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Title: Ontogeny and Metamorphosis in the Dover sole (Microstomus pacificus): A Description of the Early Life History Stages, with Comments on the Sister-group Relationship between Microstomus and Embassichthys.

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The early life history stages and metamorphosis in Dover sole (Microstomus pacificus) are described from 201 larvae and juveniles. Unlike other pleuronectids, with the possible exception of the deep-sea sole (Embassichthys bathybius), initial migration of the left eye is not associated with metamorphosis or transition from plankton to benthos. The left eye exhibits a stasis during its migration, so that premetamorphic larvae are optically asymmetrical while planktonic. Seven characters are used to quantify the progress of metamorphosis: completed eye migration, dorsal fin position, dentition, pectoral fin development, condition of posterior process of coracoid, pigmentation, and elongation of the intestine into a secondary body cavity. Other characters associated with metamorphosis are reduction in body depth associated with reductions in lengths of neural and hemal spines and associated dorsal- and anal- fin pterygiophores, decreasing interorbital distance, increasing right eye diameter and right premaxilla length,

and development of body scales. Metamorphosis appears to require 9-11 or 12 months to complete. The planktonic period of premetamorphic and metamorphic larvae is about 18-24 months. Metamorphic larvae appear to move inshore into shallower water as metamorphosis progresses. Settlement occurs from about December to April, primarily from January to March.

One of the earliest phylogenetic hypotheses about the intergeneric relationships of the pleuronectid genera Microstomus, Embassichthys, Glyptocephalus, and Tanakius was by Norman (1934), who considered Microstomus plus Embassichthys and Glyptocephalus plus Tanakius to be sister groups. An alternative hypothesis by Richardson (1981) suggested that Embassichthys larvae resembled larvae of Glyptocephalus and Tanakius based on a "leptocephalus-like" body shape, and that Microstomus was the primitive sister group of these three genera because of its dorsoventrally deepened body shape.

In order to evaluate Richardson's hypothesis of sister group relationships based on body shape, I examined morphological characters associated with eye migration and metamorphosis, and changes in body shape during ontogeny from post-flexion larvae through benthic juveniles. Richardson's character of a moderate "leptocephalus-like" body shape is an artifact of the paucity and small size range of larvae available for her examination. The body shape of larger Embassichthys larvae is also dorsoventrally deepened and the larvae appear to undergo changes in body shape during metamorphosis similar to Microstomus. Three synapomorphies that support Norman's hypothesis of an Embassichthys plus Microstomus

sister group relationship are dissociation of initial eye migration with metamorphosis, a stasis during eye migration in premetamorphic larvae, and a retention of a larval-like appearance to the eyes of newly settled specimens.

Ontogeny and Metamorphosis in the Dover sole (*Microstomus pacificus*): A Description of the Early Life History Stages, with Comments on the Sister-group Relationship between *Microstomus* and *Embassichthys*.

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CHAPTER 1

GENERAL INTRODUCTION

Metamorphosis in flatfishes entails morphological and behavioral changes associated with the transition from plankton to benthos. Most descriptions of metamorphosis in flatfishes have presented a picture of a highly canalized developmental process, with the morphological changes occurring rapidly over a limited size range (e.g. Ryland, 1966; Fukuhara, 1986, 1988). In general, the size range over which metamorphosis can occur appears to be positively correlated with the size at metamorphosis (Ahlstrom *et al.*, 1984), with nearshore species having a briefer planktonic larval period and undergoing metamorphosis across smaller size ranges than deep water species (Moser, 1981). For example, estuarine and nearshore species of *Pleuronichthys* undergo metamorphosis at smaller sizes than deepwater species (Sumida *et al.*, 1979). The rapidity of the metamorphic process in nearshore species may be a response to the vagaries of the environment, so that dispersal away from suitable settling habitat is limited and these habitats can be utilized when encountered (Sumida, Ahlstrom, and Moser, 1979; Moser, 1981). Conversely, deepwater species may protract the larval period and prolong, or delay, metamorphosis to increase the probability of locating and utilizing settling habitat as onshore transport occurs (Moser, 1981).

Dover sole (Microstomus pacificus) is a deepwater species (30-1000 m) distributed off the west coast of North America from Baja California to the Bering Sea(Miller and Lea, 1972). The early life history of Dover sole is somewhat enigmatic because large larvae (>50 mm SL) with asymmetrical eyes are found throughout the year (Pearcy et al., 1977a). The original description of the larvae characterizes the pelagic life as being prolonged and metamorphosis delayed (Hagerman, 1952). A more recent examination of the distribution and duration of larval stages suggests that settlement occurs between 30-50 mm SL and that metamorphosis occurs after about one year, with large larvae (>50 mm SL) having delayed metamorphosis possibly because they were in deeper, offshore waters during the settlement season (Pearcy et al., 1977a).

The uncertainty associated with the length of the planktonic larval period and seasonality and duration of metamorphosis should call into question estimates of the age at which Dover sole recruit into the adult fishery. An understanding of the early life history stages of Dover sole, and especially the timing and duration of metamorphosis, is needed to facilitate formulation of management and conservation plans. Chapter 1 describes the life history stages of Dover sole, focusing on developmental events associated with initiation and termination of metamorphosis and evaluates eye migration as an indicator of metamorphosis. This chapter also discusses the implications of a prolonged planktonic larval period and a protracted metamorphosis to the recruitment strategy of Dover sole.

An early phylogenetic hypothesis of relationships between Microstomus, Embassichthys, Tanakius, and Glyptocephalus recognized these genera as a "primary division" within the Pleuronectini and united them based on their more elongated bodies, and higher counts of vertebrae, fin rays, and scales in a longitudinal series (Norman, 1934). Norman's hypothesis of intergeneric relationships indicated two sister groups: Embassichthys plus Microstomus and Tanakius plus Glyptocephalus. However, he did not identify any synapomorphies to support his hypothesis. Alternatively, Richardson (1981) proposed a sister group relationship between Embassichthys, Glyptocephalus and Tanakius based on pigmentation patterns, and absence of otic spines and similarity of body shape, which she described as "leptocephalus-like". In Chapter 2, I evaluate Richardson's body shape character using a multivariate analysis of body shape change from post-flexion larvae through benthic juveniles. I also examine characters associated with eye migration and the development of five metamorphic characters found to change during metamorphosis in M. pacificus (Chapter 1) in order to identify potential synapomorphies supporting a Microstomus and Embassichthys sister group relationship.

CHAPTER 2

EARLY LIFE HISTORY STAGES AND METAMORPHOSIS IN THE DOVER SOLE (*Microstomus pacificus*)

ABSTRACT

The early life history stages and metamorphosis in Dover sole (Microstomus pacificus) are described from 201 larvae and juveniles. Unlike other pleuronectids, with the possible exception of the deep-sea sole (Embassichthys bathybius), initial migration of the left eye is not associated with metamorphosis or transition from plankton to benthos. In addition, there is a stasis during migration of the left eye, so that premetamorphic larvae between 11.7-57.2 mm SL are optically asymmetrical before metamorphosis. Seven characters are used to quantify the progress of metamorphosis: completed eye migration, dorsal fin position, dentition, pectoral fin development, condition of posterior process of coracoid, pigmentation, and elongation of the intestine into a secondary body cavity. Other characters associated with metamorphosis are reduction in body depth associated with reductions in lengths of neural and hemal spines and associated dorsal- and anal- fin pterygiophores, decreasing interorbital distance, increasing right eye diameter, increasing right premaxilla length, and development of body scales. Metamorphosis appears to require 8-12 months to complete. The planktonic period of premetamorphic and metamorphic larvae is about 18-24 months. Metamorphic larvae appear to move inshore into shallower water as metamorphosis progresses. Settlement occurs from about December to April, with peak settlement occurring primarily from January to March.

INTRODUCTION

Many species of marine fishes exhibit indirect development (Balon, 1985; Youson, 1988), wherein a planktonic larvae undergo metamorphosis into a benthic juvenile. Metamorphosis generally involves a restructuring of morphological characters, changes in behavior and physiology, and a change in niche (Youson, 1988).

Prolonging the planktonic larval period by delaying metamorphosis has been documented in several marine invertebrates (e.g. Pechenik, 1985, 1986) and two fishes (Victor, 1986; Cowen, 1991). Two commonly suggested reasons for a prolonged larval period are dispersal of young (Scheltema, 1971; Barlow, 1981; Jackson and Strathmann, 1981; Palmer and Strathmann, 1981) and unavailability of resources (e.g. suitable settling habitat) at critical periods of development (Cowen, 1991; Frogner, 1980; Jackson and Strathmann, 1981). Jackson and Strathmann (1981:16) found that marine invertebrates with a long precompetent stage ("period that [larvae are] developing into a stage capable of settling") required an equally long or longer competent stage (stage that larvae are capable of settling) for larvae to encounter suitable settling habitat.

Victor (1986) and Cowen (1991) documented delayed metamorphosis in bluehead wrasse (Thalassoma bifasciatum) and sheephead wrasse (Semicossyphus pulcher), respectively, by analyzing otolith microstructure in newly settled larvae to derive age estimates and back-calculated daily growth rates. Both studies identified larvae that had delayed metamorphosis by a period of decreased growth after a given age threshold was achieved and by their larger size at settlement. These studies also found that there was

greater variation in age of larvae at settlement than in length, a result that has been documented in empirical studies of size and age at metamorphosis in winter flounder, Pseudopleuronectes americanus (Chambers and Leggett, 1987; Cowen, 1991).

The presence of very large larvae (50-70 mm standard length [SL]) of Dover sole, Microstomus pacificus (Lockington), has been cited frequently as evidence of a prolonged larval period and delayed metamorphosis in this species (Hagerman, 1952; Ahlstrom and Moser, 1975; Pearcy et al., 1977a; Victor, 1986; Chambers and Leggett, 1987; Cowen, 1991). In the original description of Dover sole larvae, Hagerman (1952:39) characterized the pelagic life of larvae as being "prolonged for several months and metamorphosis...delayed." Hagerman (1952:42) made frequent references to specimens of differing SL in which the left eye was located on the left side or dorsal ridge of the cranium, which he considered as undergoing metamorphosis: "...available specimens indicate that the complete migration of the left eye does not take place until the total length reaches at least 35 mm. Even in a 48 mm specimen the left eye was not quite in the adult position." Hagerman (1952:42) described the ontogeny of body form as progressing from, "elongate [in larvae], to wide leaflike, and then back to the intermediate adult shape."

