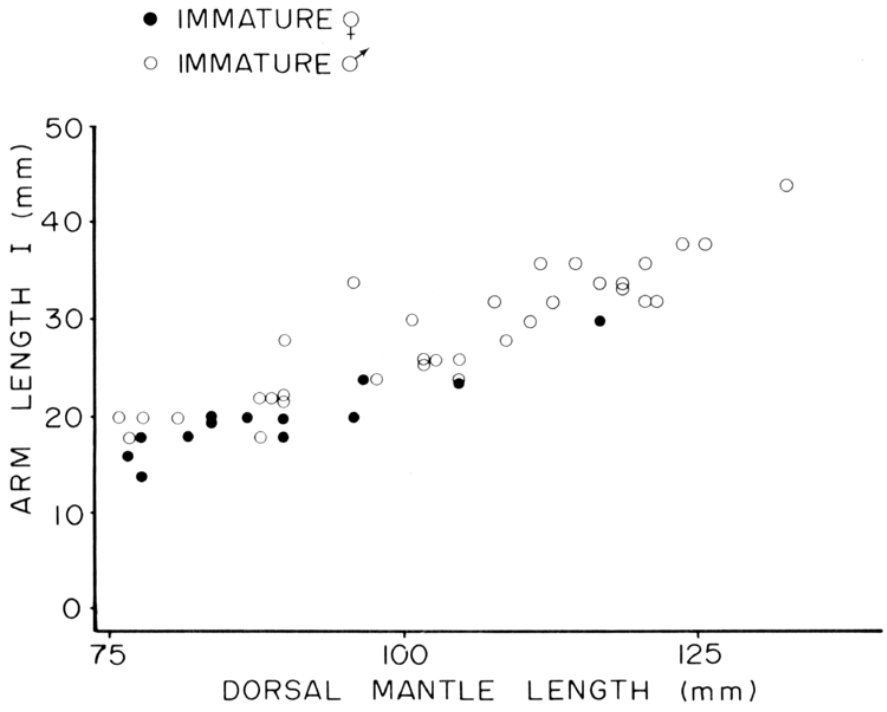


FIGURE 38. Scattergram of Arm Length I (AL I) against Dorsal Mantle Length (DML) for middle Baja California immature males and immature females.

*FIGURE **38. Scattergram of Arm Length I (AL I) against Dorsal Mantle Length (DML) for middle Baja California immature males and immature females.***

7.3.2. Maturity

The scattergrams of immature and mature males from Monterey Bay indicate possible differences in a number of measurements. The most notable differences are the longer arms in mature males (Figure 37) and longer tentacle clubs in immature males (Figure 39). Immature males also tend to have higher values for **TL, AMC,** and **MMC** compared to mature males.

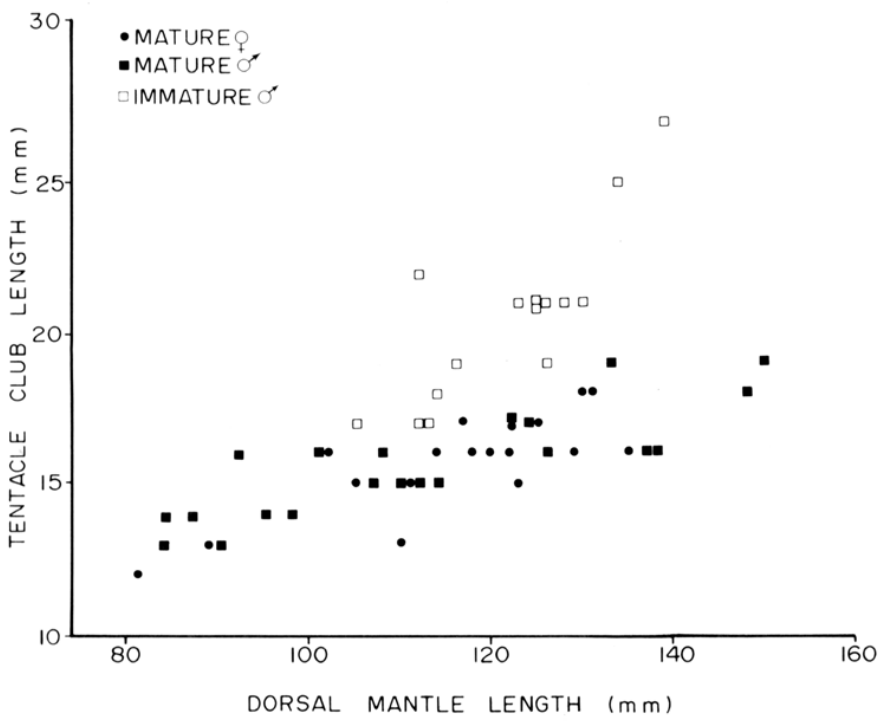


FIGURE 39. Scattergram of Tentacle Club Length (TCL) against Dorsal Mantle Length (DML) for Monterey Bay gravid females, gravid males, and immature males.

*FIGURE **39. Scattergram of Tentacle Club Length (TCL) against Dorsal Mantle Length (DML) for Monterey Bay gravid females, gravid males, and immature males.***

7.3.3. Geographical Variation

For most of the measurements plotted against **DML,** some geographical differences could be detected; the scatter of points for all areas combined was generally greater than that for the individual areas (Figures 34 and 40). In some cases the areas were fairly distinct (Figure 40), while in other cases, the areas were intermixed (Figure 34). Distinctions between areas were occasionally less pronounced at the lower end of the size range than near the center or upper end (Figure 34).

The distribution of points for squid from Puget Sound was on the upper half of the point distribution of all squid for most measurements (Figures 34, 35 and 40); the notable exception is GIF which was lower for Puget Sound squid than other areas (Figure 35). Squid from Crescent City and Monterey Bay were often found on the lower half of the total distribution of points (Figures 35 and 40); however, for some measurements, Monterey Bay squid were in the upper half. San Diego Squid generally had such scattered distributions that they could not be considered in either half of the distribution. Mid-Baja California squid tended to be on the upper half of the total distribution (Figures 35 and 38).

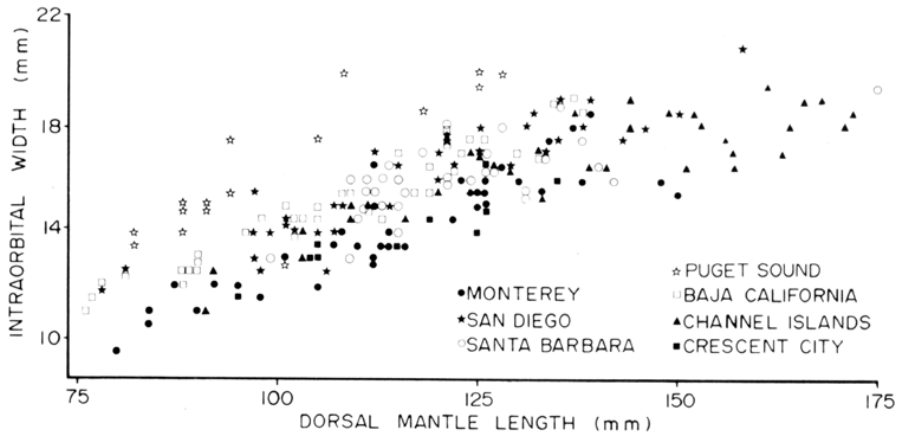


FIGURE 40. Scattergram of Interorbital Width (IOW) against Mantle Length (DML) for all males.

*FIGURE **40. Scattergram of Interorbital Width (IOW) against Mantle Length (DML) for all males.***

The most distinct geographical differences were found in **IOW** (Figure 40) and **TSW** (Figure 35). **TSW** was the most interesting plot; for larger squid, two groups could be distinguished with very few intermediate

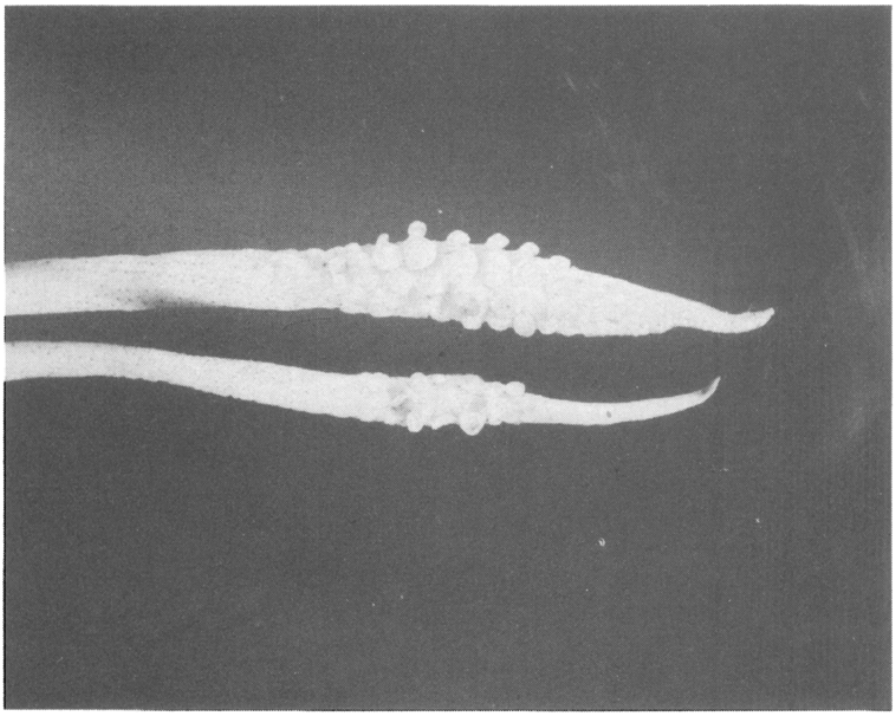


FIGURE 41. Photograph of tentacle clubs and suckers of a 122 mm DML immature male squid from mid-Baja California (upper figure) and a 123 mm DML immature male squid from Monterey Bay (lower figure).

*FIGURE **41. Photograph of tentacle clubs and suckers of a 122 mm DML immature male squid from mid-Baja California (upper figure) and a 123 mm DML immature male squid from Monterey Bay (lower figure).***

specimens (Figure 35). A group with large suckers included Puget Sound and mid-Baja California squid; a group with small suckers included squid from northern and central California and the channel islands off southern California (Figure 41). Squid from the coast of southern California fell into both groups. The few squid with intermediate sized suckers came from the Santa Barbara area and the Monterey Bay area.

7.4. DISCUSSION

of the measurements which indicate sexual dimorphism, only **AL I** and **AL II** clearly demonstrate differences. Although arms III and IV were not analyzed for this study, these arms show similar sexual differences. Cohen (1976) found indications of sexual dimorphism in all four species of Loligo in the western North Atlantic; none of the differences were great enough to be used as field characteristics in distinguishing sexes. In contrast, Fields (1965) reported that the sexes in L. opalescens could be easily distinguished in the field by arm length. Fields also describes sexual dimorphism in head size and tentacle length.

The reduced sexual dimorphism in immature squid from Baja California (Figure 38) indicates that dimorphism of the arms becomes more pronounced as the squid matures. The fact that the arms of immature males from Monterey Bay are not much longer than those of gravid females (Figure 37), further supports this observation.

The samples used to study the effects of sexual dimorphism and maturity on morphology were chosen in consideration of sample size and possible effects of geographical variation, i.e. of the areas sampled, Monterey Bay had the largest size range of gravid males and females, and mid-Baja California had the largest size range of immature males and females. Squid from other areas could have been included in these studies, but none were added since the advantages of increased sample size could be negated by the increase in variation due to geographical location.

Some differences between gravid and immature male squid from Monterey Bay may be due to the effect of spawning. Both **AMC** and **MMC** were lower for gravid males. L. opalescens apparently utilizes mantle protein as a food reserve during spawning; both mantle thickness and width become significantly reduced in spent squid (Fields, 1950 and 1965; Evans, 1976). Lower values for **AMC** and **MMC** would be expected for mature spawning squid when compared with immature animals. The higher values of **TCL** and **TL** may similarly be due to morphological changes related to spawning or may indicate that morphological characteristics for Monterey Bay squid vary between schools or year classes of squid.

The pattern of geographical variation in the morphology of L. opalescens is unclear. Although squid from Puget Sound and mid-Baja California are from opposite ends of the distribution for the species, they resemble one another in most morphological features involving the head or tentacles (**IOW, TL, TCL,** and **TSW**). Squid in the middle of the range (central and northern California and the southern California channel islands) tend to resemble one another morphologically and are distinct from Puget Sound and mid-Baja California squid. The data indicate there may be

three separate groups: Baja California, northern and central California, and Puget Sound. The main bases for distinction are **IOW, TL, TCL, TSW,** and **Gif.** Although Puget Sound and mid-Baja California squid are similar in most respects, they differ in **Gif**; it is also unlikely that they could be part of the same population since they are separated by what appears to be another population. The Santa Barbara and San Diego areas may represent areas where the Baja California and central California groups converge creating high morphological variation.

TSW is probably the most useful measurement for distinguishing the groups since very few squid have intermediate size tentacle suckers. It is interesting to note that although two groups intermix in San Diego and Santa Barbara, there are few squid with intermediate sized suckers in these areas. Squid smaller than 110 mm DML cannot be reliably placed in groups using **TSW**.

There are several possible reasons for the observed geographical variation. The differences could have been due to environmental factors. Environmental effects on the morphology of cephalopods are largely unknown. Roper (1969) correlated oxygen concentration with gill size in Bathyteuthis, yet it is uncertain whether the differences was genetic or ecophenotypic. Cohen (1976) correlated increased growth rate of gills in Loligo pealei with lower oxygen content and warmer temperatures. Little other research on the subject has been done. For fish however, much research has indicated the influence of environment on morphology (Barlow, 1961; Loch, 1974). The possibility of similar effects on cephalopods must be researched before studies of geographical variation in species can be correctly evaluated.

In this study, the only measurement showing a possible relationship to the physical environment was **Gif. Gif** was lowest for Puget Sound; however, the scatter of points was so high that few other trends can be deduced (Figure 36). Most of the highest Gif counts were from Santa Barbara, Santa Catalina Island, and San Diego squid; it was surprising that **Gif** counts in Baja California squid were not higher than those of southern California squid. Reversals in expected meristic clines for northern anchovy, Engraulis mordax, were thought to be caused by different rates of temperature increase at critical periods during larval development (McHugh, 1951). Since critical periods of morphological and meristical development for squid have not been established, similar speculations on the effects of environment on squid morphology are not possible. The patterns of variation found for **TL, TCL,** and **TSW** are difficult to explain in terms of environmental factors. For these measurements, there would have to be some factor in which Puget Sound is more similar to Baja California than it is to northern and central California.

Geographical variation may also be explained by hypothesizing genetic differences in the different regions. Voss (1977) notes that cephalopod genera from shallow water continental shelf or deep benthic areas are conducive to a high degree of speciation. Neritic squid such as Loligo opalescens would be likely candidates for subspeciation.

The variation observed could be the result of inadequate sampling. Squid for most areas were caught at one time of year during a single year.

It cannot be safely assumed that the squid sampled are representative of the area during all seasons and all years. Cohen (1976) found that samples of Loligo pealei from the same area differed more from each other than from a sample taken in another location. Puget Sound squid sampled 2 months apart showed no apparent differences; these squid are probably representative of the area for the season sampled, but there is no guarantee they are representative of other seasons or other years. Monterey Bay squid sampled 2 months apart did show differences which may indicate there is wider variation in the area with respect to time; however, the differences may also have been due to differences in state of maturity.

No statistical tests were performed because it was felt there was too much uncertainty in our having sufficiently representative samples. Until a great many more samples from the different areas at different times can be examined, the variation in morphology for the individual areas will remain unknown and the existence of subpopulations will remain unresolved. A great amount of research will also need to be done on the effects of environmental factors on the morphology of squid. In view of these limitations, the present research can only suggest morphological measurements which may be useful to determine whether or not L. opalescens is a homogeneous population. The **TSW** measurement seems to hold the most promise for such studies.

7.5. ACKNOWLEDGEMENTS

Many individuals contributed their time and energy to providing samples for this study. For arranging participation in research cruises, we would like to thank Don Gunderson and James Mason (National Marine Fisheries Service, Seattle); J. R. Raymond Ally and Kenneth Mais (California Department of Fish and Game, Long Beach); Norm Lemberg and Mark Pedersen (Washington Department of Fisheries, Seattle). We would also like to express our thanks to several individuals who aided us in our sampling program: Jay Christofferson, Konstantin Karpov, James Harvey, Douglas Vaughan, Lynne Krasnow, James Knipe, and Shirley Recksiek. We would also like to acknowledge the crew of the ALASKA, especially Frank McCumiskey, whose efforts contributed the major portion of this study's data.

8. A BIOCHEMICAL-GENETIC POPULATION STRUCTURE STUDY OF MARKET SQUID, LOLIGO OPALESCENS, ALONG THE CALIFORNIA COAST

J. R. RAYMOND ALLY
California Department of Fish and Game
Long Beach, California
and
SCOTT A. KECK
California State University, Long Beach
Long Beach, California

8.1. INTRODUCTION

Biochemical-genetic studies have been used successfully in fisheries biology to determine the number of stocks which exist in a species (de Ligny, 1969, 1972). Zone electrophoresis and histochemical stains are used to determine genetic proteinaceous variations.

Such a study was one of the methods used to determine the number of stocks which exist in market squid. This information is needed to properly manage the resource. Two laboratories were engaged in the investigation: California State College, Stanislaus (Turlock, CA), and the Department of Fish and Game (Long Beach, CA). We, at the latter laboratory, using starchgel electrophoretic procedures and mantle muscle tissue, conducted an intensive study of the enzyme phosphoglucomutase (PGM). We compared temporal and geographic PGM allelic variations of large samples.

The enzymes aconitase, fumarase, lactase dehydrogenase, nucleoside phosphorylase, glucosephosphate isomerase, and PGM were tested for polymorphism, but only PGM was found to be sufficiently polymorphic for our study.

8.2. MATERIALS AND METHODS

We used only market squid captured along the California coast in our investigation (Table 17, Figure 42). All but two samples were captured under night-lights with squid jigs and dip nets over known spawning grounds. The **Sa-Lu** sample was captured with mid-water trawl over what may or may not be a spawning ground. The **CC** sample was captured incidentally to a commercial shrimp catch.

TABLE 17
Sampling Data for *Loligo opalescens*

Location of catch	*Sample*	*Date of capture*	*Sample size*
La Jolla	LJ 1	December 1975	194
	LJ 2	February 1976	200
	LJ 3	May 1976	200
San Clemente Island	Cl	May 1976	116
Santa Catalina Island	Ca 1	December 1975	1931
	Ca 2	April 1976	200
	Ca 3	November 1976	200
Point Mugu	Mu	December 1976	200
Santa Cruz Island	Cr	March 1976	200
Santa Rosa Island	Ro 1	March 1976	200
	Ro 2	June 1976	196
Gaviota	Ga	June 1977	200
Point Conception	Co	June 1976	199
Point Sal–Point San Luis	Sa–Lu	June 1976	186
Monterey	M 1	June 1976	200
	M 2	October 1976	101
	M 3	November 1976	160
	M 4	June 1977	198
Crescent City	CC	July 1977	237

*TABLE **17***
***Sampling Data for** Loligo opalescens*

All samples were frozen immediately upon capture, taken to the laboratory, and stored at –20 C until tested.

Muscle tissue (0.3–1.0 g) from each squid was homogenized in 30% glycerol (a volume equal to 13 times the weight of tissue) with mortar and pestle. The use of glycerol was suggested by Gary Sharp (National Marine Fisheries Service, La Jolla, pers. commun.). About ½ g of purified sand was placed into the mortar to help grind the very tough tissue. This operation was done over ice. The homogenate was then centrifuged at 9000 rpm for 35 minutes at 2 C.

Electrostarch[1] (Madison, Wisconsin) and Tris-Versene-Borate (TVB) buffer (Siciliano and Shaw, 1976) were used to make a 12% starch-gel electrophoresis medium. With a few modifications, the gel was made according to Smithies (1955). The differences were 1) a wire gauze with asbestos center was placed between the flame and conical flask in the mixture heating process, 2) the contents of the flask were heated until the mixture had boiled for about 30 seconds, and 3) the process of removing air bubbles (with an aspirator attached to the laboratory water line faucet) lasted about 50 seconds. The hot starch solution was then poured into a 160 X 150 X 10 mm Plexiglas tray. The solution was allowed to cool and gel at room temperature for ½ hr; whereupon, the gel was covered with plastic wrap and kept at room temperature for 1 or 2 days. One half hour before use, the gel was cooled in a refrigerator (4 C).

Filter paper applicators (2 X 10 mm no. 1 Whatman) were immersed into the enzyme extract, blotted on clean Whatman filter paper, and placed into a slit cut in the gel 40 mm from the cathodal end. Horizontal electrophoresis was carried out at 4 C for 8 hr at a constant current of 60 milliamperes (135 volts ± 20). Direct current was supplied by Gelman

[1] 1 Trade names referred to in this paper do not imply endorsement of commercial products by the California Department of Fish and Game.