Pearcy et al. (1977a, their Table 3) examined 515 larvae 8-65 mm SL in which the left eye had either started migrating or was on the middorsal ridge of the cranium and assigned these specimens to a developmental stage according to the position of the left eye. Based on a December to February spawning season (Hagerman, 1952), a bimodal size distribution of larvae present throughout the year, and

size of benthic juveniles, they speculated that larvae settled to the benthos between 30-50 mm SL and that metamorphosis occurred after about one year. Large larvae (>50 mm SL) represented a population that had not yet settled, possibly because they were in deeper, offshore waters during the settlement season (Pearcy et al., 1977a).

Eye migration is the traditional character upon which definitions of metamorphosis in flatfishes are based and the main criterion used to judge its progress (e.g. Ryland, 1966; Hensley, 1977; Fukuhara, 1988). However, it may be an inappropriate character for determining size at, and progress of, metamorphosis in Dover sole. The objectives of this study are to: 1) describe the life history stages of Dover sole, focusing on developmental events associated with initiation and termination of metamorphosis and evaluate eye migration as an indicator of metamorphosis; 2) evaluate the hypothesis of a prolonged planktonic larval period in Dover sole based on the chronological distribution of life history stages and determine the seasonality of metamorphosis; and 3) discuss the implications of a prolonged larval period to the recruitment strategy of Dover sole.

MATERIALS AND METHODS

Materials.--Larval Dover sole were obtained from 102 midwater trawl collections taken off Oregon between 1961 to 1978. Details of sampling methods were given in Pearcy (1976) and Pearcy et al. (1977a, b). Station data was recorded from the cruise data. Offshore distribution of larvae was based on specimens collected along the Newport Hydroline, 44°40′ N latitude (Pearcy, 1976; Pearcy et al., 1977a, b). Additional material of larval Dover sole was obtained from

the Larval Fish Collection, National Marine Fisheries Service, Northwest and Alaska Fisheries Center.

Juvenile Dover sole were obtained from benthic trawls taken off the Oregon coast with a 27 m shrimp net towed for 5 min on the bottom. Juveniles were collected at depths of 74-142 m during March, 1988 and January, 1989. Additional juvenile material was obtained with a 7.5 m otter trawl towed for 30 min at depths between 40-75 m in Monterey Bay, California during March, 1988.

A list of material examined is given in Appendix A. Specimen information includes: Oregon State University Fish Collection (OS) catalog number, whether the specimen was x-rayed (XR) or cleared and stained (CS), locality, number of specimens, and standard length or standard length size range.

Methods.--I measured 201 larvae, juveniles, and adults. Only larvae that had undergone notochord flexion and had distinct hypural, epural and parhypural elements were used. Meristic data included counts of vertebrae, and dorsal-, anal-, caudal-, pectoral-, and pelvic- fin rays. All counts were defined in Hubbs and Lagler (1958). Larvae and juveniles were pat-dried and weighed to the nearest 0.01 g with a Mettler AE240 electronic balance.

Standard length, right eye diameter, interorbital width, and right premaxilla length were defined in Hubbs and Lagler (1958). Additional measurements included: body depth at anus (BD); pre-anal length, measured from anterior of premaxilla to anus; intestinal length, measured from anus to most posterior part of intestine; snout-to-intestine length (GI), sum of pre-anal length and intestinal length; length of first caudal-neural spine (CNS) and immediate

anterior (ACNS) and posterior (PCNS) pterygiophores; and, length of first hemal spine (CHS) and immediate anterior (ACHS) and posterior (PCHS) pterygiophores. Lengths of neural and hemal spines and associated pterygiophores were taken from cleared and stained specimens or x-rays. All measurements were taken to the nearest 0.1 mm. Measurements on specimens <20 mm SL were made with a camera lucida attached to a Zeiss dissecting microscope with an ocular micrometer. Larger specimens were measured with dial calipers.

Changes in body shape that occurred during ontogeny were quantified with a system of truss distances (Strauss and Bookstein, 1982) between seven anatomical landmarks (Fig. 1). These trusses form geometric patterns sensitive to changes in size and shape (Strauss and Bookstein, 1982; Strauss and Fuiman, 1985). Because of a lack of distinct, external reference points (e.g. origin of a second dorsal fin) on the body of flatfish, straight-line intersections of four landmarks (b, c, d, and e) with the midsagittal outline were used. Morphological landmarks were: a) medial tip of premaxilla; b) intersection of distal end of cleithrum with midsagittal outline; c) intersection of a straight line drawn from reference point b to the midsagittal-dorsal outline of the body; d) dorsal intersection of distal end of first caudal-neural spine with midsagittal outline; e) ventral intersection of distal end of first hemal spine with midsagittal outline; f) posterior, dorsal corner of epural; and, g) posterior, ventral corner of parhypural. Anatomical landmarks were digitized and truss distances computed as Euclidean distances (Strauss and Bookstein, 1982).

Positions of anatomical landmarks were taken from specimens that were either cleared and differentially stained with Alizarin Red S and Alcian Blue following the method of Potthoff (1984) or x-rayed. In certain cases, x-rayed specimens were also cleared and stained. Reference points on larvae <20 mm SL were transferred to paper with a camera lucida attached to a Zeis dissecting microscope. Cleared and stained larvae 20-50 mm SL were photographed and reference points transferred to paper from photographic negatives with a negative enlarger. Reference points on specimens >50 mm SL were taken directly from x-rays.

Statistical Methods.--Descriptive statistics and plotting procedures were performed in STATGRAPHICS (Statistical Graphics Corporation, 1987). Principal component (PC) analysis of truss distances was used to summarize changes in body size and shape that occurred during ontogeny. Principal components were computed from the covariance matrix of logarithmically transformed truss distances (Jolicoeur, 1963; Strauss and Fuiman, 1985). Principal components were computed with BIO∑TAT II (Pimental and Smith, 1986).

Multigroup discriminant function analysis (MDFA) has been used to compare intra- and inter- populational variation, while remaining sensitive to individual variation within a population(s) (Neff and Smith, 1979; Pimental, 1979; Albrecht, 1980; Michaux, 1989). MDFA was used to corroborate the assignment of specimens into life history stages (described in following section) by testing the relationship between developmental events with changes in body size and shape during ontogeny. MDFA was performed on the logarithmically transformed truss distances with BIO∑TAT II (Pimental and Smith,

1986). Geisser classification probabilities were also generated with BIOΣTAT II.

Ontogenetic Staging System.--A staging system describing life history stages of Dover sole was modified from the terminology of Balon (1979, 1984, 1985) and Youson (1988) in that stage referred to a given level of development, based on a metamorphic score, rather than an instantaneous point of development. The metamorphic score of a specimen was the summation of character states associated with seven morphological characters that changed during metamorphosis (Table 1). Binary characters were scored as (0) for larval and (1) for adult character states. Multistate characters were scored as (0) for larval, (1) for transitional, and (2) for adult character states. Initiation of metamorphosis was identified by a change in any of the first five metamorphic characters (Table 1) to the adult (eye position or dorsal fin position) or transitional (dentition, pectoral fin development, or posterior process of coracoid development) character state. The event terminating metamorphosis was elongation of the intestine into a secondary body cavity. Because of the continuous nature of this character, metamorphosis was considered terminated when the ratio ln(GI)/ln(SL) approximated 0.87. This value represented the midpoint in the ratio where the greatest rate of change occurred in specimens between 67 and 69 mm SL (Markle et al., 1992).

The larval period of Dover sole, excluding the preflexion phase, consisted of two phases in post-flexion larvae, premetamorphic and metamorphic. I considered all premetamorphic phase larvae to be Stage 1 larvae because of my emphasis on metamorphosis, although other stages could have been recognized within the premetamorphic phase of

the larval period (e.g. Stages II and III of Pearcy et al., 1977a). The metamorphic phase was divided into three stages (Stages 2-4) that corresponded with initiation or termination of development of the seven metamorphic characters. Metamorphic larvae were classified also by their "competency" (Jackson and Strathmann, 1981), or presumed ability to settle to the benthos. Precompetent metamorphic larvae (Stage 2) were in the initial stage of metamorphosis and all specimens were planktonic; competent metamorphic larvae (Stage 3) had developed six of the seven metamorphic characters and specimens were both planktonic and benthic; and post-competent metamorphic larvae (stage 4) had developed all seven metamorphic characters and all, except one, were collected on the bottom. The embryonic period (interval from fertilization to hatching), preflexion phase of the larval period, and adult period (sexually mature fish have been collected as small as 235 mm TL and approximately 5 years of age [Yoklavich and Pikitch, 1989]) were not considered in this study.

RESULTS

Character States of the Seven Metamorphic Characters.--The eyes of premetamorphic larvae are on small, raised stalks; larvae are either optically symmetrical, or the left eye is migrating to, or is on, the dorsal ridge of the cranium (score = 0; Fig. 2A). The anterior dorsal fin pterygiophores and fin rays of premetamorphic larvae are located posterior to the left eye (score = 0; Fig. 2A). During metamorphosis, the left eye completes its migration (score = 1) and dorsal fin pterygiophores and fin rays move forward to a position anterior to the left eye (score = 1; Fig. 2B).

Canine-like teeth are present on both sides of the premaxilla and dentary in premetamorphic larvae (score = 0; Fig. 3A). Incisors develop in the left premaxilla and dentary during metamorphosis while canines are still present on both sides of the jaws (score = 1: Fig. 3B). These canines are lost or resorbed with the eruption of the incisors (score = 2; Fig. 3C).