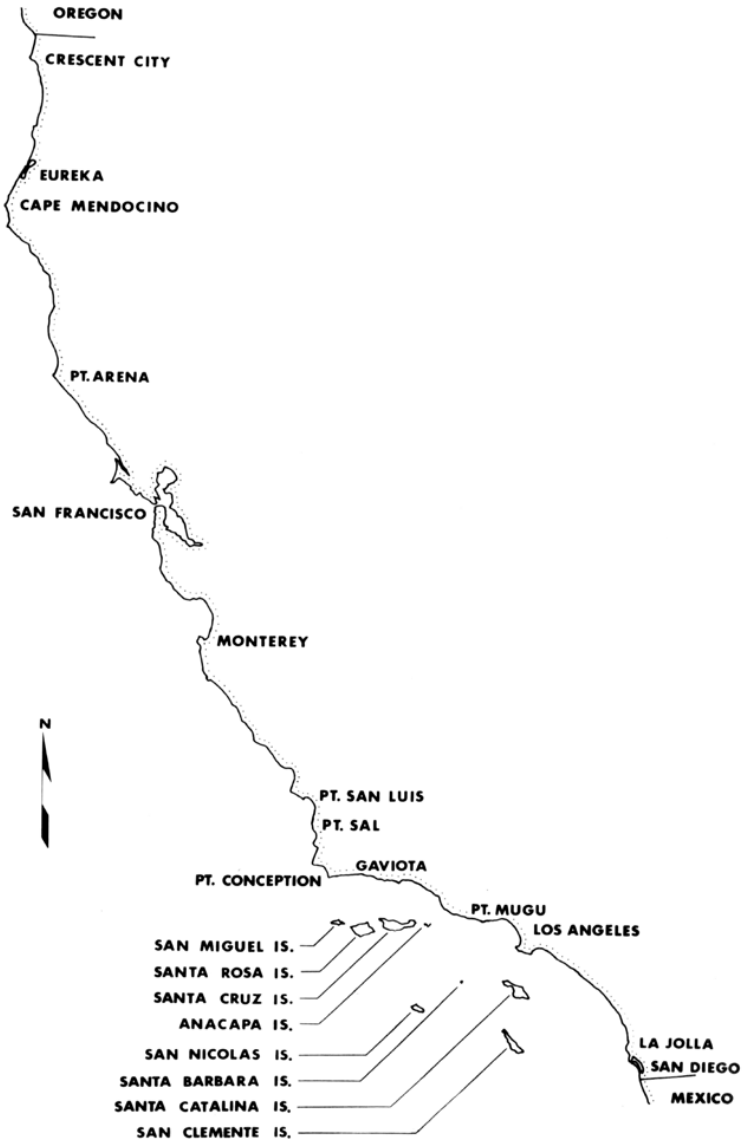


FIGURE 42. Areas from which market squid, ***Loligo opalescens,*** were collected.

*FIGURE **42. Areas from which market squid,** Loligo opalescens, **were collected.***

Electrophoresis Power Supply units. The electrode buffer used was TVB (Siciliano and Shaw, 1976). A 2 mm thick slice of the gel was then stained for PGM activity by incabating it in the dark at 37 C for 30–45 min using the following histochemical mixture obtained from Michael Soule (University of California, San Diego, pers. commun.): 25 ml of H_2O; 5 ml of 0.2 M Trizma Base (pH 8); 5 ml of 0.05 M glucose-1-phosphate; 2 ml of 3 X 10^{-4}M glucose-1,6-diphosphate; 5 ml of 0.1 M $MgCl_2$. $6H_2O$; 0.5 ml of 0.013 M NADP; 30 units of glucose-6-phosphate dehydrogenase (stock solution: 10 units per ml H_2O); 0.5 ml of 0.024 M MTT; and 0.2 ml of 0.033 M phenazine methosulfate. The gel was then fixed in a 5:5:1 solution of water-methanol-acetic acid, scored, and photographed with Kodax Plus-X 35 mm film.

The goodness-of-fit test used in our statistical analyses of the laboratory data was chi-square (X^2). The data were examined in the following manner: 1) we tested the fit of PGM phenotype frequencies of each sample by sex to the expected Hardy-Weinberg distribution; 2) we used the relative allele frequencies of the **Ca 1** sample to determine the expected allele frequencies of the other samples, then compared the allele frequencies of each sample to those of **Ca 1** (we believe that a pairwise test of goodness-of-fit between a large sample and a small sample gives more power of discrimination than does one between two, small, equal sized samples); 3) excluding **LJ 1–3** and **M 4**, we compared the sample with the highest frequency of the most common allele to that with the lowest frequency of the most common allele; 4) we compared the allele frequencies of **LJ 1–3** to one another; and 5) we tested the allelic homogeneity of samples from selected geographical areas.

Allele frequencies of males only were tested because ten samples contained too few females to test against the expected Hardy-Weinberg distribution.

We did not analyze the **Sa-Lu** sample data because the sample was composed of juveniles of indeterminable sex.

Another type of analysis was done with the **Ca 1, Ca 3, M 1,** and **M 4** samples. The relative frequency of their most common allele was compared to the mean relative frequency of the most common allele of all samples (excluding **LJ 1–3**).

8.3. RESULTS AND DISCUSSION

All but two samples were composed of spawning or about-to-spawn animals; the **Sa-Lu** sample was composed of juveniles, and the **CC** sample was composed mostly of adults with did not appear to have reached spawning condition.

In all but one sample, males outnumbered females anywhere from 1.1:1 to 49:1. Only in the **M 1** sample did females outnumber males (1.6:1). The predominance of males was not unexpected. Although both sexes are positively phototactic, the condition appears to be considerably stronger in males, probably due to their seemingly more aggressive behavior.

Zymograms of mantle muscle extracts showed one region of PGM activity occupied by five anodal bands labeled A, B, C, D, and E, in order of increasing mobility (Figure 43 and 44). We observed one and two-banded

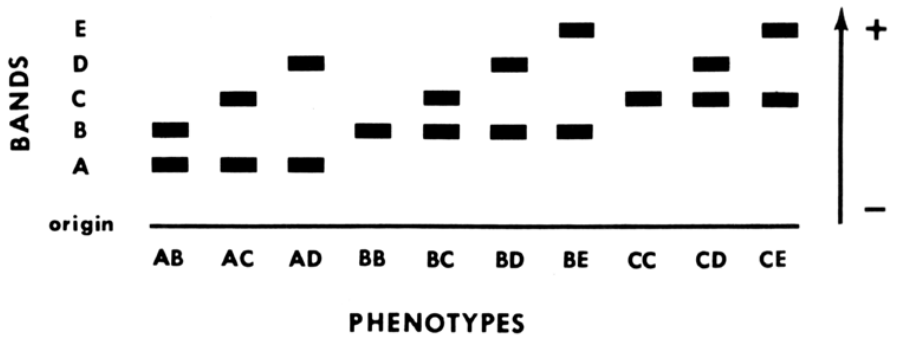


FIGURE 43. Starch-gel electrophoresis diagrammatic zymogram of market squid mantle muscle tissue extracts showing phosphoglucomutase bands and phenotypes.

*FIGURE **43. Starch-gel electrophoresis diagrammatic zymogram of market squid mantle muscle tissue extracts showing phosphoglucomutase bands and phenotypes.***

phenotypes. Satellite bands like those reported by Lush (1969) and Johnson, Utter, and Hodgins (1971) were seen occasionally.

Ten of 15 possible phenotypes were detected; BC was the most common, followed closely by CC, distantly by BB, and very distantly by the remaining phenotypes (Table 18 and 19).

The phenotype distribution for male squid in each sample, except that of **LJ 1** and **Cr**, was consistent with the expected Hardy-Weinberg distribution (Table 20). The phenotype distribution for female individuals in each testable sample, except that of **Ca 1**, followed the expected Hardy-Weinberg distribution (Table 20).

The most common allele was C, followed by B, A, D, and E in that sequence (Table 21).

In comparing the allele frequencies of **Ca 1** to those of each other sample, we found that the frequencies of **LJ 2** and **M 4** differed significantly from those of **Ca 1**; the frequencies of the other samples did not (Table 20).

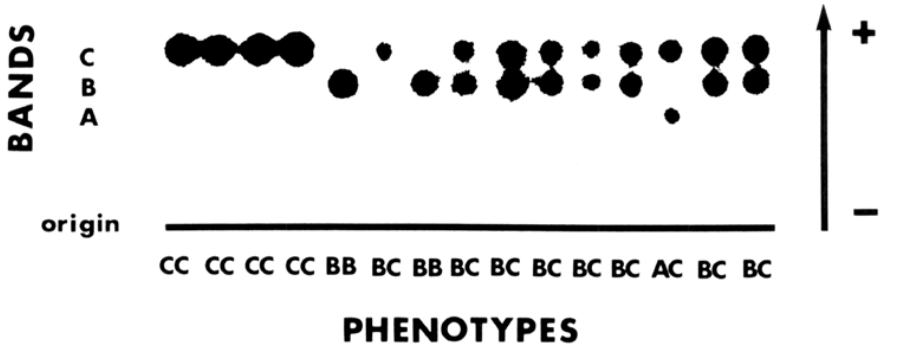


FIGURE 44. A representative starch-gel electrophoresis zymogram showing three bands of phosphoglucomutase from mantle muscle of market squid.

*FIGURE **44. A representative starch-gel electrophoresis zymogram showing three bands of phosphoglucomutase from mantle muscle of market squid.***

TABLE 18
Distribution of Phosphoglucomutase Phenotypes in Mantle Muscle Tissue Samples for Male Market Squid, *Loligo opalescens*

	Phenotypes														
Sample	*AA*	*AB*	*AC*	*AD*	*AE*	*BB*	*BC*	*BD*	*BE*	*CC*	*CD*	*CE*	*DD*	*DE*	*EE*	*Total*
LJ 1						15	96			70						181
LJ 2		1				37	92			66						196
LJ 3						24	52	1		52						129
Cl			2			15	47			39	1					104
Ca 1		7	8	1		196	653			564	2					1431
Ca 2		2	1			28	80			82	1					194
Ca 3						36	75			69						180
Mu		1	2			23	65	1		74						166
Cr			3			16	83			54						156
Ro 1		2	1			22	75	1		68						169
Ro 2			1			31	89			67						188
Ga		1				22	97			77		1				198
Co						18	48			40						106
M 1						12	35			31						78
M 2						12	37			26	1					76
M 3			1			15	52			41						109
M 4		1	1			36	78	1		52	1					170
CC			2			20	73			46						141

*TABLE **18***
Distribution of Phosphoglucomutase Phenotypes in Mantle Muscle Tissue Samples for Male Market Squid, *Loligo opalescens*

TABLE 19
Distribution of Phosphoglucomutase Phenotypes in Mantle Muscle Tissue Samples for Female Market Squid, *Loligo opalescens*

	Phenotypes														
Sample	*AA*	*AB*	*AC*	*AD*	*AE*	*BB*	*BC*	*BD*	*BE*	*CC*	*CD*	*CE*	*DD*	*DE*	*EE*	*Total*
LJ 1						2	4			7						13
LJ 2						1	2			1						4
LJ 3			3			10	25			33						71
Cl						4	4			4						12
Ca 1		6	1			50	256	1		186						500
Ca 2						3				3						6
Ca 3						1	9			10						20
Mu		1				1	12			20						34
Cr		1				2	16	1		24						44
Ro 1						5	13			12	1					31
Ro 2						1	5			2						8
Ga							1			1						2
Co			1			13	44	1		34						93
M 1						18	64		1	39						122
M 2						5	12			8						25
M 3			1			11	18			21						51
M 4						2	16			10						28
CC			3			13	45	1		34						96

*TABLE **19***
Distribution of Phosphoglucomutase Phenotypes in Mantle Muscle Tissue Samples for Female Market Squid, *Loligo opalescens*

TABLE 20

Statistical Test Results on Phosphoglucomutase Data from Mantle Muscle Tissue Samples of Market Squid, *Loligo opalescens*

	Hardy-Weinberg Test		*Allele Frequency Test comparing each sample to* **Ca 1** *Sample*	*Allelic Homogeneity Test of Samples from selected geographical areas*
	$P(X^2_{1df} > 3.84) = 0.05$ +		$P(X^2_{1df} > 3.84) = 0.05$	
Sample	X^2	X^2	X^2	X^2 *Rejection level*
	Male	Female		
LJ 1	5.15**		1.09	
LJ 2	0.34		4.86 *	5.15 $P(X^2_{2df} > 5.99) = 0.05$
LJ 3	3.18	1.00	0.47	
Cl	0.03		0.09	
Ca 1	0.87	5.13 **	—	1.85 $P(X^2_{3df} > 7.82) = 0.05$
Ca 2	1.54		0.12	
Ca 3	3.42		1.74	
Mu	2.23		0.70	
Cr	4.62**		0.02	
Ro 1	0.25	0.07	0.01	2.71 $P(X^2_{5df} > 11.07) = 0.05$
Ro 2	0.01		1.41	
Ga	0.95		0.20	
Co	0.31	0.02	0.42	
M 1	0.17	0.75	0.01	
M 2	0.09	0.02	0.72	4.64 $P(X^2_{3df} > 7.82) = 0.05$
M 3	0.11	2.58	0.03	
M 4	0.47		10.28**	
CC	1.45	0.20	1.32	

* Rejection level
** Significant

*TABLE **20***
***Statistical Test Results on Phosphoglucomutase Data from Mantle Muscle Tissue Samples of Market Squid,** Loligo opalescens*

Comparing the sample with the highest C allele frequency against that with the lowest C frequency (excluding **LJ 1–3** and **M 4**), no significant difference was found [$X^2 = 2.29$; P ($X^2_{1\ df} > 3.84$) = 0.05]. Thus, we can infer that samples with C frequencies lying between those of the two samples tested would not give a significant X^2 value when compared with one another.

Comparing the allele frequencies of **LJ 1** with those of **LJ 2**, we obtained a significant value [$X^2 = 5.13$; P ($X^2_{1\ df} \geq 3.84$) = 0.05]. No significant value was found in comparing **LJ 1** frequencies with those of **LJ 3** [$X^2 = 1.45$; P ($X^2_{1\ df} > 3.84$) = 0.05] and **LJ2** with **LJ3** [$X^2 = 0.71$; P ($X^2_{1\ df} > 3.84$) = 0.05].

In testing allelic homogeneity of samples from selected geographical areas, no significant difference was found, but the **LJ 1–3** group value approached the rejection level (Table 20).

Considering only the results of the statistical tests performed on the La Jolla samples, we concluded that homogeneity of PGM in that area is suspect. For three samples only, there are too many significant and nearly significant X^2 values to conclude otherwise. The area might be a southern transition zone for what could be a California subpopulation. Further work is needed to support this hypothesis.

TABLE 21

Relative Frequencies of Phosphoglucomutase Alleles in Mantle Muscle Tissue Samples for Male Market Squid, *Loligo opalescens*

	Alleles				
Sample	*A*	*B*	*C*	*D*	*E*
LJ 1	0.000	0.348	0.652	0.000	0.000
LJ 2	0.003	0.426	0.571	0.000	0.000
LJ 3	0.000	0.391	0.605	0.004	0.000
Cl	0.010	0.370	0.615	0.005	0.000
Ca 1	0.006	0.368	0.626	0.001	0.000
Ca 2	0.008	0.356	0.634	0.003	0.000
Ca 3	0.000	0.408	0.592	0.000	0.000
Mu	0.009	0.340	0.648	0.003	0.000
Cr	0.010	0.369	0.622	0.000	0.000
Ro 1	0.009	0.361	0.627	0.003	0.000
Ro 2	0.003	0.402	0.596	0.000	0.000
Ga	0.003	0.359	0.636	0.000	0.003
Co	0.000	0.396	0.604	0.000	0.000
M 1	0.000	0.378	0.622	0.000	0.000
M 2	0.000	0.401	0.592	0.007	0.000
M 3	0.005	0.376	0.619	0.000	0.000
M 4	0.006	0.447	0.541	0.006	0.000
CC	0.007	0.401	0.592	0.000	0.000

*TABLE **21***

***Relative Frequencies of Phosphoglucomutase Alleles in Mantle Muscle Tissue Samples for Male Market Squid,** Loligo opalescens*

When testing a parameter on a relatively large number of samples using a statistical test such as X^2, it would not be unexpected for one of the samples to show a significant value, nor would it be necessary to conclude that this sample is unlike the others. The significant value could be due to change; this change is 1 in 20 (5%) as stated in our significance level. Considering all samples except those from La Jolla, we believe the preceding is a reasonable explanation for the failure of the **Cr** sample to pass the X^2 test as applied to the expected Hardy-Weinberg phenotype distribution. However, with respect to the statistical test comparing each sample to **Ca 1**, we believe that the magnitude of the significant X^2 value of **M 4** is too great to have occurred by chance alone. Therefore, we concluded that this sample probably came from a different population than did **Ca 1**. It is unclear why the phenotype distribution for female individuals in **Ca 1** did not follow the expected Hardy-Weinberg distribution.

Ca 1 and **Ca 3** were captured in the same locality 1 year apart, as were **M 1** and **M 4**. We examined the relative frequency of the C allele of these samples (Table 21). **Ca 1** and **M 1** frequencies were above the mean of all samples (excluding those of **LJ 1–3**), while those of **Ca 3** and **M4** were below the mean. This suggests possible temporal isolation of two independent populations with alternating spawning seasons. This situation is found in pink salmon, Oncorhynchus gorbuscha (Fry, 1973). Statistical evaluation of allele frequencies does not support the above hypothesis, allele frequencies of **Ca 1** were not significantly different from those of **Ca 3** [$X^2 = 1.58$; P ($X^2_{1df} > 3.84$) = 0.05], nor were those of **M 1** from **M 4** [$X^2 = 2.83$; P ($X^2_{1df} > 3.84$) = 0.05]. Perhaps larger samples would have shown statistically significant differences. of the four samples, only **Ca 1** and **M 4** allele frequencies were significantly different from one another [$X^2 = 9.20$; P ($X^2_{1df} > 3.84$) = 0.05]. However, if further study supports the hypothesis

of temporal isolation, close monitoring of the resource would be necessary to avoid destroying one or both gene pools. This hypothesis also implies a 2 year life cycle for the market squid.

8.4. CONCLUSION

In itself, our PGM investigation to date is inconclusive regarding the question of stock discrimination of market squid. Although our results suggest possible geographically and temporally structured populations, further study is necessary to substantiate these hypotheses. On the basis of this study, the biochemical-genetic investigation is being continued, and upon its completion the results will be evaluated together with those of other market squid stock discrimination studies currently in progress. These should give us a firmer understanding of the temporal and geographical population structure of the market squid.

8.5. ACKNOWLEDGMENTS

We wish to express our sincere gratitude and appreciation to the following individuals for their help in this study: John Fitch, Eugene Fleming, Herbert Frey, Paul Gregory, William Lambert, Robin Lewis, Alec MacCall, Eugene Witeck, and the crews of the ALASKA and KELP BASS of the California Department of Fish and Game, Long Beach, California; Paul Charleston and Jay Christofferson of California State College, Stanislaus, Turlock, California; Charles Allender of California State University, Long Beach, California; Gary Sharp of the Inter-American Tropical Tuna Commission, Southwest Fisheries Center, La Jolla, California (now at FAO, Rome); Thomas Thompson (deceased), Conrad Recksiek, Douglas Vaughan, and Jerry Kashiwada of Moss Landing Marine Laboratories, California; Robert Holzinger of the University of California, Los Angeles, California; Michael Soule and John Senner of the University of California, San Diego, California; David Wallace of the commercial fishing vessel KILEY, Crescent City, California; Dennis Hedgecock of the University of California, Bodega Marine Laboratory, Bodega Bay, California; Fred Utter of the National Marine Fisheries Service, Seattle, Washington; and to all other colleagues who gave of their time.

9. AN ELECTROPHORETIC STUDY OF SELECT PROTEINS FROM THE MARKET SQUID, LOLIGO OPALESCENS BERRY

J. P. CHRISTOFFERSON,
A. FOSS,
W.E. LAMBERT,*
and
B. WELGE
California State College, Stanislaus
Turlock, California

9.1. INTRODUCTION

There have been predictions that the fishery in California for market squid, Loligo opalescens, will grow (Frey, 1971; Gulland, 1971). In order to establish reasonable management procedures, it is necessary to determine the market squid population structure in California waters. One assumption is that the squid composes a freely interbreeding homogenous population. If this is true, certain areas in which concentrated fishing occurs would be restocked by a central pool. If more than one stock exists in the population, it may be possible to overharvest one segment of the population while another segment might be virtually unutilized. A limited morphometric study by Fields (1965) suggested that there were two populations of squid spawning in Monterey Bay. A smaller sized squid is present in the spring, with larger animals appearing in the summer. Nothing further has been done to determine the population makeup of this species.

A combination of electrophoretic and histochemical techniques has enabled the separation and visualization of several types of biochemical molecules, most notably proteins (Siciliano and Shaw, 1976). Proteins, which are under direct genetic control, offer an opportunity to distinguish genetic loci. Allele frequencies and heterozygosity can be and have been used to elucidate the population structure of many animal species (Gooch and Schopf, 1970; Tracey, *et al*, 1975).