Pectoral fins of premetamorphic larvae are round and paddle-like in appearance with a thin base; precursors of fin rays are developing in the finfold (score = 0; Fig. 4A). Pectoral fin rays develop initially between 15-20 mm SL and the adult complement is formed between 30-40 mm SL. Pectoral fins remain somewhat rounded in shape during metamorphosis, the base becoming more rectangular, and cartilaginous radials are present (score = 1; Fig. 4B). Pectoral fins of juveniles are oval in shape with ossified fin rays and radials; the bases of the fins have a rectangular appearance (score = 2; Fig. 4C).

The posterior process of the coracoid also changes during ontogeny of the pectoral girdle. This process is long and slender in premetamorphic larvae and projects posteriorly above the visceral cavity, just underneath the skin (score = 0; Fig. 4A). The posterior process begins to degenerate in metamorphic larvae, recognizable by the distorted shape and poor quality of staining by Alcian Blue (score = 1; Fig. 4B). Ultimately, the entire process is resorbed (score = 2; Fig. 4C).

Planktonic premetamorphic larvae have no pigmentation in the mid-lateral area of the myomeres. However, there are a series of dashes along the base of the dorsal and anal fin rays and outer margins of the epaxial and hypaxial musculature (Fig. 5A, margin of

hypaxial musculature). This pattern of dashes will ultimately develop into a solid outline (Fig. 5A, margin of epaxial musculature; Pearcy et al., 1977a). A transitional pigmentation pattern, in which melanophores develop along the myosepta, forms prior to settlement (Fig. 5B). The larval and transitional pigmentation patterns are scored as 0 because both patterns are found on planktonic premetamorphic larvae. The initial benthic pigmentation pattern consists of circular aggregations of melanophores along the mid-lateral surface of the myomeres (score = 1; Fig. 5C).

The final character to change during metamorphosis is the intestine, which elongates into a secondary body cavity. The intestine of premetamorphic larvae is arranged in a coiled pattern (score = 0; Fig. 6A). After settlement, the intestine elongates past the first anal fin pterygiophore (score = 1; Fig. 6E). Metamorphosis is terminated when the ratio ln(GI)/ln(SL) approximates 0.87 (score = 2; Fig. 18).

Life History Stages

Premetamorphic larvae (Stage 1) (Fig. 6A)

Ninety-nine specimens between 9.6-57.2 mm SL are classified as Stage 1 larvae (metamorphic score = 0). During caudal fin flexion, the left eye begins migration (discussed below), dorsal- and anal- fin rays form, and BD increases (Fig. 7). Following caudal fin flexion, Stage 1 larvae attain adult numbers of vertebrae and dorsal-, anal-, caudal- and pelvic- fin rays; the stomach and intestine coil and three to four pyloric cecae develop; prominent cone-shaped otic spines develop; and the initial pigmentation pattern of dashes develops into a solid outline on specimens 35-40 mm SL.

The left eye of Stage 1 larvae migrates shortly after notochord flexion, around 10-15 mm SL (Pearcy and Richardson, 1977). In this study, 14 larvae (11.7-23.4 mm SL) have the eye migrating towards, but not on, the dorsal ridge of the cranium. The smallest larvae with the left eye on the dorsal ridge is 15.4 mm SL. The left eye remains in this position until metamorphosis. Consequently, Stage 1 larvae are optically asymmetrical in the plankton until metamorphosis is initiated in Stage 2, requiring an additional 19.4-47.9 mm of growth.

Precompetent, metamorphic larvae (Stage 2) (Fig. 6B)

Thirty specimens between 42.8-59.6 mm SL are classified as Stage 2 larvae (metamorphic score = 1-8). Metamorphosis is initiated with the development of any of the first five metamorphic characters (Table 1): development of incisors in the right premaxilla and dentary; completion of left eye migration; forward shift of dorsal fin to a position anterior to left eye; degeneration of posterior process of coracoid; development of intermediate pectoral fin shape. The transitional coloration pattern develops also.

Other characters associated with Stage 2 include: reduction in BD (Fig. 7), reduction of otic spines, decreasing interorbital distance (Fig. 8), increasing right eye diameter (Fig. 9) and right premaxilla length (Fig. 10), and initial formation of zygopophyses on the vertebral centra. Scales develop on Stage 2 larvae with metamorphic scores of 7 or 8. Two to three rows of scales develop around the lateral line on the anterior body, posterior cranium, and caudal peduncle.

Competent, metamorphic larvae (Stage 3) (Fig. 6C)

Forty specimens between 44.5-64.8 mm SL are classified as Stage 3 larvae (12 planktonic, 28 benthic). Stage 3 larvae have a metamorphic score of 9, indicating that metamorphic characters from Stage 2 are in the adult character state and that benthic pigmentation is beginning to form. Morphometric changes initiated in Stage 2, including reductions in BD (Fig. 7) and interorbital distance (Fig. 8), and increasing right eye diameter (Fig. 9) and right premaxilla length (Fig. 10), continue in Stage 3. Pelvic fin rays and radials begin to ossify during this stage and the asymmetrical coloration pattern becomes more pronounced.

Post-competent, metamorphic larvae (Stage 4) (Figs. 6D-E)

Twenty-eight specimens between 41.1-62.6 mm SL are classified as Stage 4 larvae (metamorphic score = 10). The beginning of this stage can be identified by the elongation of the intestine past the first anal fin pterygiophore (score = 1; Figs. 6D-E). Reductions in BD and other morphometric changes initiated in Stage 2 are completed during Stage 4. The axial skeleton is completely ossified and the vertebral centra have well developed apophyses.

Juvenile (Stage 5) (Fig. 6F)

Four specimens between 78.5-124.3 mm SL are classified as Stage 5 or juveniles (metamorphic score = 10). Juveniles have a ln(GI)/ln(SL) ratio $\geq$0.87 (Markle et al., 1992), the overall appearance of adults, mottled benthic coloration, and well developed scales. Stage 4 larvae and Stage 5 juveniles have resumed a more linear growth relationship between BD and SL (Fig. 7).

Ordination of Life History Stages.--The first two MDF analysis axes account for 97.5% of between group variation. Discriminant function

axes contrast changes in body size (DF1) with changes in body depth (DF2) during ontogeny (Fig. 11). DF1 is correlated ($\geq$0.20) with truss distances associated with increases in head and body length and body length + width measures (Table 2). DF2 is correlated ($\geq$0.30) with all truss distances, except the two head length distances (truss distances 1 and 2; Table 2).

An overall Geisser classification probability of 95% is achieved with the truss distances in corroborating the assignment of a specimen into one of the five life history stages. Discrimination of life history stages is greatest for stages 1 (96%), 2 (100%), 4 (96%), and 5 (100%). The remaining 4% of Stage 1 larvae are classified as Stage 2. These four specimens are among the largest of all Stage 1 larvae (between 51.2-57.2 mm SL) and are from collections made in late May (2) and July (2), July being a month when Stage 2 larvae have the lowest metamorphic scores (discussed below). Stage 3 larvae have the lowest correct classification rate at 88%; 10% (N = 4) are misclassified as Stage 2 larvae and 3% (N = 1) as Stage 4 larvae. Larvae incorrectly classified as Stage 2 larvae are from planktonic collections made from December to March, the period of settlement to the bottom. The specimen classified as a Stage 4 larvae is 44.5 mm SL and is from a benthic collection taken during March. Finally, 96% of Stage 4 larvae are correctly classified. One benthic specimen, collected in March, has an approximately equal probability of being either a Stage 3 or Stage 4 larvae.

Because Geisser classifications assign group membership based on proximity to group centroids (Pimentel, 1979), the high classification probabilities for Stages 1 and 5 can be explained

primarily by their non-overlapping SL size ranges (Fig. 7) and, for Stage 5, by small sample size. In addition, the out-lying distance of the group centroids for Stages 1 and 5 tends to increase the distances between the group centroids for Stages 2-4, which also contributes to the high classification rate for these three stages.

In order to assess this effect, an additional MDFA was performed in which Stage 1 larvae $\leq$39.0 mm SL and Stage 5 juveniles were eliminated. As in the first analysis, the first two axes accounted for a very high level of between group variation (97.7%). However, the canonical structure of the analysis changed to reflect the importance of decreasing body depth during metamorphosis, rather than overall body size (Table 2). Distances between group centroids also decreased, resulting in a decrease of the Geisser classification probabilities: Stage 1 - 89%; Stage 2 - 90%; Stage 3 - 82%; and Stage 4 - 93%.

Morphometric changes during metamorphosis.--The first two principal components axes describe 97.7% of total variation in the truss distances between the five life history stages. The first principal component (PC1) accounts for 93.6% of total variation and eigenvector loadings are positive for all truss distances (Table 3). Based on the high positive correlations between the truss distances and PC1 and the large amount of variation associated with each truss distance, I interpret PC1 as an allometric size axis (Fig. 12; Shea, 1985; Strauss and Fuiman, 1985; Tissot, 1988).

Principal component 2 (PC2) accounts for 4.1% of total variation. Eigenvector loadings are both positive and negative, with the highest loadings associated with truss distances 8 and 1 (Table

3). Positive correlations are found between truss distances 8 (equivalent to maximum BD), 4, 10, and 11 (length plus body depth) and PC2. Negative correlations exist between PC2 and truss distances measuring head length (truss distances 1 and 2) and trunk length plus trunk depth (truss distances 5, 6, 12, and 13; Table 3), both of which are related to decreasing body depth during metamorphosis (Fig. 12).

A bivariate plot of BD against SL reveals a pattern similar to that of the PC analysis (Fig. 7). Stage 1 larvae increase rapidly in BD relative to SL (BD = 1.72 + 0.57(SL), F-test $P<<0.0001$, df = 1, 97), followed by dramatic decreases in BD during Stages 2-3. Following metamorphosis, Stage 4 larvae and Stage 5 juveniles resume a more linear relationship between BD and SL (Fig. 7; BD = 4.12 + 0.23(SL), F-test $P<<0.0001$, df = 1, 30).

Reductions in BD are accomplished by decreasing lengths of neural and hemal spines and dorsal and anal fin pterygiophores (Table 4). For example, mean length of CNS in specimens 40.0-49.9 mm SL decreases by half during metamorphosis from 6.59 mm (se = 0.22) in Stage 1 larvae to 3.47 mm in Stage 4 larvae (se = 0.06; Table 4; Fig. 13A). Mean length of CHS decreases from 10.20 mm (se = 0.23) in Stage 1 larvae to 4.42 mm (se = 0.12) in Stage 4 larvae (Table 4; Fig. 14A). Similar changes in length occur in the immediate anterior and posterior pterygiophores of both spines (Table 4; Figs. 13B-C, 14B-C).

The diameter of the right eye of Stage 1 larvae increases at a relatively slow rate while larvae are asymmetrical in the plankton (Fig. 9; right eye = 0.35 + 0.03(SL), F-test $P<<0.0001$, df = 1, 96). During metamorphosis, the right eye begins to increase without concordant increases in SL (Fig. 9). However, the right eye

maintains its larval appearance until Stage 4, when it begins to increase rapidly in diameter (right eye = -0.86 + 0.10(SL), F-test $P<<0.0001$, df = 1, 29) and develops a more adult appearance (Fig. 6d).

Similarly, right premaxilla length of Stage 1 larvae increases at a relatively slow rate during the planktonic period (right premaxilla = 0.79 + 0.02(SL), F-test $P<<0.0001$, df = 1, 87), and there is also an increase in length without concordant increases in SL during metamorphosis (Fig. 10). The asymmetrical shape of the right premaxilla and dentary develops during metamorphosis, after which the right premaxilla of Stage 4 larvae and Stage 5 juveniles begins to increase in length (right premaxilla = -0.27 + 0.05(SL), F-test $P<<0.0001$, df = 1, 30).

Dover sole larvae decrease in weight during metamorphosis, from an average of 2.73 g (se = 0.11) in Stage 2 larvae to 2.03 g (se = 0.09) in Stage 3 larvae to 1.49 g (se = 0.10) in Stage 4 larvae. At the minimum length for metamorphosis (approximately 40 mm SL), the minimum weight of a 42.8 mm SL Stage 2 larva is 1.39 g. This larva, collected in January, has a metamorphic score of 7 and has been presumably losing weight since initiation of metamorphosis in June or July (discussed below). The smallest Stage 3 larva, collected in March, is 44.5 mm SL and weighs 1.12 g; the smallest Stage 4 larva, also collected in March, is 41.1 mm SL and weighs 0.72 g. Therefore, the estimated weight threshold for metamorphosis is probably greater than the minimum weight of 1.39 g for Stage 2 larvae. Further, the similarity of minimum size of Stage 2-4 larvae (42.8, 44.5, and 41.1 mm SL) and size range of these stages (see life history stage

descriptions) suggests that there is little, or no, increase in length during metamorphosis.

Chronological, Longitudinal, and Depth Distributions of Premetamorphic and Metamorphic Larvae.--Because larvae were chosen for their suitability for morphological analysis, rather than chronological or spatial distribution, a bias existed in the chronological, longitudinal, and depth distributions. However, several general patterns were apparent. Stage 1 larvae occurred in samples from throughout the year (Table 5; Fig. 15), attaining their highest frequency in June and July when the frequency of sampling was highest. Stage 2 larvae were most abundant in samples from September (8) and December (9), although they occurred in samples from June to February (Table 5; Fig. 15). Metamorphic scores for Stage 2 larvae demonstrated a clear progression throughout the year, with specimens from June to September having metamorphic scores of 1-6 and specimens from October to February having scores of 4-8 (Table 5). Stage 2 larvae with metamorphic scores of 8 were most abundant in December samples (Table 6), although they were found as early as October and as late as February, indicating that approximately 6-7 months were required to progress through this stage. One notable exception to this pattern was a specimen collected on 31 July 1965 with a metamorphic score of 6, suggesting that either metamorphosis began earlier than June, or that metamorphosis was rapid and larvae with benthic characters remained planktonic until settlement. Planktonic Stage 3 larvae were found from December to March, predominantly from January to March (Table 5; Fig. 15), which coincides with the peak period of settlement (Markle et al., 1992). Aquarium observations on

recently settled Stage 3 larvae suggest that these larvae were on the bottom about one month before elongation of the intestine. Additional aquarium observations on a single Stage 4 larva, collected as a Stage 3 larvae prior to the elongation of the intestine, indicated that 43 days were required for this larva to progress through Stage 4. Based on the estimated duration of Stage 2 (6-7 months), peak settlement occurring over a three month period (January to March), and approximately one to two additional months to progress through Stage 4, it appears that a minimum of 8 months, and possibly as long as 12 months, are required for larvae to progress through Stages 2-4.

A general trend of onshore movement was apparent in the offshore distribution of Stage 2 and 3 larvae collected along the Newport Hydroline (44°40′ N latitude). Stage 1 larvae had the greatest offshore distribution, being collected 30-145 km offshore (Table 7; Fig. 16). The majority (72%) of Stage 2 larvae were collected 50-65 km offshore between July and February; larvae found further offshore were collected in June (145 km) and September (80 and 100 km) (Table 7; Fig. 16). Planktonic Stage 3 larvae appeared to have the narrowest offshore distribution of the three stages, being collected 25-65 km offshore (Table 7; Fig. 16)

Premetamorphic and metamorphic larvae (Stages 2 and 3) collected along the Newport Hydroline were caught primarily in trawls fished between 0-600 m (Table 8; Fig. 17). However, most Stage 1 and 2 larvae were collected between 0-300 m, where 66.3% of 2,468 midwater trawls were conducted (Markle et al., 1992). Stage 1 larvae were collected in tows between 0-1,000 m (Table 8; Fig. 17), although the majority (83%), including 18 of 23 specimens >40 mm SL, were collected

in tows between 0-300 m. Stage 2 were collected in tows from 0-2,075 m, but almost half (48%) were found in tows to 200-300 m (Table 8; Fig. 17). Similarly, most planktonic Stage 3 larvae (92%) occurred in tows to 450 m, although they ranged in depth from 0-970 m (Table 8; Fig. 17). A single planktonic Stage 4 larvae (53 mm SL), with an elongated intestine containing sand grains, was collected off the mouth of the Columbia River on 19 April 1963 in a midwater trawl towed at 73 m in 125 m of water. Additional planktonic Stage 3 and 4 larvae were collected in midwater trawls fished primarily between 0-30 m (total range of collections 0-110 m) over bottom depths of 33-1462 m (bottom depths of Stage 3 collections, 73-1462 m; Stage 4 collections, 33-91 m) between Monterey Bay and San Francisco, California (W. Lenarz, NOAA, NMFS, Tiburon, California, Unpublished data).

Bottom trawl surveys conducted during 1989 indicated that Stage 3 larvae settled across a broad depth range (55-377 m) from January to April (Markle et al., 1992). As larvae developed and metamorphosis was completed, the distribution of Stage 4's and Stage 5's became somewhat more restricted, being collected between 40-170 m and 75-188 m, respectively, with the densest aggregations centering around 125 m (Markle et al., 1992).

DISCUSSION

Metamorphosis in Dover sole.--Unlike any other pleuronectid, with the possible exception of the closely related deep-sea sole, Embassichthys bathybius (Chapter 2), the migrating left eye of Dover sole remains on the dorsal ridge of the cranium across a 17-45 mm SL size range. It is possible that this position, coupled with both eyes being on small, raised stalks, imparts a limited binocularity and increases the volume

of the field of vision (Weihs and Moser, 1981). Such a specialization may enhance the probability of detecting prey and predators. Another possibility is that eye migration and increasing BD are synchronized developmental events. The migratory stasis of the left eye may be the result of selection for the large, round, deep-bodied larval shape. This suggests that, in conjunction with their predominance in deeper (0-600 m), offshore (50-65 nautical miles) tows, large body size may provide a size-based refuge from predation prior to inshore movement and settlement.

The size range of metamorphic larvae (41.1-64.8 mm SL) demonstrates that the "holdover" larvae of Pearcy et al. (1977a) do, in fact, metamorphose. Further, similarities between the minimum size of the three metamorphic stages (Stage 2, 42.8 mm SL; Stage 3, 44.5 mm SL; Stage 4, 41.1 mm SL) suggests that little or no growth occurs during metamorphosis. A cessation in growth of body length before and during metamorphosis is known from experiments on other flounders (Fukuhara, 1986, 1988). Body length begins to increase again during Stages 4 and 5 (Fig. 7).

The primary morphometric change that Dover sole larvae undergo during metamorphosis is a decrease in BD, which contrasts with the results of other studies on pleuronectiforms that have reported increases in BD during metamorphosis (Fukuhara, 1986, 1988; Rosenberg and Laroche, 1982). Two- to three- fold reductions in length of the neural and hemal spines and dorsal- and anal-fin pterygiophores occur during Stages 2-4. Consequently, metamorphosing specimens 40-50 mm SL have neural and hemal spines and pterygiophores comparable in length to those of 20-30 mm SL larvae. The neural and hemal spines and

associated pterygiophores may serve as a reservoir for calcium and other minerals needed during metamorphosis, similar to that hypothesized for eel leptocephali (Hulet, 1978). Minerals could be released during metamorphosis from the spines and pterygiophores by a regressive process (sensu Youson, 1988), wherein the spines and pterygiophores undergo autolysis and their minerals and other components are released. The observation that complete ossification of the axial skeleton and presence of well developed zygopophyses on vertebral centra in Stage 4 larvae and Stage 5 juveniles would seem to support this hypothesis.

Seasonality of Spawning, Metamorphosis and Recruitment.--Dover sole are thought to spawn in winter, primarily from December to February (Hagerman, 1952). However, peak abundances of eggs and small larvae (<13 mm SL) occur in April and May, and eggs and larvae are found as late as August (Molina-Urena, 1989). Consequently, the spawning period of Dover sole also appears to be a protracted event, probably occurring from December to July.