Electrophoretic examination of cephalopod proteins has not been extensive. Serum proteins have been separated in L. peallii (Woods, *et al*, 1958); Todarodes pacificus (Motochiro and Inoue, 1970); and L. vulgaris, Sepia officinalis, and Sepiola atlantica (Decleir and Richards, 1970). The eye lens proteins of Notodarus hawaiiensis were investigated by Smith (1969b). The number of isozymes for several mantle muscle enzymes of the squid Symplectoteuthis ovalaniensis are briefly mentioned in a publication edited by Hochachka (1975).

In this study, select proteins from blood, eye lens, digestive gland, mantle, and tentacular muscle of L. opalescens were separated by electrophoresis. The polymorphic locus of glutamate oxaloacetate transaminase (GOT) and several esterase loci were described and analyzed.

9.2. MATERIALS AND METHODS

Samples of L. opalescens were obtained at 11 locations along the coast of California from La Jolla to Crescent City (Table 22). The sample taken by midwater trawl, between Point Sal and Point San Luis, was composed of juveniles. Squid from waters off Crescent City were captured incidentally in a commercial shrimp trawl. The other samples were netted or jigged at night-light stations over spawning grounds. Each squid was measured, sexed, placed into a separate labelled plastic bag, and frozen at -20 C for analysis later. Blood samples were drawn with a syringe from the branchial heart of some squid. The syringe was then labelled and frozen.

Tissue samples (other than blood) were later removed, ground, extracted, centrifuged, electrophoresed, and visualized using histochemical methods. Specific procedures are outlined for each protein.

Blood samples were thawed and expelled from the syringes into 400 microcentrifuge tubes and centrifuged at 10,000 rpm for 5 minutes at 0 C. The supernatant was drawn into capillary tubes and expelled onto Whatman I chromatographic paper wicks. The wicks were then inserted into a slit made in a 12% starch-gel. A 0.05 M tris-glycine, pH 8.6 buffer diluted 1:2 was used to make the gel. The gel and wicks were then electrophoresed

TABLE 22
Sampling Data for *Loligo opalescens*

Location of Sample	*Sample*	*Date of Capture*	*Sample Size* *
	LJ 1	December 1975	176
La Jolla	**LJ 2**	February 1976	194
	LJ 3	May 1976	179
	LJ 4	December 1977	196
San Clemente Island	**C1**	May 1976	111
	Ca 1	November 1974	33
	Ca 2	December 1975	556
Santa Catalina Island	**Ca 3**	April 1976	199
	Ca 4	November 1976	1616
	Ca 5	December 1977	199
Point Mugu	**Mu**	December 1976	193
Santa Cruz Island	**Cr**	March 1976	195
Santa Rosa Island	**Ro 1**	March 1976	189
	Ro 2	June 1976	199
Gaviota	**Ga**	June 1977	192
Point Conception	**Co**	June 1976	191
Point Sal—Point San Luis (juveniles)	**Sa-Lu**	June 1976	199
	M 1	September 1974	33
	M 2	July 1975	52
Monterey Bay	**M 3**	October 1976	179
	M 4	November 1976	108
	M 5	June 1977	196
Crescent City	**CC**	July 1977	383

* Sample size indicates the maximum number of squid analyzed in any one of the procedures found in the materials and methods section.

*TABLE **22***
***Sampling Data for** Loligo opalescens*

at 25 mA for 6 hours at 10 C using a horizontal system similar to that of Smithies (1955). Polyacrylamide disc electrophoresis, using 7.5% gels made with a 0.05M trisglycine buffer diluted 1:10, was also used on some blood samples. The samples were electrophoresed using the procedure of Whitaker (1967) at 2.5 mA/tube until the bromophenol blue dye reached the end of the tube.

The presence of hemocyanin in the gel (polyacrylaminde or starch) was indicated by a positive reaction, at the same location (on duplicate gels), for protein (0.25% coomassie brilliant blue), copper (0.1% rubeanic acid in 70% acetic acid), peroxidase and tyrosinase activity (Shaw and Prasad, 1970), and carbohydrate (Periodic Acid-Schiff's reaction, Kapitany and Zebrowski, 1973).

The effect of freezing and thawing on the hemocyanin molecules was determined by freezing and thawing blood samples three to seven times either quickly (dry ice and 50 C water bath) or slowly (freezer and 24 C). Then the samples were electrophoresed as previously outlined and the hemocyanin patterns scored.

9.2.1. Eye Lens Proteins

The eye lenses of L. opalescens were removed and frozen at -20 C. When a lens was thawed, the cortex and the nucleus were separated; the tissues were ground separately, extracted in distilled water or 0.14% NaCl and electrophoresed in cellulose acetate according to the procedure of Smith (1969), or in polyacrylamide gels using the method previously mentioned. Both anionic (3¾%—pH 8.3, and 7.5%—pH 8.9 gels) and cationic (7.5%—pH 4 gels) systems were utilized in this study. Proteins were then stained with 0.25% coomassie brilliant blue.

9.2.2. Tissue Enzymes

Digestive gland, mantle muscle, and tentacle tissues were separately ground with a glass rod and a small amount of sand (washed and ignited) in a 400 microcentrifuge tube. The mascerated tissue was then extracted for 16 to 48 hours in 40% glycerol (1:2 weight:volume) at 10 C. The use of glycerol was suggested by Gary Sharp (National Marine Fisheries, La Jolla, pers. commun.). The extract was centrifuged at 13,400 rpm at 0 C for 15 minutes. The supernatant was drawn up into capillary tubes and expelled onto Whatman III chromatographic paper wicks. The wicks were inserted into a slit made in a 12% (11% for esterases) starch gel slab and electrophoresed at 10 C, using the horizontal electrophoresis method of Smithies (1955). The enzymes were electrophoresed at 35 mA for 8 hours except for esterases which were at 25mA for 6 hours.

Two mm slices of the gels (except the top slice) were then stained using the procedures outlined in Siciliano and Shaw (1976) unless otherwise indicated (Table 23). Esterase substrates used were alpha-naphthyl acetate, alpha-napthyl butyrate, alpha-naphthyl propionate, alpha-naphthyl laurate, alpha-naphthyl myristate, alpha-naphthyl caproate, alpha-naphthyl caprylate, beta-naphthyl acetate, beta-naphthyl butyrate, beta-naphthyl propionate, beta-naphthyl caproate, indoxylacetate, and naphthol

AS-D acetate. Peptidase substrates were glycyl-L-leucine, glycyl-L-tyrosine, L-leucyl-L-alanine, L-leucyl-glycine, and L-leucyl-glycyl-glycine.

Inhibitor studies were conducted during the esterase experiments according to the procedures of Flowerdew and Crisp (1975). The inhibitors EDTA—ethylene-diamine tetra-acetic acid, eserine sulfate, and p-hydroxy-mercuribenzoate were used. Reduced enzyme activity in the presence of EDTA or p-hydroxymercuribenzoate indicates arylesterases while eserine sulfate inhibits certain cholinesterases (Gooch and Schopf, 1970; Flowerdew and Crisp, 1975). The substrates used were alpha-naphthyl acetate and alpha-naphthyl butyrate. During each trial, one slice of gel was not inhibited prior to staining and served as a control. The pattern and degree of staining observed with the control was used to visually determine the amount of inhibition which has occurred in each of the other duplicate gel slices.

Glutamate oxalocetate transaminase (GOT) was visualized according to the method of Shaw and Prasad (1970) or as follows: a stock solution was made up of 0.146g of alpha-ketoglutarate, 0.532g L-aspartic acid, 2.00g polyvinylpyrolidione, 0.200 g EDTA, 5.680 g $Na_2HPO_4 \cdot 7\ H_2O$, and 200 ml of water adjusted to pH 7.4; the stain consisted of 24 ml of stock solution, 25 ml of water, and 125 mg of Fast Blue BB adjusted to pH 7.6. The formula for this solution was obtained from Michael Soule (University of California, San Diego, pers. commun.). The stain solution was poured over the gel slice and the contents were incubated in the dark at 37.5 C for 2 hours.

9.3. RESULTS AND DISCUSSION

9.3.1. Hemocyanin

Blood proteins have been successfully used to investigate populations in vertebrates (Fujino, 1967; Vrooman and Paloma, 1975). However, to our knowledge, these proteins have not been used in an analysis of invertebrate populations. Hemocyanin is considered to be the most common protein in cephalopod blood (Prosser, 1973). This molecule forms very large (mw 3.8×10^6) extracellular aggregates of subunits, depending on environmental calcium concentration and pH (Prosser, 1973).

Zyumograms of L. opalescens blood show three patterns (Figure 45). The most common pattern is two bands (Pattern I), which agrees with the results of Decleir and Richards (1970) for L. vulgaris and Sepia officinalis. The three (Pattern II) and four (Pattern III) band patterns were usually not as common. Multiple bands (more than two) were found by Decleir and Richard (1970) in larval and juvenile S. officinalis. Patterns II and III could sometimes be produced by freezing and thawing the blood five or more times. It is not known whether the observed multiple bands are produced by the experimental procedure or indicate genetic and/or a physiological difference in the squid.

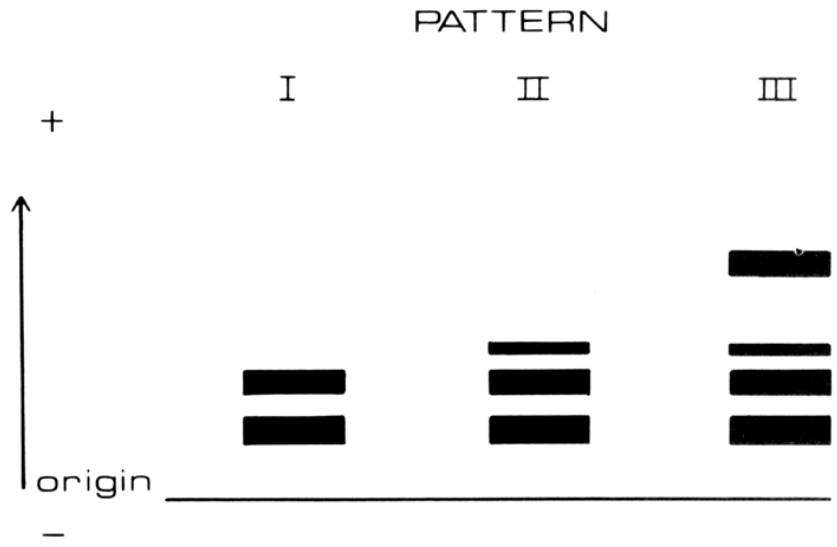


FIGURE 45. Starch-gel zymogram of *Loligo opalescens* blood hemocyanin patterns.

*FIGURE **45. Starch-gel zymogram of Loligo opalescens blood hemocyanin patterns.***

9.3.2. Eye Lens Proteins

Eye lens tissue from fish has been an important source of polymorphic proteins. These proteins have been used by Smith (1969a) for the analysis of population structure. Smith (1969b) examined the lens tissue of the squid, Notodarus hawaiiensis, and found several protein patterns. We were, however, unable to resolve any distinct patterns in the lens tissue of L. opalescens. Our results support those of Bon, Dohrn, and Batink (1967) and Swanborn (1971). They found that both decapod and octapod lens proteins were very easily denatured, especially if they were frozen.

9.3.3. Digestive Gland Esterases

of the 26 digestive gland tissue enzymes tested for, 23 were observed (Table 23). Digestive gland esterases and mantle muscle glutamate oxaloacetate transaminase were studied in detail. Fructose-biphosphatealdolase; fructose 1, 6-dephosphatase; glyceraldehyde 3-phosphate dehydrogenase; glycerol-3-phosphate dehydrogenase; and malate dehydrogenase (NADP +) may be polymorphic. The results were not clear with our procedure. The remaining enzymes appear to be monomorphic.

When a general esterase staining procedure was used, numerous esterase isozymes were observed. These esterase isozymes are selective for short chain esters (Table 24). They are sensitive to specific inhibitors (Figure 46).

TABLE 23

***Loligo opalescens* Enzymes Assayed *, the Buffer Systems Used, and Tissue Sources**

Enzyme	*Buffer ** system*	*Tissue Source †*			
		Juvenile DG	*DG*	*Adult MM*	*TM*
acid phosphatase	A	+ (1)	+ (1)	0	0
adenylate kinase	A	–	–	+ (1)	–
alcohol dehydrogenase	A	–	0	0	0
alpha-galactosidase	A	–	+ (2)	0	0
beta-glucuronidase	B	–	+ (1)	0	0
beta-glucosidase	B	–	+ (1)	0	0
catalase	B	0	+ (1)	–	–
carbonic anhydrase	E	–	0	0	–
creatine kinase	C	–	0	+ (1)	0
esterase	E	+ (6)	+ (6)	–	–
fructose-biophosphate aldolase	B	–	+ (1–2)	+ (1)	+ (1)
fructose 1, 6-diphosphatase	E	–	+ (1)	+ 1–2)	–
glutamate oxaloacetate transaminase	A	–	+ (1)	+ (1)	+ (1)
glyceraldehyde 3-phosphate dehydrogenase	A	–	+ (1)	+ (3–4)	+ (3)
glycerate-2 dehydrogenase	A	–	0	+ (1)	+ (1)
glycerol-3-phosphate dehydrogenase	A	–	+ (1)	+ (4)	+ (2)
leucine amino peptidase	E	–	+ (3–4)	0	0
malate dehydrogenase	A	–	+ (7)	+ (7)	+ (7)
malate dehydrogenase ($NADP^+$)	A	–	+ (1–2)	+ (1)	+ (1)
octanol dehydrogenase	D	–	+ (1–2)	0	0
peptidase	B				
Substrates					
glycyl-L-leucine		+ (2)	+ (2)		–
glycyl-L-tyrosine		–	+ (1)	–	–
L-leucyl-L-alanine		–	+ (2)	–	–
L-leucyl-glycine		–	+ (2)	–	–
L-leucyl-glycyl-glycine		+ (2)	+ (2)	–	–
peroxidase	A	–	0	–	–
2, 3-phosphoglycerate mutase	B	–	+ (1)	+ (1)	+ (1)
pyruvate kinase	B	–	0	+ (1)	+ (1)
superoxide dismutase	B	–	0	+ (3)	–
triose phosphate isomerase	B	–	+ (1)	+ (1)	+ (1)

* Bands of activity are indicated by the numbers in parentheses. Symbols:
+ = activity observed; 0 = no activity observed; – = tissue not analyzed.

** Buffer systems: A = Tris-citrate pH 7 (Siciliano and Shaw 1976).
B = Tris-EDTA-boric acid, pH 8.0 (Siciliano and Shaw, 1976), C = Poulik, pH 8.1 (Schaal and Anderson, 1974), D = Tris-borate, pH 9.0 + ETDA (Schaal and Anderson, 1974), E = Boric acid, pH 8.0 (Shaw and Prasad, 1970), F = Tris-citrate, pH 8.0 (Shaw and Prasad, 1970)

† Tissue source: DG = digestive gland, MM = mantle muscle, TM = tentacle muscle

*TABLE **23***

Loligo opalescens ***Enzymes Assayed, the Buffer Systems Used, and Tissue Sources***

The six loci (A, B, C, F, G, H) are characterized by any of the following: substrate selectivity, sensitivity to select inhibitors or the distance of migration. Isozymes D and E were not present in tissue samples used for the inhibitor or substrate experiments. Isozyme A hydrolyzes alpha- and beta-naphthyl acetate but not butyrate; isozyme B hydrolyzes alpha-naphthyl butyrate but not acetate (found only in juvenile squid); isozyme C appears to represent a separate locus by its migration rate, isozymes D and E (which are more common in juveniles) may also be part of this locus; isozyme F hydrolyzes alpha- and beta-naphthyl butyrate, alpha-naphthyl caproate, and is inhibited by mercuribenzoate; isozyme G is inhibited by eserine sulfate; isozyme H is considered to be separate by its migration rate.

TABLE 24

Substrate Specificity of Digestive Gland Esterases from *Loligo opalescens* *

Substrate	Isozyme A	C	F	G	H
3×10^{-3} M					
alpha-naphthyl acetate	++	+++	−	+	++
alpha-naphthyl butyrate	−	++	++	++	++
alpha-naphthyl propionate	+−	+++	+−	−	++
alpha-naphthyl laurate	−	−	−	−	−
alpha-naphthyl myristate	−	−	−	−	−
alpha-naphthyl caproate	−	−	+	−	−
alpha-naphthyl caprylate	−	−	−	−	−
beta-naphthyl acetate	++	+++	−	+	++
beta-naphthyl butyrate	−	++	+++	++	++
beta-naphthyl propionate	+−	++	+−	−	+
beta-naphthyl caproate	−	−	−	−	−
5×10^{-3} M					
indoxylacetate	+	++	−	−	−
naphthol AS-D acetate	+−	−	−	−	−

* The degree of activity is estimated: − = no reaction; +− = questionable activity; + = slight activity; ++ = medium activity; +++ = strong activity.

*TABLE **24***

***Substrate Specificity of Digestive Gland Esterases from** Loligo opalescens*

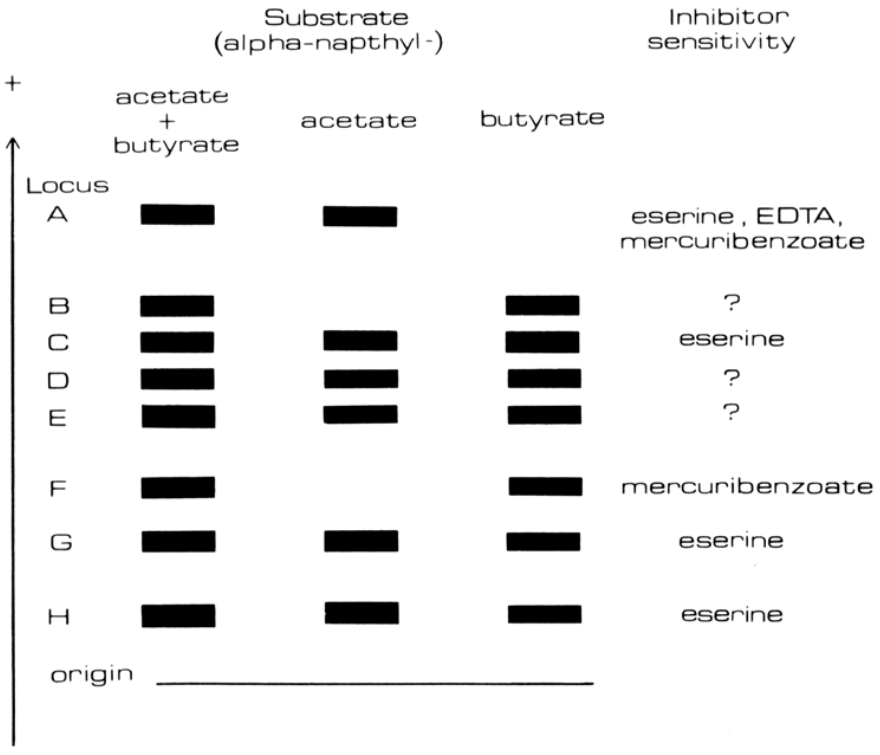


FIGURE 46. Zymogram of digestive gland esterase isozymes (A-HO) from *Loligo opalescens*. Tentative loci and inhibitor sensitivity for each isozyme is indicated (? = isozyme was not present in study). Inhibitors used: EDTA = ethylene-diamine tetraacetic acid, eserine sulfate, p-hydrozymercuri-benzoate.

*FIGURE **46. Zymogram of digestive gland esterase isozymes (A-HO) from** Loligo opalescens. **Tentative loci and inhibitor sensitivity for each isozyme is indicated (? = isozyme was not present in study). Inhibitors used: EDTA = ethylene-diamine tetraacetic acid, eserine sulfate, p-hydrozymercuri-benzoate.***

In summary, there appear to be six loci (Figure 47). These isozymes preferentially hydrolyze short chain esters with no specificity for the alpha or beta forms. The results of the inhibitor study indicate that isozymes A, C, G, and H have cholinesterase activity while isozymes B and F have arylesterase activity.

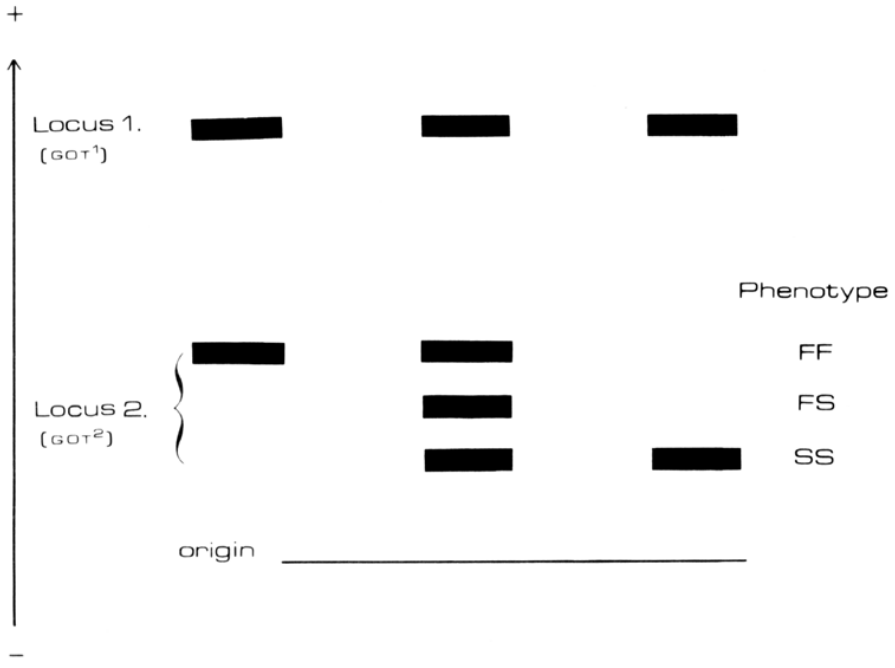


FIGURE 47. Zymogram of mantle muscle glutamate oxaloacetate transaminase (GOT) isozymes from *Loligo opalescens.* The three phenotypes observed in the GOT 2 locality are indicated.