The chronological distribution of metamorphic larvae indicates that metamorphosis begins as early as June and Stage 2 and 3 larvae remain planktonic until settlement the following January to April. If larvae grow to 20-30 mm during their first year, as estimated by Pearcy et al. (1977a), then metamorphosis for larvae spawned in December could begin at 18 or 19 months, assuming an additional 10-20 mm of growth from January to June of the second year. These larvae would be 25 months at settlement the following January. For larvae spawned in July, assuming they could grow to 40 mm SL by the following June, metamorphosis could begin at 11 or 12 months and

settlement the following January would occur at 18 months. The potential 18-25 month duration of the planktonic period of Dover sole larvae is greater than that reported for any other pleuronectid in the Northeast Pacific (Matarese et al., 1989), with the possible exceptions of Embassichthys and Glyptocephalus.

It has been hypothesized that the eggs and early larvae of Dover sole are transported offshore during upwelling events in spring and summer and metamorphic larvae are returned onshore during downwelling events in winter (Hayman and Tyler, 1980; Parrish et al., 1981). An implicit assumption of this hypothesis is that metamorphic larvae should be found in the upper 40 m of the water column during winter, where most onshore flow occurs (Huyer, 1983). Offshore distribution of Stage 2 larvae with metamorphic scores of 8 and Stage 3 larvae along the Newport Hydroline do indicate a somewhat more restricted distribution of these larvae in inshore waters in comparison to Stage 1 larvae. However, occurrence of the majority of Stage 2 and 3 larvae in tows fished to 0-600 m and 0-400 m, respectively, indicates that they are found in waters with deeper bottom depths than newly settled Stage 3 and planktonic Stage 4 larvae. Although nets with opening-and-closing devices would be required to corroborate their exact distribution in the water column, this suggests that planktonic Stage 2 and 3 larvae may occur deeper in the water column. The broad distribution (55-377 m) of recently settled Stage 3 larvae in bottom trawls in January and March (Markle et al., 1992) suggests that planktonic metamorphic larvae are opportunistic in settling, utilizing onshore transport events, such as winter storms or periodic winter upwelling (Huyer, 1983), to

facilitate inshore movement. Within a month or so of settlement, the distribution of Stage 4 larvae and Stage 5 juveniles becomes more restricted, centering around an apparent nursery ground between 40-188 m. The restricted distribution following settlement indicates that larvae outside of the nursery grounds either migrate into this area or suffer mortality. The occurrence of recently settled Stage 3 and 4 larvae in the plankton provides evidence of a potential behavioral mechanism that would facilitate movement into the nursery grounds following initial settlement.

One assumption of the model currently used to manage Dover sole fisheries is that larvae settling to the bottom in a given year are 1 year old fish, and ultimately these fish recruit into the adult fishery at age 6 (Methot et al., 1990). These results suggest that settlement does not occur until two years after spawning, which should call into question the validity of assumptions concerning the absolute age of adult Dover sole.

Delayed vs. protracted metamorphosis.--Theoretical and empirical studies of the early life history of marine invertebrates (Jackson and Strathmann, 1981; Palmer and Strathmann, 1981; Pechenik, 1985, 1986) and vertebrates (Victor, 1986; Cowen, 1991) have stressed delayed metamorphosis as the mechanism by which the planktonic larval period may be prolonged. For the two species of wrasses in which this phenomenon has been documented, metamorphosis was delayed beyond a designated minimum body size (Victor, 1986) or age (Cowen, 1991) threshold by a reduction in growth rate. Several recent studies of metamorphosis in flatfishes have found that a minimum body size and weight threshold must be achieved prior to metamorphosis (Policansky,

1982, 1983; Chambers and Leggett, 1987; Chambers, Leggett, and Brown, 1988) and that a positive correlation exists between size and age at metamorphosis, i.e. larvae that metamorphose at larger sizes do so after a longer larval period (Chambers and Leggett, 1987; Chambers, Leggett, and Brown, 1988). Based on these two criteria, Stage 1 larvae that are greater than 40 mm SL and 1.39 g that are present throughout the year would be considered older and slower growing than Stage 2 larvae that initiated metamorphosis around 40 mm SL, and, thus, to have delayed metamorphosis.

However, the clear progression of metamorphic scores from June to December, the occurrence of planktonic Stage 3 larvae from December to March, and continued metamorphic changes following settlement argue that metamorphosis in Dover sole is protracted, not delayed. The offshore distribution of metamorphic larvae beyond the nursery grounds and the highly seasonal settlement period suggests that initiation of metamorphosis may be influenced more by seasonal cues than by attainment of a given a body size and weight threshold or environmental cues associated with the settlement area. The large size range over which premetamorphic larvae are found throughout the year can be accounted for by differences in spawning dates and individual growth rates. It is possible that over the 18-25 month planktonic period some premetamorphic larvae may grow faster due to favorable oceanographic conditions, and, so, metamorphose at larger sizes. These two scenarios could be evaluated for Dover sole if either different year-classes are identified among recently settled Stage 3 larvae (delayed metamorphosis) or, if Stage 3 larvae could be identified as early versus late metamorphic individuals and estimates

of their age and growth rate at metamorphosis obtained (differences in growth rates).

One prediction of the theory of saltatory ontogeny (Balon, 1979, 1984, 1985) is that transitions between developmental "steps" (or life history stages) should occur rapidly because intermediate life history stages are thought to be maladapted to their environment and subject to greater risk of predation. In general, metamorphosis in flatfishes would seem to support this prediction, with the transition from plankton to benthos, and the associated morphological and behavioral transformations. occurring anywhere from a few hours (Houde et al., 1970) to a few days (Rosenberg and Laroche, 1982). A rapid metamorphic process may be one mechanism that limits dispersal away from restricted nursery areas such as estuaries. However, flatfishes with protracted planktonic periods are subject, possibly, to long distance transport away from nursery areas and must be ready to take advantage of suitable settling habitat when encountered. Delayed or protracted metamorphosis may be similar in that both processes provide flexibility in response to short-term oceanographic events by extending the time period over which larvae can settle. For Dover sole, one additional advantage of protracted metamorphosis may be the retention of the large and slightly rounded body shape by benthic Stage 3 larvae, which may provide newly settled larvae with a size-shape based refuge from predation.

CHAPTER 3

COMMENTS ON THE SISTER GROUP RELATIONSHIP BETWEEN *Microstomus* AND *Embassichthys* BASED ON CHARACTERS ASSOCIATED WITH METAMORPHOSIS

ABSTRACT

Norman (1934) recognized the pleuronectid genera Microstomus, Embassichthys, Glyptocephalus, and Tanakius as a "primary division" within the Pleuronectini based on their more elongated bodies, and higher counts of vertebrae, fin rays, and scales in a longitudinal series. He suggested that Microstomus plus Embassichthys was a sister group and considered Glyptocephalus to be "close to" Tanakius. An alternative hypothesis by Richardson (1981) suggested that Embassichthys larvae resembled larvae of Glyptocephalus and Tanakius based on a "leptocephalus-like" body shape, and that Microstomus was the primitive sister group of these three genera because of its dorsoventrally deepened body shape.

In order to evaluate Richardson's hypothesis of sister group relationships based on body shape, I examined morphological characters associated with eye migration and metamorphosis, and changes in body shape during ontogeny from post-flexion larvae through benthic juveniles. Richardson's character of a moderate "leptocephalus-like" body shape is an artifact of the paucity and small size range of larvae available for her examination. The body shape of larger Embassichthys larvae is also dorsoventrally deepened and the larvae appear to undergo changes in body shape during metamorphosis similar to Microstomus. Three synapomorphies that support Norman's hypothesis of an Embassichthys plus Microstomus sister group relationship are dissociation of initial eye migration with metamorphosis, a stasis during eye migration in premetamorphic larvae, and a retention of a larval-like appearance to the eyes of newly settled specimens.

INTRODUCTION

Norman (1934) recognized the pleuronectid genera Microstomus, Embassichthys, Glyptocephalus, and Tanakius as a "primary division" within the Pleuronectini based on their more elongated bodies, and higher counts of vertebrae, fin rays, and scales in a longitudinal series. Although he considered this arrangement to be artificial and doubted that these genera form a natural group, he did suggest a sister group relationship between Microstomus and Embassichthys and thought that Glyptocephalus was "close to" Tanakius. A recent analysis of phenetic data also indicated that Embassichthys and Microstomus were more similar to each other than to Glyptocephalus (sensu lato) or Tanakius, although no synapomorphies were identified (Sakamoto, 1984). Chiu (1987) identified five synapomorphies that supported the monophyly of these four genera and referred to the group as the "glyptocephaline" flounders. He also identified six synapomorphies from the premaxilla, upper gill arch, pelvic fin, and caudal fin that supported an Embassichthys plus Microstomus sister group relationship.

Alternatively, Richardson (1981) proposed that Embassichthys larvae resembled Glyptocephalus and Tanakius larvae based on strong, postanal pigment banding, lack of otic spines, and a "leptocephalus-like" body shape. She also suggested a polarity to the body shape character that can be tested; she characterized larval Glyptocephalus and Tanakius as having "pronounced leptocephalus-like shapes", larval Embassichthys as having a "moderate leptocephalus-like shape" and larval Microstomus, which she considered to be the primitive sister group to the other three genera, as having "much

less tendency toward [a] long leptocephalus-like shape with dorsoventral deepening of body instead."

Richardson (1981) examined six Embassichthys larvae between 9.8-16.2 mm standard length (SL), the largest of which was optically symmetrical and undergoing notochord flexion. Microstomus pacificus larvae of comparable lengths may be optically symmetrical or asymmetrical and are beginning to increase in body depth and become rounder in body shape (Chapter 1). Thus, the "leptocephalus-like" shape of Embassichthys may be an artifact of the paucity and small size range of larvae examined.

The discovery of additional larval material of Embassichthys and a recent examination (Chapter 1) of metamorphosis and ontogeny of body shape in M. pacificus provided the stimulus to test the hypothesis of a Microstomus plus Embassichthys sister group relationship. My examination is based on characters associated with eye migration, the development of five metamorphic characters found to change during metamorphosis in M. pacificus (Chapter 1), and a multivariate analysis of body shape change from post-flexion larvae through benthic juveniles.

MATERIALS AND METHODS

Materials.--I examined the following numbers of larvae and juveniles: Microstomus pacificus - 201, Embassichthys bathybius - 12, Glyptocephalus zachirus - 94, and Parophrys vetulus - 42. Larvae of M. pacificus, E. bathybius, and G. zachirus were obtained from 102 midwater trawl collections taken off Oregon between 1961 and 1978. Details of sampling methods are given in Pearcy (1976), Pearcy et al. (1977a), and Pearcy et al. (1977b). Additional material of larval M.

pacificus was obtained from the Larval Fish Collection, National Marine Fisheries Service, Northwest and Alaska Fisheries Center.

Juvenile M. pacificus were obtained from benthic trawls off the Oregon coast with a 27 m shrimp net towed for 5 min on the bottom. Juveniles were collected at depths of 74-142 m during March, 1988 and January, 1989. Additional juvenile material was obtained with a 7.5 m otter trawl towed for 30 min at depths between 40-75 m in Monterey Bay, California during March, 1988.

Juvenile G. zachirus were collected in a benthic trawl taken off Willapa Bay, Washington during September, 1987 with a 7.5 m otter trawl towed for 30 min at a bottom depth of 115 m.

Larval and juvenile P. vetulus and juvenile E. bathybius were obtained from specimens deposited in the fish collection at Oregon State University (OS).

A list of material examined is given in Appendix A. Specimen information includes: Oregon State University Fish Collection (OS) catalog number, whether the specimen was x-rayed (XR) or cleared and stained (CS), locality, number of specimens, and SL or SL size range.

Methods.--Taxa were united based on the distribution of shared, derived characters (synapomorphies) following the methodology of Hennig (1966). Parophrys was selected as an outgroup (Watrous and Wheeler, 1981) based on a recent phylogenetic hypothesis of relationships between Microstomus, Embassichthys, and Glyptocephalus and Tanakius (Fig. 18; Chiu, 1987). Based on this hypothesis, it was possible to compare the distribution of a character among the four species and determine if that character evolved in the presumed common

ancestor and persisted, or if it evolved independently in one or more species (Funk and Brooks, 1990).

I recognized the initiation of metamorphosis by a change to the adult or transitional character state in any of the five metamorphic characters listed in Table 9. I selected metamorphic characters that were common to two or more of the taxa, thus autapomorphies such as elongation of the intestine in Microstomus (Chapter 1) were excluded. Binary characters were scored as (0) for larval and (1) for adult character states. Multistate characters were scored as (0) for larval, (1) for transitional, and (2) for adult character states. Scoring of the transitional character state for dentition was modified for Parophrys and Glyptocephalus to include the development of a row of recurved canines. Development of incisors was not observed in either Parophrys or Glyptocephalus. Specimens were classified as premetamorphic larvae (metamorphic score = 0), metamorphic larvae (1-7), or juveniles (8) based on a metamorphic score that was the summation of character-state values.

Size at initial eye migration and size range over which eye migration occurred was determined by coding the position of the left eye during migration as (0) symmetrical, (1) left side migrating, (2) dorsal ridge of cranium, and (3) adult position. Initial eye migration was not used in calculating the metamorphic score because of the dissociation of eye migration with metamorphosis in M. pacificus (Chapter 1).

Body shape analysis.--Only larvae that had undergone notochord flexion and had distinct hypural, epural and parhypural elements were used. Standard length, body depth at anus (BD), and right eye diameter were

recorded. All measurements were taken to the nearest 0.1 mm. Measurements on specimens <20 mm SL were made with a camera lucida attached to a Zeiss dissecting microscope with an ocular micrometer. Larger specimens were measured with dial calipers.

A system of truss distances (Strauss and Bookstein, 1982; Strauss and Fuiman, 1985) between seven anatomical landmarks (Fig. 1) was used to summarize changes in body shape that occurred during ontogeny. Because of a lack of distinct, external landmarks (e.g. origin of a second dorsal fin) on the body of flatfish, the straight-line intersection of the landmark (e.g. distal end of first caudal neural spine) with the midsagittal outline was used. This procedure was followed with landmarks b, c, d, and e. Anatomical landmarks were: a) medial tip of premaxilla; b) intersection of distal end of cleithrum with midsagittal outline; c) intersection of a straight line drawn from landmark b to the midsagittal-dorsal outline of the body; d) dorsal intersection of distal end of first caudal-neural spine with midsaggital outline; e) ventral intersection of distal end of first hemal spine with midsagittal outline; f) posterior, dorsal corner of epural; and, g) posterior, ventral corner of parhypural. Landmarks were digitized and truss distances computed as Euclidean distances (Strauss and Bookstein, 1982).

Position of anatomical landmarks were taken from specimens that were either cleared and differentially stained following the method of Potthoff (1984) or x-rayed. In certain cases, x-rayed specimens were also cleared and stained. Landmarks on larvae <20 mm SL were transferred to paper with a camera lucida attached to a Zeis dissecting microscope. Cleared and stained larvae between 20-50 mm

were photographed and reference points transferred to paper from photographic negatives with a negative enlarger. Reference points on specimens >50 mm were taken directly from x-rays.

Statistical Methods.--Descriptive statistics, simple linear regression, outlier-rejection regression and plotting procedures were performed in STATGRAPHICS (Statistical Graphics Corporation, 1987). Outlier-rejection regression was performed on each species to determine if one, or more, regressions would best describe the relationship between BD and SL for premetamorphic larvae, metamorphic larvae and juveniles. Specimens with metamorphic scores of 0 were the initial group and I progressed by adding specimens with progressively higher scores until the best fit of the model was achieved, as indicated by minimizing the mean square error associated with the residuals and maximizing the coefficient of determination.

Principal component (PC) analysis of truss distances was used to summarize changes in body size and shape that occurred during ontogeny. Principal components were computed from the covariance matrix of logarithmically transformed truss distances (Jolicoeur, 1963; Strauss and Fuiman, 1985) with BIOΣTAT II (Pimental and Smith, 1986).

RESULTS

Eye migration and metamorphosis.--Initiation of eye migration (eye position = 1) between 10-25 mm SL is considered to be the primitive condition in pleuronectiformes (Ahlstrom et al., 1984). Parophrys, Microstomus, and Embassichthys initiate eye migration within this size range (Table 10). In contrast, Glyptocephalus larvae initiate eye migration between 37.6-68.1 mm SL (Table 10). The smallest optically

asymmetrical Glyptocephalus larvae (37.6 mm SL) is 3x larger than the minimum size at which eye migration is initiated in Microstomus, and larvae as large as 61.7 mm SL remain optically symmetrical (Table 10). Thus, increased size at initial migration of the left eye appears to be an advanced character of Glyptocephalus.

Published accounts of metamorphosis in flatfishes generally describe development of metamorphic characters as being concurrent with initial eye migration (Ryland, 1966; Fukuhara, 1986, 1988), which I consider to be the primitive character state for flatfishes. The advanced character state is dissociation of eye migration with development of metamorphic characters, as described for M. pacificus (Chapter 1). Both Parophrys and Glyptocephalus exhibit concurrent, or nearly so, development of metamorphic characters (metamorphic score = 1) with initial eye migration (eye position = 1; Tables 11 and 12). In contrast, Embassichthys also appears to exhibit the advanced character state; all four Embassichthys larvae (20.2-35.6 mm SL) have initiated eye migration (Table 10) and have metamorphic scores of 0 (Table 11).

Eye migration in flatfishes is considered to be a continuous process that proceeds without pause from ocular symmetry (eye position = 0) to complete asymmetry with the eye in the adult position (eye position = 3; Ryland, 1966; Fukuhara, 1986, 1988; Chambers and Leggett, 1987). I consider continuous migration of the left eye to be a primitive character and arrested eye migration, with the eye remaining on the dorsal ridge of the cranium (eye position = 2) across a large size range, to be an advanced character. Both Parophrys and Glyptocephalus exhibit continuous eye migration, with

Parophrys accomplishing eye migration across a limited size range (approximately 16-22 mm SL; Table 10). Glyptocephalus larvae also appear to have continuous eye migration, based on the small size range (12 mm) between the smallest larvae with eyes in positions 1 and 3, in comparison with the large size range (approximately 30 mm) for Microstomus larvae (Table 10). Additionally, there is a paucity of larvae with the left eye located on the dorsal ridge of the cranium in Pearcy's midwater trawl collections. Only 12 of 363 Glyptocephalus larvae examined by Pearcy et al. (1977) have the eye on the dorsal margin of the cranium, suggesting that this stage is transitory and that these larvae may be moving toward the benthos to settle.

In contrast, eye migration in Microstomus is arrested once the left eye has reached the dorsal ridge of the cranium (Table 10) and it remains in this position until metamorphosis (Chapter 1). Three Embassichthys larvae (30.0-35.6 mm SL) have the left eye on the dorsal ridge of the cranium (Table 10), but have not developed any metamorphic characters (Tables 11 and 12), suggesting that eye migration is also arrested in Embassichthys. Acquisition of metamorphic characters in Embassichthys presumably is synchronous with the resumption of eye migration, similar to Microstomus (Chapter 1).