*FIGURE **47. Zymogram of mantle muscle glutamate oxaloacetate transaminase (GOT) isozymes from** Loligo opalescens. **The three phenotypes observed in the GOT 2 locality are indicated.***

The presence or absence of isozymes D and/or F, and G was examined in both female and male squid by locality and date of capture (Tables 25 and 26). There is considerable variability between locations and dates of capture. Nevertheless, there appear to be trends, i.e., the three samples from La Jolla and the two from Monterey Bay indicate that there are temporal differences in the squid taken at these locations. It is difficult to interpret the results; it appears that some loci turn on and off. This might suggest an environmental influence.

Age influences the enzyme composition of L. opalescens. Juvenile squid have a digestive gland acid phosphatase isozyme and peptidase isozyme not present in adult squid. Catalase activity, which occurs in the adult digestive gland, was not found in juveniles (Table 23).

TABLE 25

Number and Percentage by Locality of *Loligo opalescens* Individuals Having Isozyme D *

Sample	*Isozyme D*	*Percentage*	*Sample size*
LJ 1	72	43	167
LJ 2	51	40	128
LJ 3	21	12	179
Ca 2	13	29	45
Ro 2	3	2	179
Ga	1	4	21
Co	12	6	185
M 1	4	12	33
M 2	22	33	66
M 3	18	10	179
CC	2	9	21

* Substrate: alpha naphthyl acetate.

*TABLE **25***

Number and Percentage by Locality of** Loligo opalescens **Individuals Having Isozyme D

9.3.4. Mantle Muscle GOT

Glutameate oxaloacetate transaminase (GOT) is produced at two loci in mantle and tentacle muscle tissue while only the product of a single locus is observed in the digestive gland tissue (Figure 47). The slower of the two proteins (GOT 2) is polymorphic. Heterozygeous individuals have a three band pattern which is consistent with a dimeric protein.

The distribution of GOT 2 phenotypes by location and date of capture varied (Table 27). Allele frequencies determined from these phenotypes fit the Hardy-Weinberg equilibrium formula except in the case of the Pt. Mugu (**Mu**) sample (Table 28). There was no significant difference between any of the samples when compared to the large (1,616 individuals) Santa Catalina (**Ca 4**) sample. The difference of allele frequencies between geographically pooled samples was not significant.

TABLE 26

Number and Percentage by Locality of *Loligo opalescens* Individuals Having Both Isozyme F and G

Sample	*Isozyme F and G*	*Percentage*	*Sample size*
LJ 1	2	1	176
LJ 2	16	12	135
LJ 3	45	25	180
Ca 2	0	0	48
C 1	12	11	111
Ro 2	10	5	199
Ga	1	5	21
Co	111	58	191
Sa-Lu *	36	18	199
M 2	35	67	52
M 3	6	3	177
CC	16	80	21

* Juveniles

*TABLE **26***

Number and Percentage by Locality of** Loligo opalescens **Individuals Having Both Isozyme F and G

TABLE 27
Distribution of Glutamate Oxaloacetate Transaminase (GOT²) Phenotypes from Mantle Muscle of Adult Male and Female *Loligo opalescens* by Location of Capture

	Sample											
Phenotype	*LJ3*	*LJ4*	*C1*	*Ca3*	*Ca4*	*Ca5*	*Mu*	*Cr*	*Ga*	*M4*	*M5*	*CC*
FF	59	57	31	57	505	68	64	65	58	41	49	108
FS	91	97	48	67	784	93	82	83	91	51	106	200
SS	25	42	23	31	327	38	47	31	43	16	41	75
Total	175	196	102	155	1616	199	193	179	192	108	196	383

*TABLE **27***
Distribution of Glutamate Oxaloacetate Transaminase (GOT 2) Phenotypes from Mantle Muscle of Adult Male and Female** Loligo opalescens **by Location of Capture

In summary, analysis of the esterase results suggests that there may be some population changes. It is not, however, possible to state if these results have a genetic basis. This was particularly evident in the first three La Jolla (**LJ 1, 2, 3**) samples (Tables 25 and 26). These results tend to support a hypothesis that there is a subpopulation of L. opalescens off the coast of Mexico which periodically, possibly because of oceano-graphic conditions, migrates northward. Unfortunately, there was no analysis for GOT 2 in those samples.

With these data it is not possible to conclude that there are or are not subpopulations of L. opalescens along the coast of California.

TABLE 28
Statistical Test Results on Glutamate Oxaloacetate Transaminase (GOT²) Data from Mantle Muscle Samples of Adult Male and Female *Loligo opalescens*

Sample	*Hardy-Weinberg Test* $P(X^2_{1df} > 3.84) = 0.05$ * X^2	*Allele Frequency Test comparing each sample to Ca 4 Sample* $P(X^2_{1df} > 3.84) = 0.05$ * X^2 (males only)	*Pooled Allele Frequency Test of samples from selected geological areas* X^2 *Rejection Level* (males only)
LJ 3	1.14	0.17	$0.72\ P(X^2_{1df} > 3.84) = 0.05$
LJ 4	0.003	0.98	
C1	0.29	0.49	—
Ca 3	1.89	0.17	
Ca 4	0.51	—	$0.33\ P(X^2_{2df} > 5.99) = 0.05$
Ca 5	0.38	0.25	
Mu	3.98 †	0.67	$0.002\ P(X^2_{1df} > 3.84) = 0.05$
Ga	0.40	0.67	
Cr	0.26	0.42	—
M 4	0.0004	1.10	$2.05\ P(X^2_{1df} > 3.84) = 0.05$
M 5	1.37	1.02	
CC	1.0266	0.63	—

* = Rejection level
† = Significant at the 0.05 level

*TABLE **28***
***Statistical Test Results on Glutamate Oxaloacetate Transaminase (GOT 2) Data from Mantle Muscle Samples of Adult Male and Female** Loligo opalescens*

9.4. ACKNOWLEDGEMENTS

The authors wish to especially express appreciation to Raymond Ally, Herbert Frey, Scott Keck, and the crews of the ALASKA and KELP BASS of the California Department of Fish and Game, Long Beach, California. Carolyn Argo, Warren Brandle, Paul Charleston, Jeanne L. Christofferson, and Craig Foster of California State College, Stanislaus, Turlock, California; Gary Sharp (now at FAO, Rome) of the Inter-American Tropical Tuna Commission, Southwest Fisheries Center, La Jolla, California; Thomas Thompson (deceased), Conrad Recksiek, and Jerry Kashiwada of Moss Landing Marine Laboratories, Moss Landing, California; Michael Soule of the University of California, San Diego, California; David Wallace of the commercial fishing vessel KILEY, Crescent City, California; and to all other colleagues who assisted in this study.

* **Present Address: California Department of Fish and Game Long Beach, California**

10. AN ACOUSTIC INVESTIGATION OF MARKET SQUID, LOLIGO OPALESCENS

DOUGLAS LEE VAUGHAN
and CONRAD W. RECKSIEK
Moss Landing Marine Laboratories
Moss Landing, California

10.1. INTRODUCTION

Acoustic sensing offers potential to survey market squid efficiently. It has been employed throughout the commercial fishing industry to increase catch efficiency and is emerging as an important tool to estimate size of fish stocks (Vestne, 1971; Forbes and Nakken, 1972).

Echolocation of fishable squid concentrations is used in several fisheries. Examples include the Japanese fisheries (Flores, 1962) for Todarodes pacificus (surume-ika), Sthenotensis bartrami (kaka-ika), Loligo budo (budo-ika), Doryteuthis kensaki (kensaki-ika), and Doryteuthis bleakeri (yari-ika); and the California market squid, Loligo opalescens, fishery of southern and central California.

Studies of squid aggregations using echo sounders have been carried out in Japanese waters. Shibata and Flores (1972) described echogram traces produced by 50 and 200 kHz echo sounders of various squid species during commercial fishing operations. Echo sounders have been used in describing T. pacificus school size, diel behavioral changes, and depth distribution (Kawaguchi and Nazumi, 1972; Suzuki, Tahiro, and Yamagichi, 1974).

This study had three objectives: 1. to describe the echogram traces that vertical sounding echo sounders produce when insonifying market squid; 2. to interpret behavior and dispersion patterns of market squid, based on echogram analysis; 3. to determine market squid school formations most conducive to acoustic market squid abundance and biomass estimations.

10.2. MATERIALS AND METHODS

Echograms containing traces of squid were collected from four separate echo sounders, which varied in frequency from 38 to 200 kHz, throughout the period of May 1976 to December 1978. These machines differed in frequency, pulse length, pulse repetition rate, paper speed, type of paper, beam angle, and time-varied gain capability (Table 29). They were used at various locations between Santa Catalina Island and Santa Cruz, California (Figure 48). The manual gain and white line settings for each instrument at each location were adjusted for optimal reading of the echograms. Verification that the traces observed were caused by L. opalescens was accomplished through surface observations, midwater trawling, jig fishing under lights, an underwater video system, drop camera photography, or scuba diver observations.

TABLE 29
Specifications for Echo Sounders Used in Detection of *Loligo opalescens* *

Echo sounder	*Vessel*	*Frequency (kHz)*	*Pulse length (msec)*	*Pulse repetition rate*	*½ Beam angle at −3 dB loss*	*Paper speed (mm/min)*	*Type paper*	*Time-varied gain*	*Mounting*
Gemtronics GT-105	ALASKA	200	0.85	180/min.	unavailable	8	dry	no	side
Simrad EK-38	ALASKA	38	3.0–0.3	96/min.	20° alongship; 13° athwartship	25	wet	yes	hull
Japan Radio Co. NJA-310	PACIFIC RAIDER	50	1–3	unavail-able	22°	≈10	wet	no	hull
Japan Radio Co. NJA-320A	OCONOSTOTA	200	1.2	200/min.	7.5°	10–29	dry	no	side

* These machines may possess multiple specifications for parameters listed. Specifications in this table hold for echograms discussed in this paper.

*TABLE **29***
***Specifications for Echo Sounders Used in Detection of** Loligo opalescens*

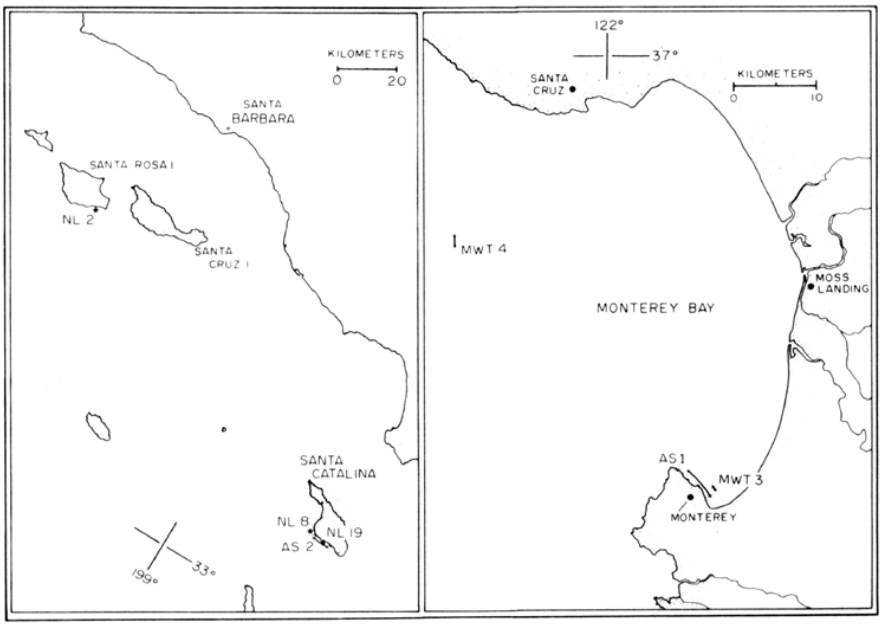


FIGURE 48. Areas where echosounders were used to detect squid. NL=night-light stations; MWT=midwater trawl; AS=acoustic search.

*FIGURE **48. Areas where echosounders were used to detect squid. NL=night-light stations; MWT=midwater trawl; AS=acoustic search.***

10.3. RESULTS

Echograms were taken simultaneously with the Gemtronics GT-105 (200 kHz) and the Simrad EK-38 (38 kHz) when the Department of Fish and Game ALASKA was searching for squid at an estimated speed of 2 knots next to Santa Rosa Island. The mark on the echogram (letter A) was made at the same time for both units (Figure 49A). The primary trace represented a concentration of animals approximately 0.5 km long extending from 2.3 to 14.5 m off the bottom. The ship's position at the time of observing the trace was approximately 33° 55.7' N and 120° 0.5' W (Location NL 2, Figure 48). After passing over the school the vessel doubled back over it, anchored, and the night-light was switched on. Squid were caught soon after jigs were lowered to the bottom. The latter occurred at 0340 on 8 June 1976 over a depth of 27.4 m.

After the light was on for approximately 5 minutes, plumelike traces on the Gemtronics appeared to rise toward the surface (Figure 50A). By 0410 this sounder was recording a continuous bottom trace about 3.7 m in height and numerous "splotch-like" traces (Figure 50C). These "splotches" were recorded as almost saturating the water column. At this time adult squid were being jigged at a relatively fast rate; 500 were caught in about 2 hours. They were also in heavy concentrations at the surface, and many were seen copulating. Other marine organisms visible were spiny dogfish, other unidentified sharks, and pelagic polychaetes.

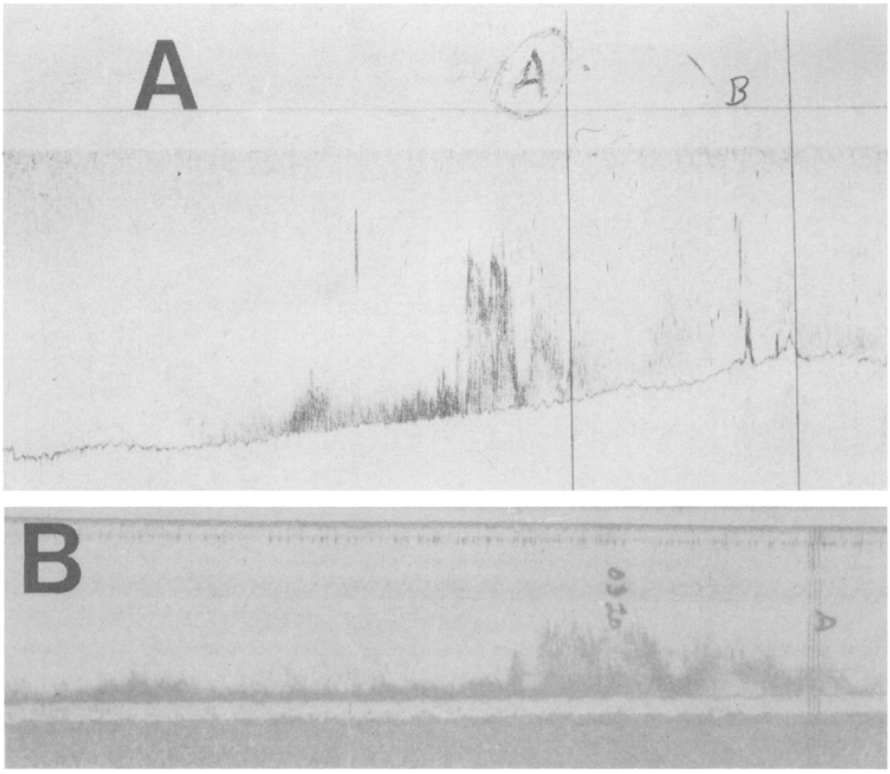


FIGURE 49. Echogram traces representing market squid taken underway at approximate speed of 2 knots while searching for squid near Santa Rosa Island 8 June 1976. Mark 'A' produced simultaneously on both echograms (ALASKA). A. Echogram taken by 200 kHz echo sounder (0310-0326). B. Echogram taken by 38 kHz echo sounder (0314-0322).

*FIGURE **49. Echogram traces representing market squid taken underway at approximate speed of 2 knots while searching for squid near Santa Rosa Island 8 June 1976. Mark 'A' produced simultaneously on both echograms (ALASKA). A. Echogram taken by 200 kHz echo sounder (0310–0326). B. Echogram taken by 38 kHz echo sounder (0314–0322).***

The EK-38 recorded dark bottom traces intermittently during the station and very faint traces in the water column above the bottom (Figure 50B). Traces off the bottom were resolved to a much lesser degree than Gemtronics traces taken at the same time. The continuous traces next to the bottom between echogram marks (E and F) were assumed to represent market squid.

As the dawn approached fewer traces were recorded until almost none was on the echogram (Figure 50D); fewer squid were caught with the fishing gear and fewer were observed at the surface. By sunrise squid were no longer detected under the vessel.

Daytime traces of market squid were collected with the Gemtronics GT-105 and Simrad EK-38 echo sounders (Figure 51) in Monterey Bay on 20 June 1976. These traces were recorded while the ALASKA was towing a midwater trawl (described in Mais, 1974), moving at a speed of approximately 3 knots (36° 37.3' N, 121° 53.5' W; to 36° 37.1' N, 121° 53.4' W; depth 31–37 m; Location MWT 3, Figure 48). The trawl's head rope was at an

estimated depth of 11 m and its foot rope was estimated at 29 m. The catch was approximately 1,600 market squid, one Torpedo californica, one Pelagia sp., Chrysaora sp., three unidentified jellyfish, and one unidentified crab. The market squid had a mean dorsal mantle length of 117.6 mm (s = 12.6 mm). The Gemtronics echogram for the trawl showed three large, dark, plumelike traces (Figure 51A). The first one to appear made a trace that began at 7 m and continued to 14 m. Because of the high catch of squid and low numbers of other species found in the net, these traces were assumed to represent squid. The Simrad EK-38 recorded one major plume between 15 and 27 m (Figure 51B). The target making this trace was assumed to be the same as that causing the middle trace on the Gemtronics. Both occurred at a similar depth range and both took place approximately 3 minutes after the onset of the trawl.

Echograms with traces that very probably represented market squid were taken from a 50 kHz Japan Radio Company (JRC) zoom echo sounder (Model NJA 310) during a midwater trawl operation and from a Furuno net sounder (Model FNR 400) which was operating simultaneously (Figure 52). The net sounder placed on the head rope of the midwater trawl

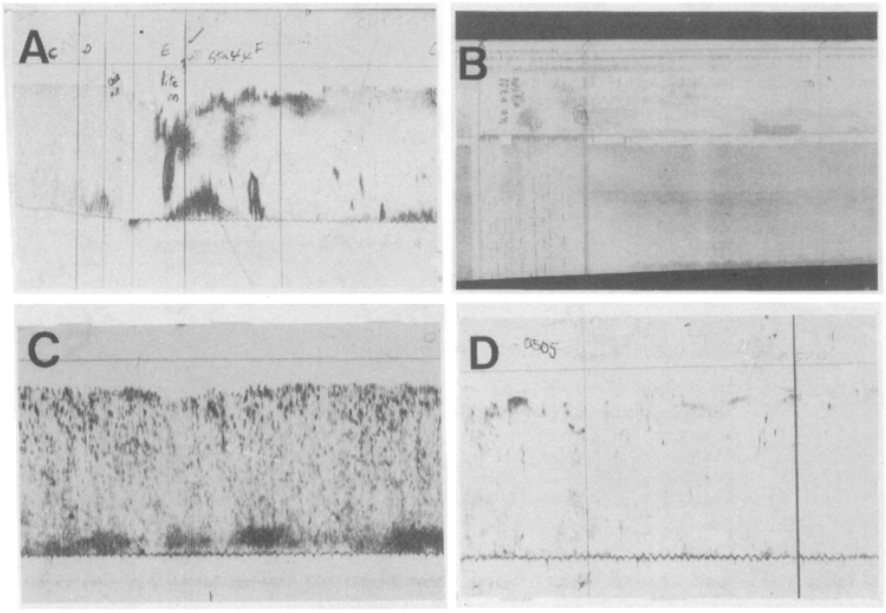


FIGURE 50. Echogram traces representing market squid at night-light station near Santa Rosa Island 8 June 1976 (ALASKA). A. A 200 kHz echogram showing traces throughout the water column. Light on at approximately 0340 (mark 'E'), mark 'F' made at approximately 0344. Plume traces after mark 'F' represent squid rising toward surface. B. A 38 kHz echogram. Continuous traces next to bottom between marks 'E' and 'F' assumed to be squid. C. A 200 kHz echogram taken approximately 0405–0430 showing 'splotch' traces almost saturating water column; a continuous trace along bottom. D. A 200 kHz echogram taken 0449–0510 showing fewer traces with approach of daylight.