Another character shared between Microstomus and Embassichthys is retention of a larval-like appearance to the eyes of recently settled specimens. Mean right eye diameter in Microstomus appears to increase gradually between planktonic and newly settled benthic juveniles (planktonic, mean = 3.18 mm, N = 23; benthic, mean = 4.14 mm, N = 55), and the appearance of the eyes resembles that of planktonic, metamorphic larvae (Fig. 19). The eyes of one

Embassichthys juvenile (59.7 mm SL), collected in a bottom trawl from 450-500 fathoms, are distinctly larval in appearance and are only slightly larger (right eye diameter = 3.51 mm) than the mean right eye diameter of planktonic Microstomus with metamorphic scores of 8 (mean = 3.18 mm, N = 23; Fig. 19). The right eyes of two slightly larger Embassichthys juveniles (65.4 and 66.1 mm SL) have increased in diameter to 5.46 and 4.94 mm, respectively, and have a more adult appearance (Fig. 19). In contrast, the average right eye diameter of benthic Glyptocephalus larvae (metamorphic score = 7) is 4.21 mm (N = 14), which is 1.5x as large as planktonic, metamorphic larvae (mean = 2.6 mm, N = 13, metamorphic score = 1-6), and the eyes have developed a distinctly adult-like appearance (Fig. 19).

Body shape changes.--The first two principal component axes describe more than 95% of the total variation in the truss distances for the four species. The first principal component (PC1) accounts for variation in body size during ontogeny, based on positive eigenvector loadings, highly positive correlations between the truss distances and PC1, and large amount of variation associated with each truss distance (Table 13; Fig. 20; Shea, 1985; Strauss and Fuiman, 1985; Tissot, 1988). PC2 describes primarily variation associated with changes in body depth during ontogeny, based on the high eigenvector loading and large amount of variation accounted for by truss distance 8, which approximates maximum body depth (Table 13; Fig. 20).

The "pronounced leptocephalus-like" shape of Glyptocephalus is illustrated by a 68 mm SL larvae of G. zachirus (Fig. 22a). The ontogeny of body shape in Glyptocephalus resembles that of Parophrys, where larvae increase rapidly in BD relative to

body size during metamorphosis, after which there is a decrease in the amount of variation attributable to BD until, in larger specimens, most variation is associated with increasing body size (Figs. 20 and 21). Both Parophrys and Glyptocephalus maintain relatively slender, elongate body shapes throughout their ontogeny, although Glyptocephalus increase in BD at a significantly greater rate than Parophrys (comparison of slopes- $t = 13.7$, $df = 116$, $P < 0.001$; Fig. 23).

Premetamorphic larvae (metamorphic score = 0) of Microstomus (Fig. 22b) and Embassichthys (Fig. 22c) are very deep bodied, reflected by their higher PC2 scores, in comparison with Parophrys and Glyptocephalus premetamorphic larvae (Figs. 20 and 21) and increased linear regression intercept, which is significantly different from that of Parophrys ($t = 40.5$, $df = 133$, $P < 0.001$; Fig. 24). Premetamorphic Microstomus larvae exhibit almost linear increases in BD relative to SL prior to metamorphosis, and the three Embassichthys larvae appear to demonstrate a similar relationship (Fig. 24). During metamorphosis in Microstomus, BD decreases without any apparent concomitant changes in SL (Chapter 1), after which BD and SL once again begin to increase, although the slope of the relationship has greatly decreased (Fig. 24). Lower PC2 scores for Embassichthys juveniles and adults relative to the three larger premetamorphic larvae suggest that reductions in BD occur during metamorphosis, similar to Microstomus (Figs. 20 and 21). Significant differences ($t = 10.0$, $df = 81$, $P < 0.001$) in the slope of the regression of BD against SL exist between Microstomus juveniles (Fig. 24) and Embassichthys juveniles ($\ln BD = 0.25 + 0.72 \ln(SL)$, $N = 8$, R^2

= 86.5, $P < 0.002$); however, there is no significant difference ($t = 1.5$, $df = 97$, $P > 0.10$) in the intercepts, indicating that following metamorphosis juvenile Embassichthys increase in BD at a slightly increased rate.

DISCUSSION

Richardson (1981) hypothesized a relationship between Embassichthys, Glyptocephalus and Tanakius based on a lack of otic spines, strong, mediolateral postanal pigment banding and a "leptocephalus-like" body shape. The pigmentation character is somewhat equivocal because the 16.2 mm SL Embassichthys larvae illustrated by Richardson (1981) possess bands of pigmentation along the margins of the epaxial and hypaxial musculature, somewhat similar to Microstomus larvae. In addition, the moderate "leptocephalus-like" body shape character of Embassichthys is an artifact of the small size range of larvae examined. Larger Embassichthys larvae are deep bodied and clearly similar in overall body shape to Microstomus. In fact, the three larger Embassichthys larvae I examined are so similar to Microstomus larvae that initially they were identified in the Oregon State University collection as M. pacificus and 16 M. pacificus larvae were misidentified as E. bathybius larvae in the British Columbia Provincial Museum. Characters separating Embassichthys from Microstomus are lack of otic spines and relatively longer dorsal and anal fin rays (Fig. 25).

Four early life history characters that support an Embassichthys and Microstomus sister group relationship are deep bodied, round body shape of premetamorphic larvae, dissociation of initial eye migration with metamorphosis, stasis during eye migration

in premetamorphic larvae, and retention of a larval-like appearance to the eyes in newly settled specimens. Although there is no direct evidence of body depth reductions during metamorphosis in Embassichthys, the decrease in variance along PC2 from premetamorphic larvae to juveniles and a growth pattern that seems to consist of two separate periods, similar to Microstomus, suggest that similar reductions in BD occur during metamorphosis. However, it is also possible that Embassichthys larvae maintain their deep-bodied shape during metamorphosis, similar to Glyptocephalus maintaining its elongate body shape, and that a single linear regression could describe the BD versus SL relationship. Additional material of larger (>40 mm SL) premetamorphic and metamorphic Embassichthys larvae is required to evaluate this character.

Increased minimum size at metamorphosis and large body size of premetamorphic larvae appears to be synapomorphies uniting Glyptocephalus, Microstomus and Embassichthys. Although no metamorphic Embassichthys larvae are available for examination, I estimate the range over which metamorphosis occurs to be >35.6 (largest premetamorphic larvae) and <59.7 mm SL (juvenile with larval-like appearing eyes). Okiyama and Takahashi (1976) illustrate a 23.1 mm total length Tanakius kitaharae larvae undergoing metamorphosis with the left eye near the dorsal margin of the cranium and fin rays developing in the pectoral finfold. Thus, Tanakius may be the most primitive member of the group, being most similar to Parophrys in undergoing metamorphosis at smaller sizes and retaining the association of eye migration with development of metamorphic characters.

Calder (1984) suggests that the deviation of a character from the ancestral allometric trajectory requires a compensation or "counterbalancing" by other characters to maintain developmental integrity. The growth trajectory of premetamorphic Microstomus and Embassichthys larvae, in comparison with the single growth trajectories exhibited by all stages of Parophrys and Glyptocephalus, may be such a character. Harris (Chapter 1) suggests that eye migration and increasing BD are synchronized developmental events in Microstomus and that arrested eye migration may be the result of selection for the large, round body shape of premetamorphic larvae, facilitated by the upward shift of the BD intercept. This suggests that eye migration is associated with increasing BD in the common ancestor of Microstomus and Embassichthys. Increasing BD does seem to be associated with metamorphosis in Parophrys (Fig. 20) and empirical studies of flatfish development have documented an increase in BD during metamorphosis (Fukuhara, 1986, 1988). Reductions in BD during metamorphosis in Microstomus and Embassichthys apparently serve as the compensatory mechanism that converges the large, round body shape of premetamorphic larvae with that of the more elongate form of adults.

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Table 1. Characters used to quantify metamorphosis in Microstomus pacificus.

Character	Character State
Eye position	0 - Left side of head or dorsal ridge. 1 - Right side of head, adult position.
Position of dorsal fin on head	0 - First ray posterior to orbit of left eye. 1 - First ray equal with posterior of, or is anterior to, left eye.
Dentition	0 - Canines 1 - Canines present, incisors developing 2 - Incisors
Pectoral fin	0 - Round, paddlelike shape, < adult compliment of rays, no radials formed. 1 - Intermediate shape, adult compliment of rays, cartilaginous radials. 2 - Rectangular base, ossified radials and rays.
Posterior process of coracoid	0 - Straight, angled posteriorly. 1 - Resorption beginning, tip curled into hook. 2 - Resorption complete, process absent.
Pigmentation	0 - Planktonic coloration. 1 - Benthic coloration.
Intestine	0 - Coiled arrangement. 1 - Initial elongation of intestine past first anal fin pterygiophore. 2 - ln(snout-to-intestine)/ln SL = 0.87.

Table 2. Component correlations and percent variation of 13 truss distances from two multigroup discriminant functions analyses on 201 Dover sole. Analysis One - Stages 1-5; Analysis Two - Stages 1-4, with Stage 1 larvae <39.0 mm SL and Stage 5 juveniles excluded.

	Analysis One				Analysis Two			
	Component correlations		Percent variation		Component correlations		Percent variation	
Truss Distance	DF1	DF2	DF1	DF2	DF1	DF2	DF1	DF2
1	0.28	0.25	96.52	2.26	0.14	-0.01	96.14	1.91
2	0.27	0.17	73.80	24.15	0.13	0.27	26.47	71.54
3	0.16	0.41	99.36	0.58	-0.26	-0.26	99.87	0.04
4	0.12	0.33	93.24	5.48	-0.43	-0.02	75.05	23.35
5	0.23	0.40	86.79	12.26	-0.09	-0.32	5.32	37.82
6	0.22	0.41	56.76	27.63	-0.11	-0.36	3.79	96.18
7	0.14	0.32	25.98	34.80	-0.30	-0.07	71.74	9.45
8	0.04	0.40	99.56	0.23	0.70	-0.25	71.01	0.02
9	0.29	0.44	19.24	75.30	0.12	-0.43	6.84	91.33
10	0.09	0.36	99.86	0.00	-0.59	-0.12	99.80	0.17
11	0.12	0.40	97.14	0.09	-0.49	-0.27	69.29	29.46
12	0.22	0.40	92.44	7.05	-0.12	0.33	3.77	24.88
13	0.22	0.41	67.52	22.49	-0.13	-0.36	4.83	95.13
Eigenvalues	15.25	1.53			7.74	1.53		
% variation	88.70	8.88			81.61	16.09		

Table 3. Eigenvectors, component correlations, and percent variation of 13 truss distances from a principal component analysis on 201 Dover sole.