*FIGURE **50. Echogram traces representing market squid at night-light station near Santa Rosa Island 8 June 1976 (ALASKA). A. A 200 kHz echogram showing traces throughout the water column. Light on at approximately 0340 (mark 'E'), mark 'F' made at approximately 0344. Plume traces after mark 'F' represent squid rising toward surface. B. A 38 kHz echogram. Continuous traces next to bottom between marks 'E' and 'F' assumed to be squid. C. A 200 kHz echogram taken approximately 0405–0430 showing 'splotch' traces almost saturating water column; a continuous trace along bottom. D. A 200 kHz echogram taken 0449–0510 showing fewer traces with approach of daylight.***

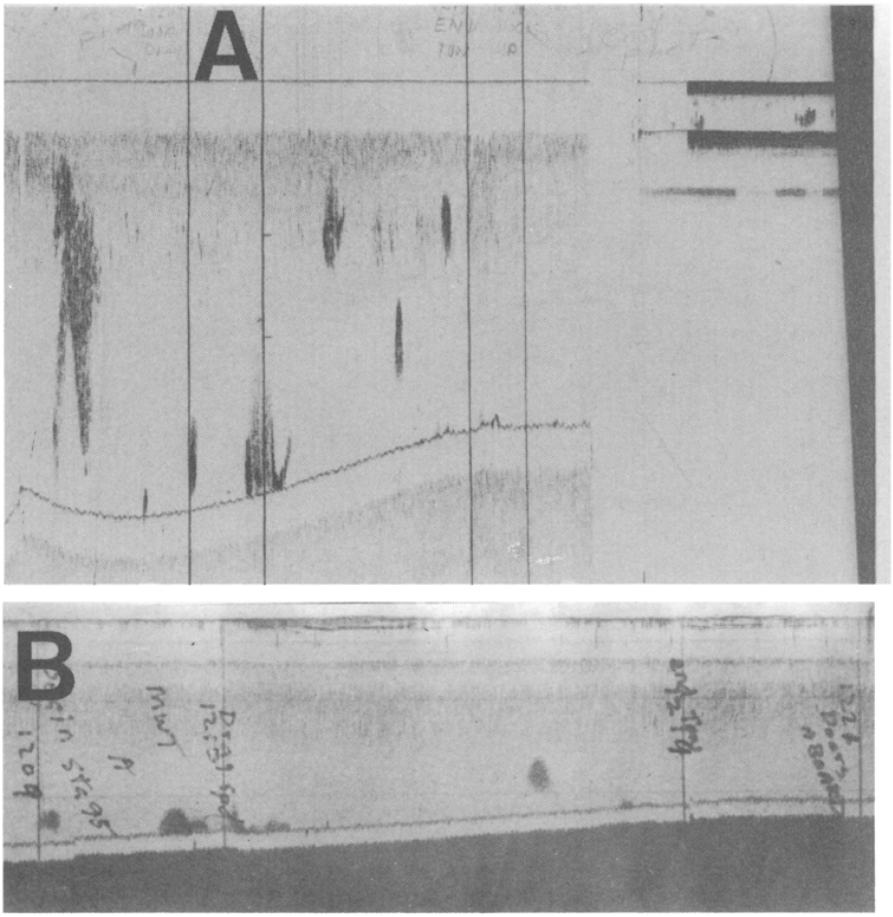


FIGURE 51. Simultaneous echograms taken in Monterey Bay during midwater trawling at an approximate speed of 3 knots, 20 June 1976 (ALASKA). Marks correspond to fishing net stages: mark I shotting (1209); marks II–III trawl speed; mark IV hauled (1226). A. A 200 kHz echogram. Three large plumelike traces between marks II and III assumed to represent market squid. B. A 38 kHz echogram. Large plume trace within marks II and III recorded at same time as middle plume trace in 200 kHz echogram.

FIGURE ***51. Simultaneous echograms taken in Monterey Bay during midwater trawling at an approximate speed of 3 knots, 20 June 1976 (ALASKA). Marks correspond to fishing net stages: mark I shotting (1209); marks II-III trawl speed; mark IV hauled (1226). A. A 200 kHz echogram. Three large plumelike traces between marks II and III assumed to represent market squid. B. A 38 kHz echogram. Large plume trace within marks II and III recorded at same time as middle plume trace in 200 kHz echogram.***

consisted of an upward and a downward facing transducer which operated at 75 kHz and a transmission transducer which operated at 50 kHz. Consequently targets that passed into the net were detected at 75 kHz and received by the recording unit at 50 kHz. Both sounders were used aboard the National Marine Fishery Service chartered PACIFIC RAIDER while midwater trawling in Monterey Bay 24 August 1976 between 0934 and 0942 (36° 50.5' N, 122° 9.5' W; to 36° 49.8' N, 122° 9.5' W, over depths of 256 to 293 m; Location MWT 4, Figure 48). During the tow the net sounder recorded a large dark trace entering the net (Figure 52A). At the time this

trace was recorded the net was assumed to be fishing at 229 m. The portion of the JRC echogram that represents the fishing depth did not have a well defined trace. However, a small "splotch" in that area may represent the net's catch (Figure 52B). The entire catch consisted of more than 200 squid, with dorsal mantle length averaging 83.7 mm (s = 8.0 mm) and four unidentified medusae. A continuous trace along the bottom extending between 20 and 60 m above bottom was recorded by the echo sounder as well.

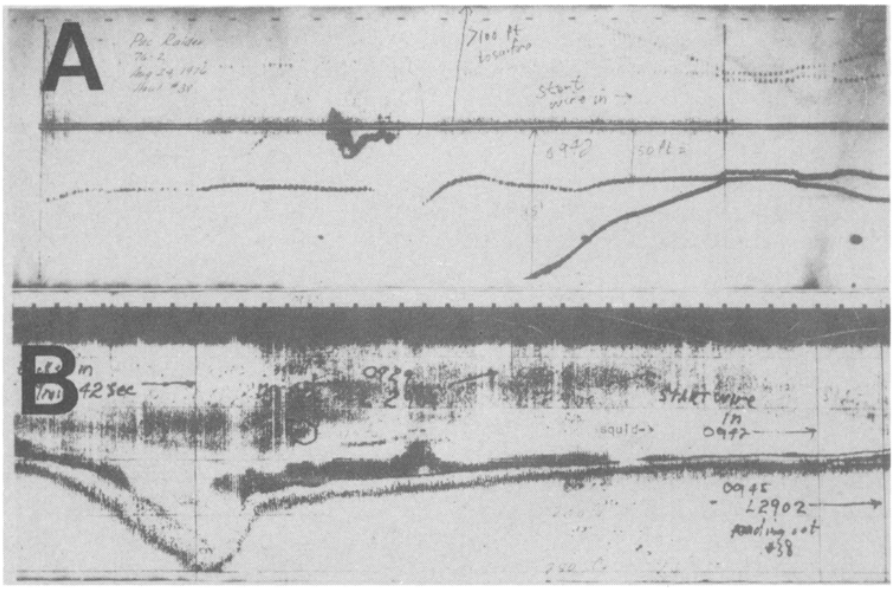


FIGURE 52. Echogram taken while midwater trawling in Monterey Bay 24 August 1976 (PACIFIC RAIDER). A. Echogram taken from net sounder. Head rope recorded as double black line toward middle of echogram. Foot rope of trawl recorded as black line under head rope trace; ocean bottom seen as rising trace at lower right of echogram. Black trace toward middle of picture, between head rope and foot rope trace, assumed to represent squid. B. Echogram taken by a 50 kHz echo sounder. Splotch labeled 'squid' is thought to represent same school as one entering net.

*FIGURE **52. Echogram taken while midwater trawling in Monterey Bay 24 August 1976 (PACIFIC RAIDER). A. Echogram taken from net sounder. Head rope recorded as double black line toward middle of echogram. Foot rope of trawl recorded as black line under head rope trace; ocean bottom seen as rising trace at lower right of echogram. Black trace toward middle of picture, between head rope and foot rope trace, assumed to represent squid. B. Echogram taken by a 50 kHz echo sounder. Splotch labeled 'squid' is thought to represent same school as one entering net.***

Echograms containing traces of market squid were collected in the southern bight of Monterey Bay 12 May 1977 during an acoustic search which lasted from 2031 to 2330. They were also obtained at a subsequent light station. These traces were collected on the OCONOSTOTA by a Japan Radio Company zoom echo sounder (Model NJA 320A) operating at a frequency of 200 kHz (Table 29). An acoustic search for squid was conducted from a position next to the United States Coast Guard jetty (36° 36.6' N, 121° 53.3' W) to a position near Point Pinos (36° 38.3' N, 121° 54.7' W) between the 20 and 70 m contours (Location AS 1, Figure 48). A total distance of 7.5 km was searched at about 2.5 knots. Traces occurred roughly between the mile buoy and the tip of the Coast Guard jetty (a distance of 370 m) within the 20 to 40 m depth contours. The configuration of traces

was of two types: 1. single plumes, and 2. traces that each contained several hyperbolic curves (Figure 53A). The latter traces appeared for the most part in the first 10 m above the bottom. The individual schools represented by the traces ranged in horizontal extent from about 8 to 90 m, and in vertical extent between 2 and 8 m. At 2330 the vessel stopped on these traces and a light was switched on. Within 2 minutes a dense aggregation of squid was observed from the surface. By 0020 on 13 May 1977, the echo sounder was recording traces throughout the water column which made the echogram appear mostly blackened (Figure 53B). These traces remained until the ship left the station. At 0030 a drop camera set to shoot one frame per minute was lowered to 20 m. Pictures of squid were taken as the camera was descending, while it was at depth, and as it was ascending. At 0100 divers were sent into the water to observe squid activity. Dense concentrations were seen from the surface to the bottom. Large numbers of females were laying eggs and many clusters of egg cases were observed. Several dead squid were observed. This night-light, drop camera, and dive lasted until 0300 13 May 1977, at which time squid were still numerous and traces on the echo sounder still appeared to mostly blacken the echogram.

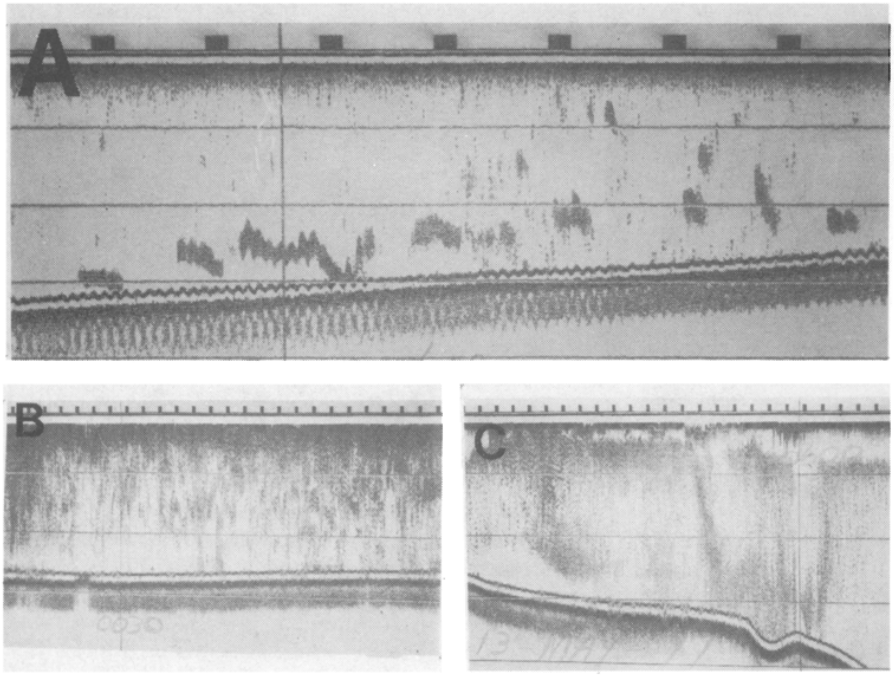


FIGURE 53. Echogram (200 kHz) traces representing market squid in southern bight of Monterey Bay (OCONOSTOTA). A. Traces recorded underway (approximately 2.5 knots) during acoustic search 2254–2260, 12 May 1977. B. Traces recorded from drifting vessel with night-light on 0024–0049, 13 May 1977. C. Traces recorded underway (2.5 knots) 0538–0604, 13 May 1977.

*FIGURE **53. Echogram (200 kHz) traces representing market squid in southern bight of Monterey Bay (OCONOSTOTA). A. Traces recorded underway (approximately 2.5 knots) during acoustic search 2254–2260, 12 May 1977. B. Traces recorded from drifting vessel with night-light on 0024–0049, 13 May 1977. C. Traces recorded underway (2.5 knots) 0538–0604, 13 May 1977.***

At 0600 after sunrise squid still appeared to be in large concentrations within the same area. Traces recorded by the echo sounder were still blackening most of the echogram (Figure 53C). These traces were known to be squid because the Monterey commercial fishing fleet was catching large quantities of squid simultaneously within the same area. Squid traces appeared to be localized. No traces were observed in the Monterey southern bight area outside the jetty-mile buoy area, and no commercial boats were catching squid anywhere else.

Thick traces at 38 kHz were recorded from a slow-moving vessel next to China Point, Santa Catalina Island (Location NL 8, Figure 48), on 10 December 1977 at approximately 2030. Traces were varied in appearance and were throughout the water column; water depth varied from 49 to 59 m (Figure 54A). Continuous traces 20 m in vertical extent were recorded both from the ocean bottom and next to the surface. "Splotches" and plumelike traces were also interspersed throughout the echogram. Traces appeared to almost blacken parts of the echogram after the ship anchored (0.8 miles WNW of China Point, 33° 19.8' N, 118° 28.9' W) and the night-light was turned on (2100; Figure 54B). While these traces were being recorded, an underwater television camera (Hydro Products underwater video system) was lowered in the water. Squid were observed on the viewing screen throughout the water column in dense aggregations, with average density estimated to be 7.3 squid/m^3 without night-lights on and 99.6 squid/m^3 with the lights on. (The methodology in obtaining these estimates is reported in Vaughan, 1978.)

On 14 December 1977 at approximately 1330, pilot whales, Globicephala macrorhynca, and a concentration of marine birds were observed midway between China Point and Salta Verde Point (Location NL 19, Figure 48), Santa Catalina Island. Upon moving over this area, traces were recorded with the EK-38 and Gemtronics GT-105 echo sounders. The traces resembled many "splotches" spaced close together and indicated the targets were located throughout the water column (Figure 55A). These traces were recorded for an approximate distance of 270 m with each pass the ship made over the area. Acoustic transects were made to determine the dispersion of squid within the general area. These transects totaled approximately 10 km searched and were run between the 37 and 55 m contours (Location AS 2, Figure 48). The only traces recorded that seemed to be squid were those that occurred in the area where we had first observed traces and the mammal-bird activity (33° 19.9' N, 118° 26.7' W). The vessel anchored and the television camera was lowered in the water at approximately 1600, at which time two small schools were observed. After sunset (2136) the television camera was again lowered and squid were viewed in heavy concentrations on the screen. The Gemtronics echogram revealed thick concentrations of squid next to the surface and close to the bottom, and smaller concentrations in midwater (Figure 55B). Their estimated densities ranged from 3.1 to 27.0 squid/m^3 (Vaughan, 1978). They were spawning, and clusters of egg cases were viewed on the bottom.

FIGURE 54. Echogram (38 kHz) traces representing market squid near Santa Catalina Island 10 December 1977 (ALASKA). A. Echogram traces recorded from slowly-moving vessel (approximately 2030–2040). B. Echogram traces recorded with night-light on (approximately 2100–2115).

*FIGURE **54. Echogram (38 kHz) traces representing market squid near Santa Catalina Island 10 December 1977 (ALASKA). A Echogram traces recorded from slowly-moving vessel (approximately 2030–2040). B. Echogram traces recorded with night-light on (approximately 2100–2115).***

10.4. DISCUSSION AND CONCLUSIONS

Schools of market squid were shown to produce four types of traces on echograms. The first was a feather plumelike trace which appeared when schools were pelagic (Figures 50A, 51A, 53B, 54A, 55A).

The second type was a more or less continuous trace which appeared when schools were on or near the bottom (Figures 49A, 49B, 50A, 50B, 50C) and within the water column (Figure 54A). The continuous trace recorded along the bottom at station MWT 4 (Figure 52B) may be squid as well. In the latter case a midwater trawl was fishing close to the shoal represented by this trace and 200 squid were captured. Squid aggregated too close together to be resolved as individuals within the continuous and plume traces; most of the surface area on the echogram within these "known to be squid" traces was darkened.

The third type of trace, observed three times, consisted of "splotches" scattered throughout the water column. It was first observed as squid were attracted toward the surface at a night-light station when the ship was anchored (Figure 50C). At the time these "splotches" were recorded squid were viewed from the surface in large numbers, some copulating. Therefore each individual splotch probably represented individual squid, a copulating pair, or a small aggregation. The second time these traces were observed was in the daytime from a slow-moving vessel (Figure 55A). At this time many pilot whales and birds were feeding throughout the immediate area and may have been responsible for breaking a larger shoal into small aggregations. The third time was within the same area when the night-light was illuminating the water (Figure 55B).

The fourth type of trace was a general blackening of the portion of the echogram representing the water column. This trace appeared at two night-light stations and from a slow-moving vessel during the early morning

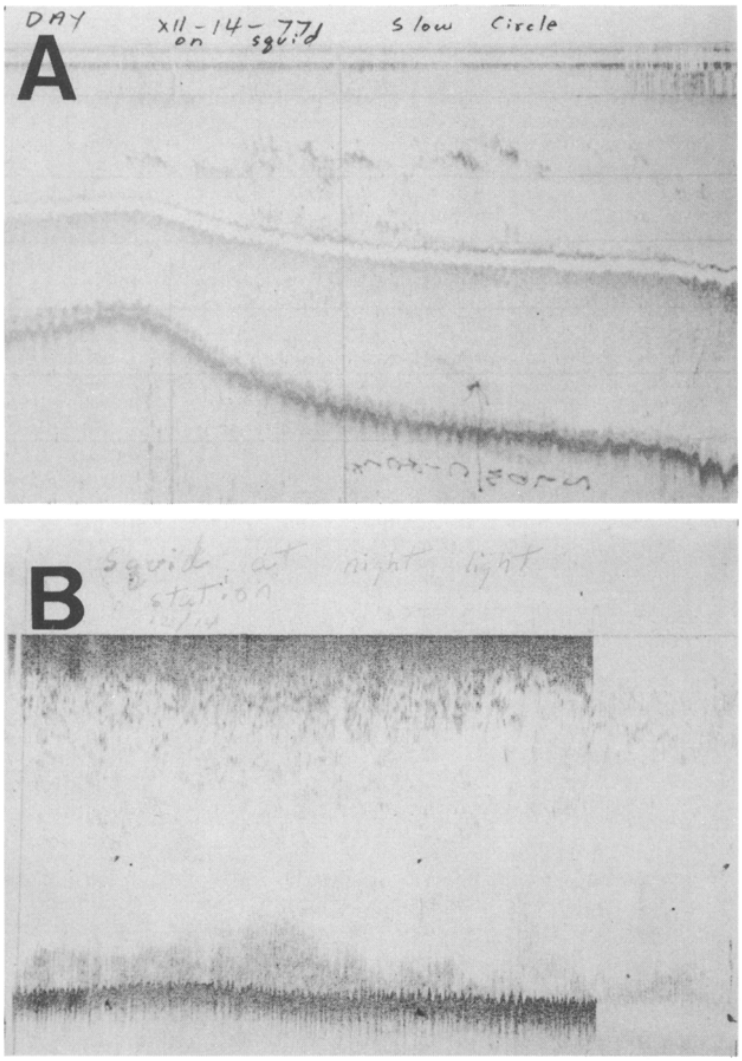


FIGURE 55. Echogram traces caused by market squid near Santa Catalina Island 14 December 1977 (ALASKA). A. A 38 kHz echogram recorded from slowly-moving vessel, 1430–1500. B. A 200 kHz echogram recorded from anchored vessel with night-lights on, approximately 2040–2100.

*FIGURE **55. Echogram traces caused by market squid near Santa Catalina Island 14 December 1977 (ALASKA). A. A 38 kHz echogram recorded from slowly-moving vessel, 1430–1500. B. A 200 kHz echogram recorded from anchored vessel with night-lights on, approximately 2040–2100.***

hours in the southern bight of Monterey Bay (Figure 53C). This type of trace represented squid in thick concentrations throughout the water column.

When traces caused by squid were observed from a moving vessel in the absence of light or predation effects, the basic patterns were either 1. pelagic, plumelike; 2. bottom-associated, continuous; or 3. pelagic, continuous configurations. Plumelike traces on echograms are caused by schools being less than the diameter of a cross-section of the acoustic beam (Cushing, 1973). A continuous trace is made when targets remain under an echo sounder beam for a relatively long period of time. This trace can be caused by the ship being stationary over a stationary target or a ship moving on top of a large school or scattering layer (Cushing, 1973). Usually the horizontal distance of the school is much larger than the cross-sectional diameter of the acoustic beam at that depth. Consequently this study detected squid aggregating either in large numbers close to the bottom or in the water column, and in smaller numbers in pelagic schools.

Traces representing market squid in this study resemble those found by other researchers. Shibata and Flores (1972), using 200 kHz and 50 kHz echo sounders off a moving boat and when squid were rising to a night-light, have shown plumelike traces representing various species of squid caught in Japanese waters. Kawaguchi and Nazumi (1972) have shown similar plumelike traces taken in the day for T. pacificus with 200 and 75 kHz echo sounders. Mais (1974), using the 38 kHz sounder, has shown L. opalescens to form a more or less continuous layer which appears next to the bottom.

Based on analysis of echograms made with the 38 and 200 kHz echo sounders, it is speculated that the higher frequency machine can better detect small pelagic schools of squid. The "splotch-like" traces that appeared on the 200 kHz Gemtronics echogram during a night-light station (Figure 50C) were not apparent on the Simrad EK-38 echogram when these units were operating simultaneously. In addition, more plume traces of market squid appeared on echograms of the Gemtronics than the Simrad EK-38 when both were operating simultaneously. The Gemtronics recorded three plume traces during the midwater trawl in Monterey Bay (Figure 51A), while the EK-38 recorded only one (Figure 51B).

It is difficult to state definitively, when two machines were operated simultaneously, why one echogram showed a single plume trace and the other showed three. Perhaps frequency, and other machine parameters or placement of the transducers may have been responsible. The transducer of the Simrad EK-38 was located on the hull, whereas the transducer of the Gemtronics was located amidships on the port side during midwater trawling operations and amidships on the starboard side under the light during night-light stations. It is likely that the Gemtronics picked up more squid plumes because squid schools were more likely to be insonified at light stations by a transducer that is next to an attracting light, squid schools were closer to the Gemtronics' transmission axis during the midwater trawl, and small squid aggregations were better detected with the higher frequency echosounders. Szuki *et al.* (1974) found that a 200 kHz echo sounder recorded squid more clearly than a 75 kHz sounder; and

Kawaguchi and Nazumi (1972) concluded that the optimum specifications of an echo sounder for squid detection were a frequency between 75 and 200 kHz, narrow beams, and a minimum pulse length.

Two stations where large aggregations of market squid became attracted to overhanging lights at night were studied the following mornings (waters next to Santa Rosa Island on 8–9 June 1976 and Monterey on 12–13 May 1977). At dawn next to Santa Rosa Island, the light lost its effectiveness to attract squid (Figure 50D). Therefore either the squid scattered, moved as a school to a different location, or moved so close to the bottom that they became undetected by the sounders. At dawn near Monterey squid were still in heavy concentrations throughout the water column (Figure 53C). It is unclear what is responsible for this dichotomy in results. The stations were separated by considerable time and distance. One speculation is the squid around Santa Rosa Island were at a different phase of their life cycle and may have been exhibiting behavior appropriate to their reproductive state.

Squid aggregations were represented by a diversity of traces which presumably signified different patterns of schooling. Certain patterns provide a better opportunity than others for acoustical surveying. One problem in conducting an acoustic survey for L. opalescens is the inability to identify traces on echograms as definitely representing squid without using some type of ground truth, e.g. midwater trawl, underwater video system, divers, etc. The pelagic plume traces observed appear to represent small, perhaps feeding groups of swiftly moving animals. If squid were aggregated in this way it would be difficult to conduct an extensive acoustic survey because identification of insonified targets would be slow and difficult to accomplish. Plume-like traces (Figure 51) were identified as squid only because the research vessel was fishing a mid-water trawl at the same time that traces occurred on the echo sounder. On the other hand, spawning squid schools produced traces that offer the prospect of being surveyed. These schools were large and stationary enough to be detected easily and identified during an acoustic investigation.

Spawning squid schools were never found to have a horizontal extent over 500 m during any acoustic transect. This observation indicates that spawning occurs in isolated schools. Each school consists of a heavy concentration of squid within a relatively small area rather than loosely scattered individuals over a wide area. Two such aggregations were observed around Santa Catalina Island. In the southern bight of Monterey Bay and along the southeastern side of Santa Rosa Island during the nights of the acoustic surveys, only one such aggregation was observed for each area. Since the scope of the acoustic search was limited within all general areas surveyed, more spawning squid aggregations may have existed and yet remained undetected.

11. THE TARGET STRENGTH OF INDIVIDUAL MARKET SQUID, LOLIGO OPALESCENS

DOUGLAS LEE VAUGHAN
Moss Landing Marine Laboratories
Moss Landing, California

11.1. INTRODUCTION

Target strength values for marine organisms specify the quantity of sound intensity or pressure that is reflected from an individual organism or a group of organisms and are related to the total scattering cross section and the backscattering cross section respectively.

The target strength for any nonresonant marine organism is dependent upon its size, the aspect which receives incident sound, the relative density and elasticity of the marine organism as compared to sea water, and the frequency of the sound wave (Clay and Medwin, 1977). Measurements are taken in the field by first recording the echo level (in decibels) of reflected sound returned to the transducer; this echo level is a function of the returned sound pressure. Besides target strength the returned sound pressure is a function of the transducers power output (source level), transmission loss, directivity of the acoustic beam, and vessel and ambient noise. All of these variables can be determined and target strength can, therefore, be calculated.

When conducting a bioacoustic investigation, the determination of the target strength for the organisms studied should be considered an important primary step. Knowledge of target strength can provide an aid to identification of marine organisms in acoustic field studies. Cushing and Richardson (1955) demonstrated differences in both variation and intensity of received signals for cod and herring irrespective of size. Love (1971a) suggests that the more advanced forms, acanthopterygians, have distinct responses to the total scattering cross section, the length of the fish, and the wave length of the acoustic beam.

Size frequency distributions can be acoustically determined with a knowledge of the relationship of target strength and size of organism. Studies have been performed to determine the relationship between target strength, fish length and wave length (Love, 1971a; McCartney and Stubbs, 1970). Cushing (1964) graphically demonstrated that among cod, coalfish, and herring target strength increases proportionately with length of fish. Acoustic estimates of length frequencies for hake off the coast of southern Africa were made by utilizing known target strength data as related to the size of fish.

The validity of acoustic abundance estimations is enhanced with an accurate knowledge and utilization of the target strength for the marine organisms assessed. Echo counting and echo integrating systems necessarily require target strength information for acoustic assessment; and population

estimations determined from echograms can also be more accurate if target strength data are incorporated into the calculations. The volume sampled by any echo sounder during one ping within a depth interval is dependent upon target strength, the directivity of the acoustic beam and the sound intensity (source level) from the transducer. Therefore, for any given echo sounder, target strength of the marine organism(s) acoustically sampled determines sampling volume. An echo counting system will sum up the number of fish over a chosen period of time or distance; it can be used only when echoes do not overlap, and it produces results of the number of fish per unit volume (Clay and Medwin, 1977). The volume sampled with an echo counter will be greater when counting fish with larger backscattering cross sections or target strengths because of the greater range of detection away from the acoustic axis. Kelso and Minns (1975) provided an example of the use of an echo counting system during an acoustic survey. These authors used a digital echo counting system to study the effects of thermal plumes on fish abundance. Fish densities were recorded to vary between 20.8/10,000 m^3 and 1,037/10,000 m^3.

Since echo counting systems depend upon resolving individual targets acoustically, they can be used only at relatively low densities of marine organisms. At relatively high densities, echo integration systems must be used for electrical processing of echoes (Clay and Medwin, 1977). In this type of system, the amount of voltage that is put out by the integrator is proportional to the density of marine organisms and the average scattering cross section (Weimer and Ehrenberg, 1975). Therefore, a knowledge of the backscattering cross section or target strength of the organism(s) acoustically surveyed is needed before density estimations can be made from an echo integrator system. Some uses of echo integrator systems have been for hake stock assessment (Thorne, 1973), herring survey (Blanken-beckler, 1976), and experimental rockfish surveys (Gunderson and Nelson, 1977).

Population assessments determined by measuring school sizes on echograms are more accurate when the target strength of the marine organnism(s) studied is well known. Sound intensity from a transducer spreads through water in a more or less conical pattern; therefore, from a moving research vessel a school received at depth will appear larger than in shallow water. A correction factor at various depth intervals must be determined if the true volume of a school is to be estimated (Forbes and Nakken, 1972). This correction factor is dependent upon the angle from the axis of transmission at which the school will be received; and the angle is a function of the target strength of the organisms within the school, the directivity of the transducer, and the source level of the echo sounder.

Selection of the optimum equipment used to conduct an acoustic investigation is facilitated when the target strength of the organism(s) studied is known. The maximum range at which an echo sounder can detect a marine organism is dictated by the target strength of that organism and the maximum noise level. Therefore, by knowing the maximum range at which a marine organism is to be detected and its target strength, the required source level of an echo sounder to be used for an acoustic investigation can be determined. A knowledge of how frequency is related to the

target strength of a marine species under acoustic investigation can aid in determining the ideal frequency to conduct an acoustic survey. Smith (1954) has shown the target strengths of scup, Stenotomus chrysops; squid; and shrimp, Penaeus and Palaemonetes, to decline with frequency over a range of 8 to 30 kHz. Love (1971a) and McCartney and Stubbs (1970) have expressed the relationship among length, total scattering cross section, and wave length for fish in the dorsal and side aspects.

Much work has been done on the target strength of fish and of organisms in the deep scattering layers (DSL) as a function of length, abundance, and frequency; however, few measurements have been taken on soft bodied animals not in the DSL such as species of squid. In this study I determined target strengths of Loligo opalescens at 200 kHz.

11.2. MATERIALS AND METHODS

In this investigation the target strengths of eleven squid were measured in the dorsal aspect with a 200 kHz echo sounder. Their dorsal mantle lengths ranged from 45 to 160 mm. Eight squid were males and the remainder were not identified by sex. For two squid, measurements of target strength in other aspects were made to assess variations in target strength as related to position of the squid. Target strength measurements were taken in the ventral, anterior, and posterior aspects for a squid with a dorsal mantle length of 133 mm. Measurements were taken in the ventral, anterior, right lateral, and left lateral aspects for a 140 mm squid. Most target strength measurements were taken from the dorsal surface because L. opalescens will for the most part scatter sound in this aspect when detected with a downward facing transducer; and echo sounders with downward transducers have been used most often for research on and commercial fishing of this species.

Measurements of target strengths for L. opalescens were taken with a 200 kHz Japan Radio Company (No. NJA 320A) echo sounder with a 1.2 m sec pulse length and a pulse repetition rate of 200 times/min. A circular transducer 10.2 cm in diameter which possessed a piezoelectric barium titanate crystal was employed. A -3dB drop, or 50% loss, in sound intensity occurred at 7.5° from the axis of transmission for the transducer.

The study was conducted in a rectangular anechoic (without echo) tank 183 X 733 X 225 cm at the United States Naval Postgraduate School in Monterey, California, during August 1977 (Figure 56). The walls and bottom of the tank had been layered with SOAB cones in order to reduce extraneous sound scattering. The tank was filled with fresh water which was approximately 16 C.

Before target strength measurements were taken, the degree to which the incident sound intensity varied throughout the area of measurement, i.e., the amount the field was uniform, was determined. The echo sounder transducer was suspended in the water in the same location and position as it would be when taking target strength measurements of squid. A test hydrophone, consisting of a Navy E-8 transducer with a receiver voltage sensitivity of -214.7 dB reference 1 volt/micropascal, was used in conjunction with a Hewlett Packard 1220A oscilloscope to obtain voltage

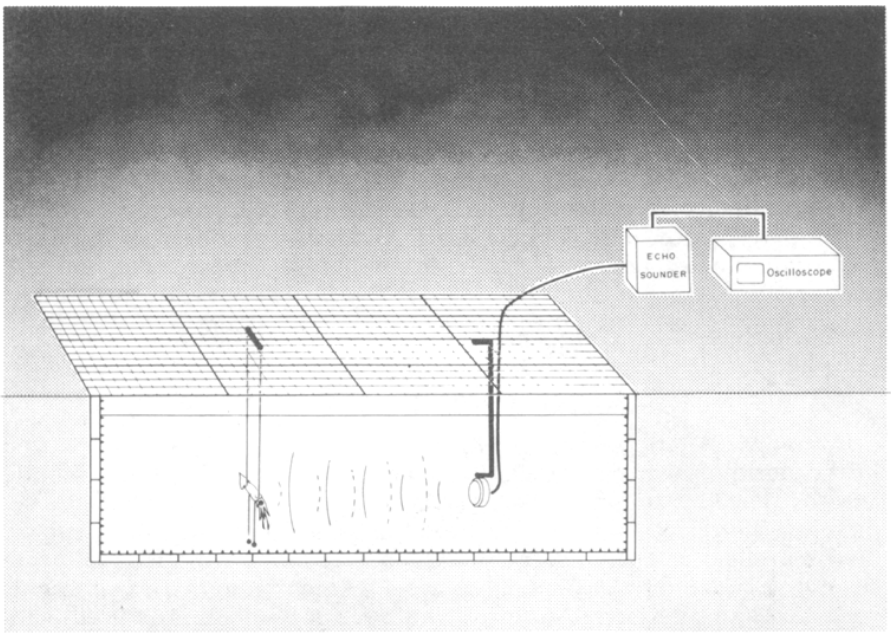


FIGURE 56. Underground side view of anechoic tank showing how squid and transducer were suspended when taking target strength measurements. Both squid and transducer drawn 3× larger than scale.

*FIGURE **56. Underground side view of anechoic tank showing how squid and transducer were suspended when taking target strength measurements. Both squid and transducer drawn 3X larger than scale.***

measurements throughout the area squid target strength measurements were to be taken (292 cm from the echo sounder transducer, in line with the center of the transducer). These readings were then converted to decibels using the following formula: Sound pressure level = 20 $\log_{10}$ E_{pp}/2 # 2 - (-214.7) dB reference 1 micropascal [1] where: E_{pp} = peak to peak voltage taken from the oscilloscope.

Sound intensity varied a maximum of 0.6 dB within the middle 200 mm from which L. opalescens target strength measurements were to be taken (Table 30). The largest squid used had a dorsal mantle length of 160 mm and a length from the beak anterior to the tip of the posterior apex of 180 mm. The tips of the tentacles of some squid did extend beyond 200 mm; however, because of the tentacles' small surface area and fleshy composition, it was assumed that these parts contributed little to the overall backscattering cross section of the entire squid. The sound field was considered to be uniform enough to make valid target strength measurements.

The midsection between the anterior of the beak and the posterior tip of the apex of each squid was lined up exactly with the acoustic axis of transmission of the transducer; the middle of the transducer and the middle of the midsection of each squid were both lowered 124 cm below the water surface of the anechoic tank and were spaced 292 cm apart (Figure 56). The farfield, i.e., the distance which is outside the maximum

TABLE 30
Horizontal Distance Between Transducer and Suspension Point * of Squid and Respective Sound Pressure Level **

Distance from transducer	*Left*			*Right*	
	20 cm	*10 cm*	*0*	*10 cm*	*20 cm*
292 cm	208.3	209.5	209.5	208.9	205.7
295 cm	–	–	209.5	–	–

* Left and right relative to facing test hydrophone; sound pressure level at 295 cm from transducer.
** In dB re 1 Pa.

*TABLE **30***
Horizontal Distance Between Transducer and Suspension Point of Squid and Respective Sound Pressure Level

range for source interference effects, for the sounder transducer was calculated to begin at 108 cm, i.e.

$$\pi a^2/\lambda = 108 \text{ cm} \quad [2]$$

where: a = radius of transducer (5.1 cm), and
λ = wave length (0.75 cm).

TABLE 30—Cont'd.

Consequently, all target strength measurements were taken in the farfield.

Target strength measurements were made on squid which were frozen immediately after death and then thawed shortly before use. After squid were suspended in the anechoic tank and before they were lowered to measurement position, they were gently shaken and squeezed to insure removal of all air bubbles, thus preventing erroneous target strength values caused by bubble scattering. Each squid was suspended on 4 lb test monofilament nylon line with size 12 fishing hooks used for attachment. Two lines hooked into the surface side of the squid and two into the opposite side. The two lines on the bottom side of the tank were weighted on the end most distal from the squid with ¾ inch steel nuts. Therefore, when the target strength of the dorsal aspect of a squid was measured, two lines strung from the surface were hooked into the left lateral side, and two weighted lines hooked into the right lateral side. The weights were suspended far enough from the axis of transmission not to cause any detectable backscatter. The sound scattering caused by the suspension apparatus (lines, hooks, and weights) without squid could not be detected above the background noise level; and this level was more than 35 dB lower than the lowest squid voltage reading. Background noise was considered negligible during the target strength measurements of L. opalescens.

Target strength measurements were derived by comparing voltage readings of L. opalescens with that of a reference which had a known backscattering cross section. The reference, a lead sphere 3.9 cm in diameter, was suspended in the same position as the squid were when measured. The backscattering cross section of a spherical reference (

EQUATION

) is given by Urick (1975) to be:

$$\sigma_{bs} = a^2/4 \text{ when } ka >> 1 \quad [3]$$

where: a = the radius of the reference in meters, and

$$k = 2\pi/\lambda \quad [4]$$

EQUATION

For the parameters in equations 3 and 4: a = 1.95 X 10^{-2}m, ka = 16.3 and is considered to be much greater than 1, and

$$\sigma_{bs}$$

EQUATION

of the reference = 9.5 X 10^{-5} m^2. The backscattering cross section of each squid measured was determined by the following ratio:

$$\sigma_{Pb}/\sigma_{L.o.} = e^2_{Pb}/e^2_{L.o.} \qquad [5]$$

EQUATION

where:

$$\sigma_{Pb}$$

EQUATION

= the backscattering cross section of the lead ball reference,

$$\sigma_{L.o.}$$

EQUATION

= the backscattering cross section of L. opalescens, e_{Pb} = the root mean square voltage of the reference, and e *L.o.* = the root mean square voltage of a single L. opalescens. Therefore, the target strength of L. opalescens is:

$$\text{T.S.} = 10 \log_{10} [\sigma_{Pb} \times e^2_{L.o.}/e^2_{Pb}] \text{ dB re 1 m}^2 \qquad [6]$$

EQUATION

where:

$$\sigma_{Pb}$$

EQUATION

is determined by equation 3, and e *L.O.* and e_{Pb} are determined from oscilloscope readings. The voltage reading caused by the scattering sound from the reference was taken several times throughout the study and consistently read as 1.7 $V_{p\text{-}p'}$ which is equivalent to 0.6 volts root mean square. Therefore, equation 6 becomes: target strength of L. opalescens = 10 $\log_{10}$ [9.5 X 10^{-5} m^2 X e^2 *L.o.* / (0.6 V_{RMS})^2] dB re 1 m^2 Linear regression equations were calculated for target strength as a function of length (dorsal mantle length, length from posterior tip of apex to anterior tip of beak, and total length) and weight (wet and dry). The regression coefficient for each linear regression equation was tested from zero by:

$$t_s = \frac{b - O}{s_b} \qquad [7]$$

EQUATION

where: t_s = the calculated student t value, b = the regression coefficient, and s_b = the standard error of the regression coefficient (Sokal and Rohlf, 1969).

11.3. RESULTS

The target strength measurements of L. opalescens in the dorsal aspect varied from a low of -49.3 dB to a high of -38.8 dB (Table 30). Squid with dorsal mantle lengths between 45 and 143 mm inclusive showed a trend of increasing target strengths correlated with increasing size; however, the three largest squid (dorsal mantle lengths of 145, 150, and 160 mm) produced the second, third, and fourth lowest readings (Table 31). No linear regression coefficient relating length or weight directly to target strength was significantly different from zero.

TABLE 31
The Length, Weight, Sex, and Respective Target Strength for the Dorsal Aspect of Individual *Loligo opalescens*

Dorsal mantle length (mm)	*Beak anterior to posterior tip of apex (mm)*	*Total length (mm)*	*Wet weight (grams)*	*Dry weight (grams)*	*Sex*	*Target strength (db re 1 m^2)*
45	60	100	2.5	0.6	?	−49.3
82	95	187	12.4	2.6	?	−43.2
85	100	185	14.4	2.8	?	−41.3
100	120	170	21.3	4.4	M	−39.3
133 •	160	270	42.3	8.5	M	−38.8
140 •	160	300	59.3	–	M	−39.7
140	155	260	41.3	7.1	M	−41.3
143	160	270	37.6	7.8	M	−38.8
145	160	270	58.5	10.6	M	−46.8
150	170	290	60.0	11.6	M	−44.0
160	180	285	59.0	12.3	M	−44.0

• Squid that were measured in other aspects besides dorsal.

TABLE 31

The Length, Weight, Sex, and Respective Target Strength for the Dorsal Aspect of Individual Loligo opalescens

Changes in the aspect of individual market squid which received incident sound created fluctuations in target strength values. A squid with a dorsal mantle length of 133 mm produced the following target strength measurements: dorsal aspect -38.8 dB, ventral -40.7 dB, anterior -44.8 dB, posterior -49.3 dB; and a squid with a dorsal mantle length of 140 mm produced measurements of: dorsal aspect -39.7 dB, ventral -41.9 dB, anterior -48.6 dB, right lateral -45.7 dB, and left lateral -41.9 dB.

11.4. DISCUSSION

Different target strength values were recorded as the size of squid was varied. However, no particular relationship between target strength and size became apparent. The three largest squid in this study produced the second, third, and fourth smallest target strengths. Smith (1954) also observed that a smaller squid (19.4 in^2) made a larger target strength measurement than a larger squid (30.2 in^2) at 14 kHz. In both experiments interference effects as described by Love (1971b) could have reduced the amplitude of the reflected sound for the larger squid. When sound energy traveling in a plane wave within a water medium arrives at a boundary of a finite medium which has a different acoustic impedance, some energy will be reflected toward the first boundary, where again the first boundary will reflect some energy and transmit the rest toward the original source. This process will continue for all boundaries encountered by the original sound wave until a steady state is initiated. In the interference region, the resultant amplitude of energy from all boundaries is the vectoral summation of all the component waves. Therefore the phases of these component waves can either increase or decrease the amplitude of the initial scattered sound energy. The correspondence of phases is accommodated by the thickness of the finite layers and the size of the wavelength. The interference region has never been determined for squid; however, Love (1971b) has calculated it for fish to be:

$$0.7 \leq L/\lambda \leq 200 \qquad [8]$$

EQUATION

where: L = the fish length, and [^] = the wave length. If equation 8 applies to squid and L = the length of squid from beak anterior to posterior apex, then all squid measured during this study were within the interference region. The smallest squid would have an L/[^] of 6 and the largest 24. In the interference region, a graph relating target strength to size would reveal a general trend toward increasing target strength related to an increase in size; nevertheless, a connecting line drawn from the various plots would show many dips and peaks.

Another explanation which could account for obscuring a functional relationship between squid size and target strength is that larger squid were possibly suspended in a manner which reduced backscattering. Squid, unlike most fish, are very flexible. It is not possible to ascertain that every squid was suspended with the aspect first exposed to incident sound to be completely straight for measurements. Slight bends which could possibly alter the backscattering cross sections may have existed; also the depth of the squid body being measured could be slightly altered with slight shifts in the suspensatory apparatus attached to the squid.

Target strength values varied with an alteration of aspect insonified. For the two male squid measured in different aspects, the dorsal surface provided the maximum target strength values. Unique to the dorsal surface, immediately internal to the dorsal mantle of L. opalescens, lies the chitonous gladius (pen) which probably contributes considerably to the overall scattering cross section of the animal. The ventral aspect produced the second highest target strength value. The difference between the target strength in the dorsal and ventral aspects was similar for both squid; both had values which were about 2 dB greater in the dorsal aspect. For the 133 mm squid, the minimum target strength (-49.3 dB) came from the posterior aspect. This aspect has the least surface area exposed to create a backscattering cross section. The right lateral aspect was 3.8 dB less in target strength than the left lateral aspect of the squid with a dorsal mantle length of 140 mm. The asymmetry of the visceral mass within the male L. opalescens is possibly responsible for this difference in measurements among the lateral sides. For example, in male market squid, the stomach is located on the right and the spermatophoric organ on the left. Since the lateral aspects of only one squid were measured, other possible explanations are that one side of the squid was suspended in a more optimal way to scatter sound back toward the transducer, and/or the difference in sound scattered between the two lateral aspects was unique only to the one squid being measured.

Since aspect does influence target strength of squid, any attempt to quantify squid schools *in situ* using acoustics should be done by insonifying the same aspect of individuals within schools as much as possible. If more than one aspect is being insonified, it will be difficult to derive a single target strength value representative of the mean target strength of individuals in a squid school sampled. L. opalescens has been observed to orient in water for the most part parallel to the horizontal plane with its dorsal surface facing the surface of the water. Therefore a vertical sounding echo sounder will insonify mainly the dorsal aspect making acoustic

quantification of squid abundance possible. Side scanning sonar, which will insonify squid in the anterior, posterior, and both lateral aspects, might not be effective in determining squid abundance. If these aspects for individual L. opalescens do not have significantly different target strength values, quantification of fish schools using this technique could produce substantially inaccurate results. Further complications associated with side scanning sonar arise from difficulties in determining sampling volume and directivity. An acoustic wave traveling horizontally in water will sometimes bend, depending upon thermal conditions of the water column (Forbes and Nakken, 1972). The range of detection and target strength for L. opalescens measured in the field would be more difficult to determine using side scanning sonar than a vertical sounding.

Acoustic sampling of L. opalescens is probably feasible only when these squid are in dense stationary aggregations; otherwise, identification of squid acoustic targets is impractical. Squid are known to exhibit such schooling behavior during spawning. Spawning schools will produce overlapping echoes for most conventional echo sounders. For example, targets insonified with a pulse of 1.2 m sec (a moderately short pulse) will produce overlapping echoes if they are spaced less than 90 cm from each other. I have observed, while diving, individuals in spawning squid schools to be less than 20 cm apart. Therefore, in order to process acoustic signals electronically, an echo integrator, opposed to an echo counter, must be used. An echo integrator can sum up overlapping echoes for a chosen depth range and determine density for marine organisms in numbers or biomass (Clay and Medwin, 1977). In order to make this evaluation, a target strength representing a known density is programmed into the integrator system, e.g. -33 dB/Kg was the value used for herring stock assessment in southeastern Alaskan waters (Thorne, 1976). Probably the optimum integrator value to use for L. opalescens (until more data are collected) would be the mean target strength from all the squid measured in this study large enough to occur in a spawning aggregation. Mature L. opalescens found in spawning aggregations have minimum dorsal mantle lengths of 72 mm for males and 81 mm for females (Fields, 1965). The mean target strength of the 10 L. opalescens in this study large enough to occur in a spawning aggregation is -41.1 dB. Therefore, upon surveying a squid spawning ground using a 200 kHz echo sounder and an echo integrator, a value of a -41.1 dB per squid could be used to give a gross estimation of numbers of L. opalescens until more experimentation can determine a more accurate value.

Other research on the target strength of squid has been carried out by Smith (1954) and Matsui, Teramoto, and Kaneko (1972). Smith used frequencies from 8 to 31 kHz on two squid of 30.2 in^2 and 19.4 in^2. For the larger squid, the target strength varied from a high of about -39 dB at 11 kHz to a low of about -43 dB at 32 kHz; and for the small squid, values varied from a high of about -40 dB at 11 kHz to a low of -49 dB at 30 kHz.

L. opalescens ranged in surface area from about 3 in^2 (45 mm dorsal mantle length) to 26 in^2 (160 mm dorsal mantle length) in my study. It is difficult to make comparisons between Smith's and my studies because

Smith made no indication of which aspect received the incident sound, length, weight, or even the species of squid used. In addition, measurements were taken over a less uniform field and frequencies used were much lower. The study by Matsui *et al* (1972) measured the target strength of a squid, Doryteuthis bleakeri, with a dorsal mantle length of 129 mm, in several aspects. They concluded that maximum target strengths were given in the dorsal-ventral roll plane. "The maximum target strength was about -45 dB at 50 kHz and -42 dB at 200 kHz. Two squid in my study produced target strengths similar to -42 dB. Both had target strengths of -41.3 dB and their dorsal mantle lengths were 85 mm and 140 mm.

The measurements of target strength of L. opalescens under laboratory conditions should be representative of individuals of this species *in situ* provided that the dead squid used in this experiment can be assumed to have the same target strength as live squid. This assumption is probably valid enough not to cause significant error. Squid, unlike many species of fish, do not have air bladders that may change in volume and/or gas composition when dead. The tissue of L. opalescens does not appear to go through any immediate chemical or structural changes that could interfere with its acoustic properties upon death or upon being frozen; when squid were thawed for measurement, the mantle tissue had the same apparent constituency (not flabby or soft) as fresh squid. The tissues that probably yield the most sound scatterings are the hard parts, the chitonous beaks and gladius which should be affected least by freezing, thawing, or death. Consequently, even if the mantle and visceral tissues from L. opalescens did undergo some changes in their acoustic properties upon death and subsequent freezing and thawing, these changes were probably insignificant compared to the overall scattering of the organism.

Before my target strength values can be used to make accurate density estimations, an additional assumption must be made that the target strength of a school of squid can be related to a multiple of the target strength of an individual squid. This assumption is much more difficult to assess. The upper layer of a school of squid could produce a shadowing effect from the incident sound on the lower layer, as witnessed by Rottingen (1976) for live saithe, Pollachius virens, and sprat, Sprattus sprattus. It is important to realize that the output of an integrator system may not be directly related to squid density when this density is above a certain threshold. Rottingen proposes that the effects of shadowing may be overcome by an application of the theory of multiple scattering. Certainly what must be done before an integrator system can be used for abundance estimations is to determine if shadowing effects exist, and if so, the extent of their error on the estimation of market squid school density.

Knowledge of target strengths for Loligo opalescens will aid further acoustic studies of this species because:

1] The minimum source level needed by an echo sounder required to detect an individual market squid can now be calculated (provided ocean noise level can be quantified). For example, the echo level received at the face of the transducer caused by an acoustic target must be above -20 dB ref 1 micropascal for detection. A squid in line with the axis of transmission

at 100 m depth with a target strength of -41.1 dB must be detected with a 200 kHz echo sounder which has a source level (at 1 m) above 115 dB re 1[u]Pa (Vaughan, 1978).

2] Individual L. opalescens can be detected with a 200 kHz echo sounder. Echograms have contained traces caused by squid aggregations but until this study, the ability to detect individuals was never before ascertained.

3] When conducting an acoustic survey, it is of value to know that the target strength of individual L. opalescens varies with the aspect exposed to incident sound. For accurate density estimation when recording *in situ* target strengths the aspect insonified must be known and kept constant (such as in vertical sounding).

4] Target strength values for market squid are now available for use with integrator strength values for market squid are now available for use in integrator systems. A range of values now exists for various sizes of market squid. Until more information on the target strength of market squid is available, especially data which determine the effects of density upon the amplitude of the return echo, the present target strengths may be used in estimating squid abundance with echo integrators.

12. CORRELATIONS BETWEEN SQUID CATCHES AND OCEANOGRAPHIC CONDITIONS IN MONTEREY BAY, CALIFORNIA

RODNEY R. McINNIS*
and
WILLIAM W. BROENKOW
Moss Landing Marine Laboratories
Moss Landing, California

12.1. INTRODUCTION

In southern California mass spawning of market squid begins in December, while near Monterey spawning begins in April and continues through November. Fields (1965) reported that egg masses were found in Monterey Bay throughout the year, indicating the presence of mature squid is not limited to a particular season. Eggs can be found from just below the intertidal zone to a lower limit of at least 180 m (Kato and Hardwick, 1975).

In Monterey Bay fishermen exploit spawning squid aggregations using lampara nets. The Monterey fleet rarely ventures more than 5 km from harbor in search of squid, since the spawning grounds appear to be concentrated in the southern bay (Figure 57) where the predominantly sand bottom slopes gently to 60 fm at the edge of the Monterey Submarine Canyon.

Between 1960 and 1974 the total Monterey market squid catch ranged from a high of 7,440 tons in 1971 to a low of 544 tons in 1973 (McInnis, 1976). No change in the market coincided with the Monterey squid fishery's collapse in 1973; therefore, other factors must have been involved.

Failure of the fishery may have been related to changes in the physical and chemical environment which can be expected to vary with time scales from a single tidal cycle to seasons, possibly to glacial epochs. Parallel variations in the distribution, reproduction, and survival of squid or their prey items might be expected. Thus it was the purpose of this study to examine statistically the relation between squid landings in Monterey Bay and available environmental data to understand better the fishery variations.

* * Present Address: Pacific Fishery Management Council
Portland, Oregon

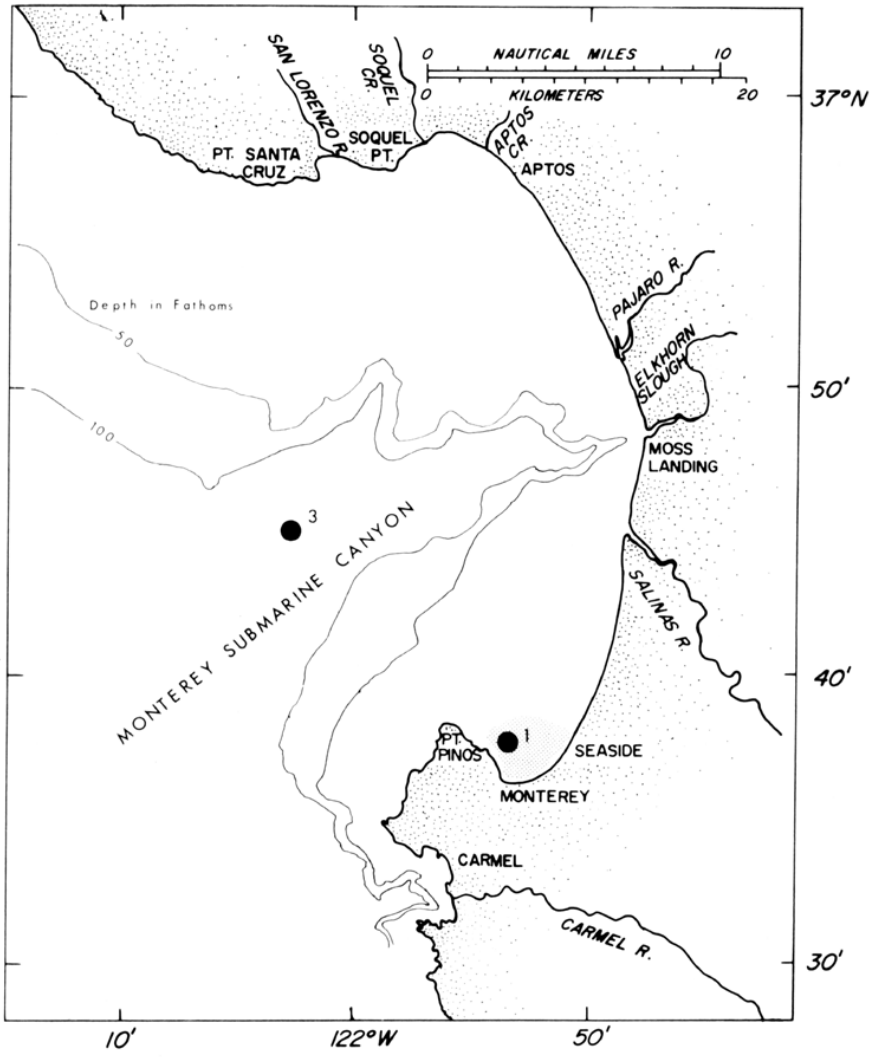


FIGURE 57. Monterey Bay region showing traditional squid fishing grounds (shaded) and CalCOFI Stations 1 and 3.

*FIGURE **57. Monterey Bay region showing traditional squid fishing grounds (shaded) and CalCOFI Stations 1 and 3.***

12.2. METHODS

Squid catch and effort data were compiled from delivery receipt summaries (J. E. Hardwick, California Department of Fish and Game). These annual summaries record the weight, price per ton, and date of delivery. Although records of total catch have been collected since 1911, delivery receipts are not retrievable for years prior to 1960. Catch per unit of effort was extracted from these data by dividing the total weight of daily squid sales by the number of boats that delivered to the processors on that day.

One boat may have apportioned its catch among two or more buyers or may have received different prices for portions of the same catch from one wholesaler. Each of these transactions was recorded separately. Consequently a boat may have shown several deliveries for only 1 day's fishing. Therefore all sales recorded on the same day are considered the product of 1 day's fishing effort. Catch per delivery day differs from other catch per effort indices in that this makes no claim to standardize a fishing day, which is impossible with the available data. Nevertheless, this effort index does reflect the fishing pressure on squid, because the composition of the fleet did not change significantly between 1960 and 1974. Delivery receipts provide no record of actual zero catch per effort, because fishing effort may or may not have been expended during periods when no deliveries were reported. Thus zero catch per delivery day values were not considered in our calculations.

The oceanography of Monterey Bay has been studied extensively, and data exist for many parameters. Available data include large scale and local wind stress indices and hydrographic observations. These records vary in length, sampling interval, and completeness, and only some were useful for the purposes of this study.

Variation in the wind field causes hydrographic changes through upwelling and changes in the California Current. One measure of the large scale wind stress is Bakun's (1973, 1975) upwelling index, which was calculated from atmospheric pressure gradients over a grid, 3° latitude by 3° longitude. The index represents the offshore Ekman transport per length of coastline. Monthly means of the upwelling index at 36° N 122° W from 1960 to 1973 were used in this study, and were based upon surface vessel reports at 6 hour intervals. Daily values are available for 1974 (Bakun, 1975), but these were computed differently from previous upwelling indices and do not give comparable results. Thus, only upwelling indices from 1960 to 1973 were used here.

The oceanographic climate of Monterey Bay has been monitored under grants from California Cooperative Oceanic Fisheries Investigations (CalCOFI) since 1950 (Hopkins Marine Station, 1958 to 1974; Broenkow, Lasley, and Schrader, 1975). An array of hydrographic stations in the bay has been sampled on a monthly or biweekly basis for temperature, salinity, dissolved oxygen, and nutrient concentrations. Completeness of these data vary. Temperature records are most complete, and as such are the most useful for the purposes of this study. A bathythermograph was used at all stations from 1954 to 1968, and during this time very few water samples were taken for further analyses. As a result, dissolved oxygen and nutrient concentrations were not available prior to 1968, and salinity data were reported from only two depths at each station. Surface to 10 m average salinity values varied over a narrow range, 32.5 to [%], and salinity was not considered a likely factor to influence the squid fishery. Temperature data from CalCOFI Monterey Bay Stations 1 and 3 (Figure 57) were used for this study, because Station 1 was located on the traditional spawning grounds and Station 2 reflects offshore conditions as described by Lynn (1967).

Monthly mean values were used in our analysis to provide data sequences with identical sampling frequencies. The use of means unavoidably compromises short term detail. To eliminate the large annual harmonic, which tended to obscure year to year variations in time series with strong seasonality, anomalies were computed as the differences between the monthly data and their 15 year (1960 to 1974) means. Cross-spectra analyses and cross correlation analyses were attempted. Cross-spectra analysis (Wastler, 1969), was invalidated by substituting zeros for missing catch-per-effort data. A cross correlation technique, similar to that used by Botsford and Wickham (1975) who investigated the relationship between upwelling index and Dungeness crab catch, provided a means of assessing the covariance of squid catch per effort with the selected environmental data. This method was useful when data were missing from the time series. The product moment correlation coefficient was calculated as

$$r_{xy}(i) = \frac{1}{n-1} \sum_{j=1}^{j=n-i} \frac{X(j)\ Y(j+i)}{S_x S_y} ,$$

EQUATION

where: r_{xy} (i) = the correlation coefficient between processes X and Y at lags of i intervals, X_j and Y_j = the values of processes X and Y at time j, S_x and S_y = the standard deviations of processes X and Y, i = the number of intervals lagged, and n = the total number of samples. In the calculation of r_{xy} (i), data pairs with zero values of catch per delivery day were not included. The statistical significance of the correlation coefficients, r_{xy}, was determined by the t-test (Rohlf and Sokal, 1969),

$$t = \frac{r\ (n-2)}{(1-r^2)} ,$$

EQUATION

where: n = the total number of samples minus the number of intervals lagged.

The 0.01 level of significance was selected to reduce the risk of accepting a random relationship as significant. When computing correlation coefficients at 20 or more lags, the 0.05 significance level is insufficient.

12.3. RESULTS

Monthly catch totals for 1960 to 1974 (Figure 58A) show yearly peaks in June or July. These peak catches seem to climb steadily from 1960 to 1967 and then slowly decrease through 1970. This pattern suggests a cycle of squid availability with a period of 14 to 16 years. The exceptionally good landings in 1971 and 1972 broke from this hypothesized pattern and indicated the existence of more complex cycles or a periodic phenomena. The sudden and nearly complete disappearance of squid from the Monterey fishing grounds in 1973 was reflected in the catch records of that year; yet the fishery recovered in 1974 to approximately the 1972 level.

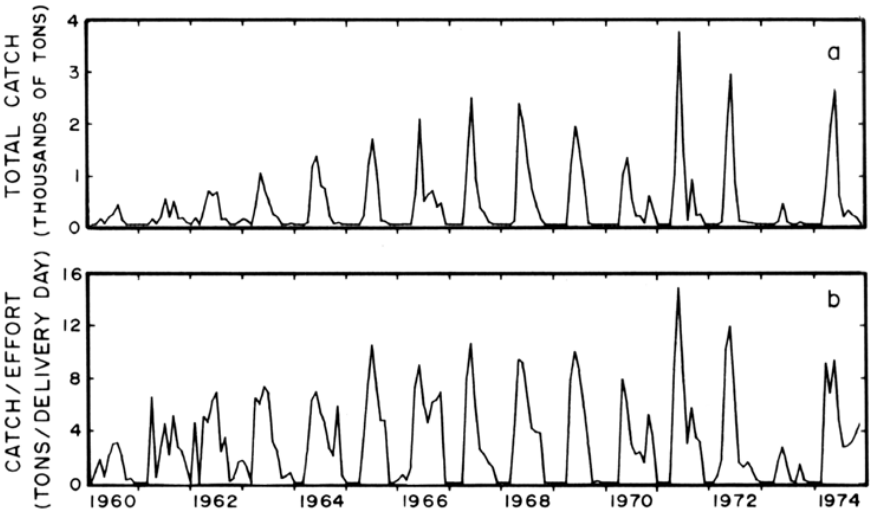


FIGURE 58. Squid landings at Monterey. A. Monthly total catch. B. Monthly catch-per-delivery day.

*FIGURE **58. Squid landings at Monterey. A. Monthly total catch. B. Monthly catch-per-delivery day.***

Mean monthly catch per effort (Figure 58B) tends to broaden the seasonal peaks and suggests relatively high availability in months other than May, June, and July. Another effect of this index is to decrease the image of a cyclic fishery. However, the disastrous proportions of the 1973 season are not obscured.

The monthly mean 10 m temperature at Station 3 (Figure 59A) exhibits a strong annual cycle. Anomalies from the 15 year average value for each month (Figure 59B) more clearly demonstrate the variability of Monterey Bay water temperature from year to year (McInnis, 1976). Prolonged periods of anomalously cold water occurred in summer and fall of 1962, most of 1971, spring of 1972, and the winter of 1973–74.

The correlations of 10 m temperature at Station 3 and squid catch per effort at several lag intervals (Figure 60) reflect the 12 month periodicity of both temperature and squid catch (Figure 61). Both series exhibit strong annual cycles which tend to mask year-to-year covariation. The 15 year (1960 to 1974) monthly averages for each of these two series show annual cycles and their temporal relationship (Figure 59). Squid catch per effort quickly peaks 1 month after the coldest temperatures and slowly decreases as summer warming occurs in the bay.

The use of temperature anomalies eliminated the annual harmonic, revealing that the correlation coefficient between 10 m temperature anomalies at Station 3 and squid catch per delivery day is highly significant [p 0.001; R = 0.38; n = 115 for an 18 month lag (Figure 62)]. Significant correlations (p 0.01) were not found at any other lag intervals, with the exception of adjacent lags at 17 and 19 months.

Other results tend to support this relationship of catch to temperature; however, they so closely parallel those presented that to include the details of each cross correlation would serve no purpose. The maximum correlaton coefficients between catch per effort and the series of environmental

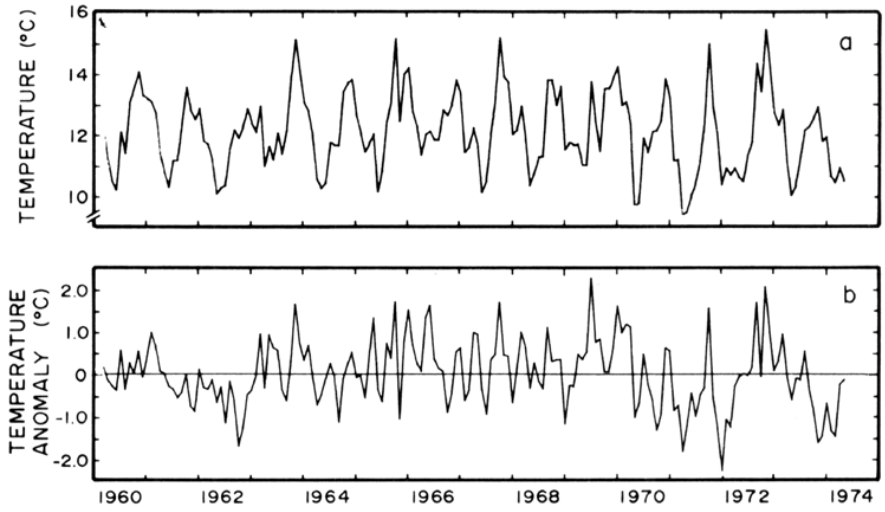


FIGURE 59. Temperature data from CalCOFI Station 3. A. Monthly 10 m Averages. B. Anomaly from 15 year (1960–1974) mean 10 m temperature values at Station 3.

*FIGURE **59. Temperature data from CalCOFI Station 3. A. Monthly 10 m Averages. B. Anomaly from 15 year (1960–1974) mean 10 m temperature values at Station 3.***

anomalies are summarized (Table 32). Significant correlations (p 0.01) occur only between subsurface temperature anomaly and catch per effort, and only at a lag of 18 months (Table 32). Surface temperature anomalies and upwelling index anomalies show no correlation coefficients which differ significantly from zero.

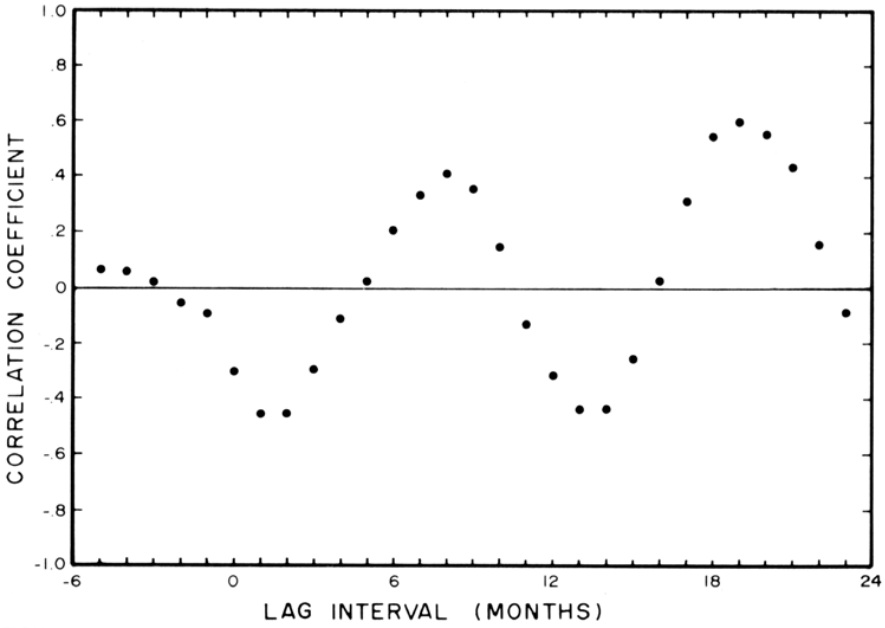


FIGURE 60. Correlation coefficients between monthly mean 10 m temperature at CalCOFI Station 3 and the Monterey squid fleet's catch-per-delivery day.

*FIGURE **60. Correlation coefficients between monthly mean 10 m temperature at CalCOFI Station 3 and the Monterey squid fleet's catch-per-delivery day.***

12.4. DISCUSSION

Aside from economic pressures, squid catch reflects two factors. One of these, stock abundance, involves long term fluctuations in the population which may be brought on by environmental changes. The second is the availability of squid to the fishermen. Many things can be detrimental to

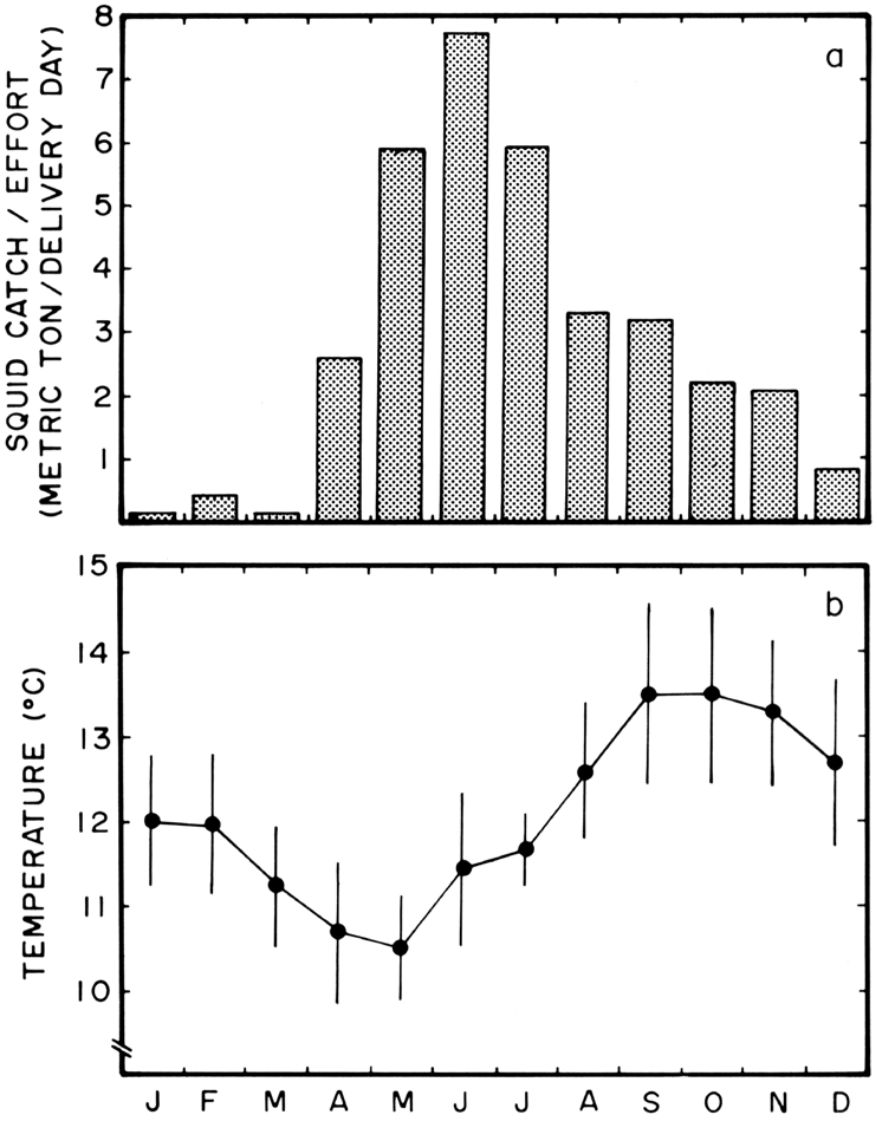


FIGURE 61. Monthly means 1960 through 1974. A. Squid catch-per-effort at Monterey. B. Monthly 10 m temperature at CalCOFI Station 3. Vertical lines indicate standard deviations of temperature means.

*FIGURE **61. Monthly means 1960 through 1974. A. Squid catch-per-effort at Monterey. B. Monthly 10 m temperature at CalCOFI Station 3. Vertical lines indicate standard deviations of temperature means.***

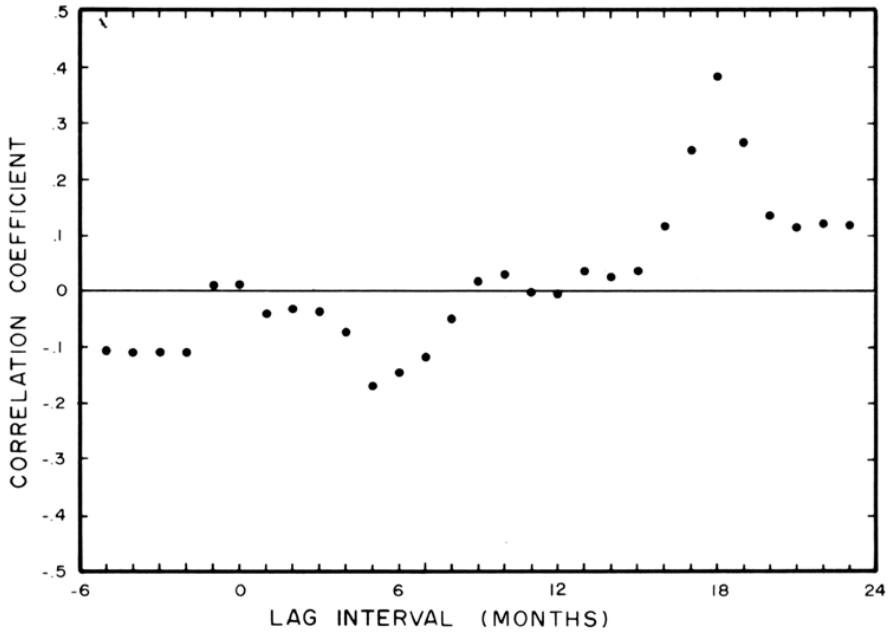


FIGURE 62. Correlation coefficients between monthly mean 10 m temperature anomalies at CalCOFI Station 3 and the Monterey squid fleet's monthly catch-per-delivery day.

*FIGURE **62. Correlation coefficients between monthly mean 10 m temperature anomalies at CalCOFI Station 3 and the Monterey squid fleet's monthly catch-per-delivery day.***

TABLE 32
Maximum Correlation Coefficients Between Data Anomaly Series and Squid Catch-Per-Delivery Day

Series	*Depth (m)*	r_{xy}	*n*	*Lag (months)*
Temperature Station 1	0	0.23	119	12
	10	0.28**	115	18
	30	0.27**	115	18
Temperature	0	–0.22	119	15
	10	0.38**+	115	18
Upwelling Index*		0.22	118	5

* Calculated at 36° N 122° W (based on 14 years data, 1960–1973)
** Correlation coefficient significant to $p < 0.01$
\+ Correlation coefficient significant to $p < 0.001$

*TABLE **32***

Maximum Correlation Coefficients Between Data Anomaly Series and Squid Catch-Per-Delivery Day

the success of fishing even an abundant stock. Movement of the spawning grounds, upon which the Monterey fleet depends, can account for a poor squid catch. Causes for a modification in spawning behavior may include long term changes such as the prolonged discharge of sewage or short term changes such as dumping dredge spoils on the traditional spawning grounds. Natural causes may also exist. For example, anomalous winds can alter the circulation and flushing time of the bay and make the southern bight temporarily incompatible with the as yet unknown requirements of spawning L. opalescens. The recovery of Monterey's squid fishery in 1974

from the poor previous year leads to speculation that short term adverse conditions led to the 1973 failure of this fishery rather than a general decay of water quality in the bay.

Seasonal temperature and salinity cycles in Monterey Bay have been described by many researchers since 1936 (Skogsberg, 1936; Skogsberg and Phelps, 1946; Bolin and Abbott, 1963). Upwelling normally begins in February and continues through early summer along the central coast of California. Onset of upwelling coincides with the cool water temperatures of March, April, and May as seen in the 15 year monthly mean 10 m temperatures at Station 3 (Figure 59). During this period prevailing northwesterly winds drive California Current water offshore by Ekman transport, allowing deeper, cool, nutrient-rich waters to surface along the coast. The cessation of northwesterly winds in the summer reduces the offshore transport, and relatively warm, low salinity California Current water moves onshore. High temperatures in September and October (Figure 59) reflect this condition. The California Countercurrent surfaces from November to February (Reid, Gunnar, and Wyllie, 1958; Reid and Schwartzlose, 1962; Wickham, 1975). The strength and timing of this countercurrent, known as the Davidson Current when it surfaces, are variable from year to year.

Peak squid catch follows the minimum temperatures by approximately 2 months on the long term average (Figures 58 and 59). The presence of squid in great numbers on the spawning grounds seems to be tied to the warming which follows cessation of upwelling. Spawning may be triggered, in fact, by this warming trend. The optic gland, which regulates the rapid maturing of male squid gonads and sperm production, may be influenced by temperature trends as well as by the length of daylight (T.M. Grieb, San Francisco State University, pers. commun.). Thus, the well known seasonality of oceanographic conditions correlates very well with the seasonal availability of market squid in the bay (Figure 61). The real question is: what causes year-to-year variations in squid abundance?

If spawning indeed is precipitated by warming trends, then sporadic episodes of upwelling may cause spawning at times other than in May, June, and July when the fleet concentrates its effort. Broenkow and Smethie have shown that in Monterey Bay episodes of strong northwesterly wind, as brief as 2 days, can induce local upwelling even in December. Because winds of such large magnitude and small temporal and spatial scales affect temperatures in the bay, Bakun's upwelling index is not perfectly suited to the purposes of this study. Therefore direct measurements of temperature which better characterize the oceanographic climate of Monterey Bay are preferred.

Year-to-year variations in the water temperature of the bay as demonstrated by temperature anomalies (Figure 59) correlated with squid catch-per-delivery day at a lag of 18 months (Figure 62). The positive sign of this correlation coefficient indicates that positive temperature anomalies precede good squid landings by 18 months. Conversely, poor squid catch followed periods of anomalously low temperatures. Monterey's annual total squid landings (Fields, 1965) and Bakun's (1973) upwelling indices from 1946 to 1959 generally support this relationship. Early years

(1946 to 1953) showed no distinct relationship between upwelling index and squid landings. However, a pattern seems to emerge since high upwelling indices, which imply low water temperature, occurred in 1955 and 1956 and in 1959 followed by poor squid catches in 1958 and 1960. Relatively low upwelling indices in 1953 and 1958 preceded good catches in 1955 and 1959. These observations do not carry the statistical weight of the more detailed analysis presented earlier, but they are in qualitiative agreement.

The time lag of 18 months between temperature and squid catch is consistent with age estimates for market squid. Estimates of the spawning age of L. opalescens range from 3 years for males and 2 years for females based on size frequency (Fields, 1965) to 2 years for both sexes based on aging of statocysts as well as size frequency studies (J.D. Spratt, California Department of Fish and Game, pers. commun.). Since squid die after spawning (Fields, 1965), this suggests that the mechanism which links temperature to stock abundance acts upon juvenile squid.

McMahon and Summers (1971) demonstrated that low temperatures slow the development in egg masses of L. pealei, and similar results have been found for L. opalescens. L. opalescens egg capsules deposited in an aquarium and maintained at 16 C hatched at a mantle length of 2.5 mm after 3 to 4 weeks (Fields, 1965). Freshly deposited egg cases kept at 13.6 C hatched at a mantle length of 2.5 mm after 30 to 35 days (McGowan, 1954). If this pattern of temperature dependent development persists beyond hatching, anomalously low temperatures would lead to prolonged juvenile stages. Higher mortality in these protracted subadult stages due to predation, for example, would contribute to poor recruitment to the fishable stock.

It is impossible to conclude that other mechanisms may not be responsible for the covariance between squid landings and temperatures in Monterey Bay. For example, food items of the juvenile L. opalescens may be adversely affected by unseasonably low termperatures. The details of the apparent temperature to squid relationship must await further study. However, since a substantial portion of the variation in squid catch can be accounted for by anomalies from the mean annual temperature cycle, this may represent an important step toward providing a predictive index of squid availability.

13. SUMMARY OF MARKET SQUID RESEARCH PROGRAM

HERBERT W. FREY
California Department of Fish and Game
Long Beach, California
and
CONRAD W. RECKSIEK
Moss Landing Marine Laboratories
Moss Landing, California

The Squid Research Program was very ambitious in the number of problems it tried to solve. As might be anticipated, the research fell short of reaching the stated objectives in a few research areas; however, considerable progress was made in the major portion of the program.

The spermatogensis and oogenesis study conclusively demonstrated that Loligo opalescens is involved in only one spawning period. For the purposes of fishery management, market squid may be considered terminal spawners.

Growth/age studies were a little less conclusive. The statolith was found to exhibit "smaller" growth rings early in the squids' life and "larger" rings in the later life periods. These studies indicate that market squid are capable of spawning at 1 year of age and all will spawn during their second year of life.

Predator studies indicate Loligo opalescens is an active, visual predator, feeding mainly during mid-day on euphausiids and other crustacea, has a rapid digestion rate, and consumes at least 14% of its total biomass daily. Prey studies clearly demonstrate that the market squid, the northern anchovy, rockfish, and euphausiids constitute the major prey species for many marine fishes, birds, and mammals in Monterey Bay. While it is certain that latitudinally, north and south, changes in food habits (largely as species substitutes) will be encountered as faunal components change, the central focal emphasis will be on these four groups of prey.

The market squid is very abundant in the waters of the California Current, and since it is a major food source for many other marine predators, it must play an important role as a vital link between zooplankton and higher trophic levels in this pelagic environment.

Population studies indicate there may be more than one stock of market squid; however, at the present time the data are too sparse to make a conclusive determination.

Some aspects of squid behavior were determined during the acoustic studies. Target strengths were ascertained which will be useful in future acoustical work. The use of acoustics by themselves may be of limited value in estimating nearshore spawning biomass; nevertheless, they may be useful in concert with other activities and equipment in accomplishing this task. Acoustics may be quite suitable for estimating the biomass of

squid during their pelagic phase. While the investigation had hoped to test this possibility, events deemed otherwise.

A relationship between temperature anomalies and catch-per-delivery day at a lag of 18 months was detected in Monterey Bay. A warmer than usual water temperature preceded good squid landings; conversely, poor squid catches followed periods of anomalously low temperatures, while it is impossible to conclude that other mechanisms may not be responsible for this apparent relationship, this may represent an important step towards providing a predictive index of market squid availability.

There are several areas where further study is needed. Additional basic data are needed to ascertain the population structure of Loligo opalescens. The techniques are developed, but additional sampling is necessary. More work is needed in developing, designing, and conducting a survey to estimate the market squid biomass.

The Market Squid Research Program is an example of a multi-funded, multi-discipline, and multi-organizational study. The funding came from a number of sources: the Marine Research Committee, the State of California (Tidelands oil revenues), the basic funding by Moss Landing Marine Laboratories, and the California Department of Fish and Game (salaries, vessels, equipment, etc.), and the University of California Sea Grant Program. Scientists in the fields of ecology, physical oceanography, invertebrate and vertebrate biology, chemistry, genetics, fishery management, ornithology, and systematics were involved in the research. Organizations representing resource management and academic institutions were involved, and an advisory panel was composed of members of the fishing industry, state and federal government, and the scientific community.

This research program represents the first large scale, multi-discipline approach to solving problems involved with the cephalopod resources of the world. The synergistic effect of scientists from different disciplines working together was a factor in the progress made by most of the projects.

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