	Eigenvectors		Component correlations		Percent variation	
Truss Distance	PC1	PC2	PC1	PC2	PC1	PC2
1	0.26	-0.48	0.902	-0.349	81.4	12.2
2	0.23	-0.29	0.939	-0.254	88.1	6.5
3	0.29	0.21	0.969	0.146	93.8	2.1
4	0.25	0.26	0.962	0.211	92.6	4.5
5	0.33	-0.14	0.993	-0.089	98.5	0.8
6	0.33	-0.11	0.993	-0.065	98.7	0.4
7	0.23	0.04	0.972	0.032	94.4	0.1
8	0.25	0.55	0.900	0.416	81.0	17.3
9	0.27	-0.26	0.966	-0.193	93.4	3.7
10	0.24	0.31	0.957	0.257	91.6	6.6
11	0.25	0.24	0.978	0.194	95.6	3.8
12	0.33	-0.11	0.995	-0.069	99.1	0.5
13	0.36	-0.10	0.995	-0.058	99.0	0.3
Eigenvalues	2.58	0.11				
% variation	93.6	4.1				

Table 4. Standard length size class (SL mm), ontogenetic stage, number (N), mean lengths and standard errors (se) for the first caudal neural spine (CNS), its immediate anterior (ACNS) and posterior (PCNS) pterygiophore, first hemal spine (CHS), and its immediate anterior (ACHS) and posterior (PCHS) pterygiophores.

SL size class	Stage	N	CNS(se)	N	ACNS(se)	N	PCNS(se)	N	CHS(se)	N	ACHS(se)	N	PCHS(se)
≤19.99	1	15	1.74(0.14)	12	2.32(0.14)	12	2.34(0.25)	15	2.80(0.18)	9	2.86(0.34)	9	2.94(0.38)
20.00-29.99	1	14	3.52(0.13)	4	5.20(0.30)	4	5.30(0.26)	14	5.48(0.19)	3	5.58(0.39)	3	5.58(0.39)
30.00-39.99	1	4	5.35(0.12)	1	7.00(0.00)	1	7.30(0.00)	4	8.39(0.30)	1	7.40(0.00)	1	7.40(0.00)
40.00-49.99	1	9	6.59(0.22)	2	9.23(0.00)	2	9.17(0.07)	9	10.20(0.23)	2	10.40(0.26)	2	10.60(0.07)
	2	2	5.98(0.00)					2	8.32(0.13)				
	3	5	4.47(0.17)	2	4.75(0.20)	2	4.88(0.20)	5	6.95(0.86)	2	5.40(0.07)	2	5.53(0.20)
	4	11	3.47(0.06)	11	3.81(0.11)	11	3.82(0.11)	11	4.42(0.12)	11	4.74(0.11)	11	4.73(0.11)
50.00-59.99	1	4	7.48(0.49)	2	10.92(0.65)	2	10.90(0.65)	4	10.79(0.74)	2	10.01(0.39)	2	10.34(0.07)
	2	17	7.67(0.16)	4	10.99(0.57)	4	11.18(0.45)	17	10.44(0.24)	4	10.60(0.46)	4	10.60(0.46)
	3	19	5.24(0.14)	3	4.81(0.91)	3	4.72(0.88)	19	6.79(0.22)	4	5.17(0.37)	4	5.23(0.39)
	4	14	4.05(0.13)	14	3.92(0.11)	14	3.99(0.11)	14	5.16(0.15)	14	5.14(0.11)	14	5.18(0.11)
60.00-69.99	3	6	5.23(0.18)					6	7.09(0.32)				
	4	2	4.36(0.20)	2	4.16(0.00)	2	4.20(0.04)	2	5.85(0.52)	2	5.53(0.20)	2	5.40(0.07)
70.00-79.99	5	1	5.85(0.00)	1	4.55(0.00)	1	4.55(0.00)	1	8.20(0.00)	1	6.60(0.00)	1	6.60(0.00)
80.00-89.99	5	1	6.11(0.00)	1	4.55(0.00)	1	4.55(0.00)	1	8.97(0.00)	1	6.11(0.00)	1	6.11(0.00)
≥90.00	5	2	9.04(0.59)	2	7.70(0.50)	2	6.58(0.73)	2	10.16(0.25)	2	9.60(0.10)	2	8.92(0.99)

Table 5. Distribution by month of planktonic larvae in Stages 1-4 collected in midwater trawls, 1961-1978. N = number of samples collected during each month.

		Stage			
Month	N	1	2	3	4
January	87	7	2	3	0
February	120	8	1	5	0
March	157	6	0	3	0
April	174	7	0	0	1
May	85	4	0	0	0
June	299	12	1	0	0
July	397	39	1	0	0
August	237	4	2	0	0
September	417	2	8	0	0
October	74	2	3	0	0
November	165	3	3	0	0
December	163	5	9	1	0

Table 6. Number of Stage 2 larvae by metamorphic score for each month, 1961-1978. N = number of samples collected during each month.

		Metamorphic Score							
Month	N	1	2	3	4	5	6	7	8
January	87							2	
February	120								1
March	157								
April	174								
May	85								
June	299	1							
July	397								
August	237	1	1						
September	417		1		4	2	1		
October	74					1	1		1
November	165							2	1
December	163							2	7

Table 7. Distance offshore (nautical miles) of planktonic larvae in Stages 1-3 collected in midwater trawls, 1961-1978, along the Newport Hydroline transect (44°40′ N latitude). N = number of samples collected at that distance.

Distance Offshore	N	Stage 1	Stage 2	Stage 3
25	66	0	0	1
30	7	1	0	0
45	110	2	0	3
50	349	18	7	4
55	13	1	2	0
60	47	5	2	0
65	1012	38	7	1
70	18	7	0	0
80	43	1	5	0
100	10	0	1	0
105	9	2	0	0
125	14	2	0	0
145	13	2	1	0

Table 8. Distribution of planktonic larvae in Stages 1-4 collected in midwater trawls, 1961-1978, along the Newport Hydroline transect, 44°40′ N latitude. Depth (m) is maximum depth fished. N = number of samples in that depth range.

		Stage			
Depth	N	1	2	3	4
0-100	314	27	0	0	1
101-200	552	5	1	2	0
201-300	208	21	9	5	0
301-400	212	1	3	0	0
401-500	153	4	0	1	0
501-600	76	5	6	0	0
601-700	22	1	1	0	0
901-1000	152	1	0	1	0
1001-1100	3	3	2	0	0
1101-1200	7	0	1	0	0
1201-1300	11	0	1	0	0
2001-2100	4	0	1	0	0

Table 9. Characters used to quantify metamorphosis in M. pacificus, E. bathybius, G. zachirus, and P. vetulus.

Character	Character State
Dentition	0 - Canines 1 - Canines present, incisors developing 2 - Incisors
Pectoral fin	0 - Round, paddlelike shape, less than adult compliment of rays, no radials formed. 1 - Intermediate shape, adult compliment of rays, cartilaginous radials. 2 - Rectangular base, ossified radials and rays.
Posterior process of coracoid	0 - Straight, angled posteriorly. 1 - Resorption beginning, tip curled into hook. 2 - Resorption complete, process absent.
Eye position	0 - Left side of head or dorsal ridge. 1 - Right side of head, adult position.
Position of dorsal fin on head	0 - First ray posterior to orbit of eye. 1 - First ray equal with posterior of orbit or in front of eye orbit.

Table 10. Standard length size ranges (mm) of larvae and juveniles <100 mm SL of M. pacificus, E. bathybius, G. zachirus, P. vetulus based on eye position. Eye position codes are (0) symmetrical, (1) left side migrating, (2) dorsal ridge of cranium, and (3) adult position.

Eye Position	N	M. pacificus	N	E. bathybius	N	G. zachirus	N	P. vetulus
0	1	9.6	0		31	28.8-61.7	4	17.4-20.2
1	14	11.7-23.4	1	20.2	25	37.6-68.1	7	16.3-22.3
2	87	15.4-58.1	3	30.0-35.6	2	57.0-57.2	3	16.6-19.5
3	97	41.1-85.6	5	59.7-99.7	30	49.5-85.6	28	16.6-74.4

Table 11. Standard length size ranges (mm) of larvae and juveniles <100 mm SL of M. pacificus, E. bathybius, G. zachirus, P. vetulus based on metamorphic score. Metamorphic scores are (0) premetamorphic larvae, (1-7) metamorphic larvae, and (8) juveniles.

Metamorphic Score	N	M. pacificus	N	E. bathybius	N	G. zachirus	N	P. vetulus
0	99	9.6-57.2	4	20.2-35.6	50	28.8-61.7	5	16.3-22.3
1-7	20	42.8-59.6	0		27	49.5-85.6	24	16.6-36.0
8	80	41.1-85.6	5	59.7-99.7	11	71.4-83.9	13	38.5-74.4

Table 12. Number of specimens <100 mm SL in the four genera classified by eye position and metamorphic score.

Eye Position	Metamorphic Score								
	0	1	2	3	4	5	6	7	8
Parophrys									
0	2	2	0	0	0	0	0	0	0
1	3	4	0	0	0	0	0	0	0
2	0	2	2	0	0	0	0	0	0
3	0	0	0	0	0	1	11	2	13
Glyptocephalus									
0	30	0	0	0	0	0	0	0	0
1	20	6	0	0	0	0	0	0	0
2	0	0	1	1	0	0	0	0	0
3	0	0	0	0	0	3	2	14	11
Microstomus									
0	1	0	0	0	0	0	0	0	0
1	15	0	0	0	0	0	0	0	0
2	82	2	2	0	1	0	0	0	0
3	0	0	0	0	3	3	3	6	80
Embassichthys									
0	0	0	0	0	0	0	0	0	0
1	2	0	0	0	0	0	0	0	0
2	2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	5

Table 13. Eigenvectors, component correlations, and percent variation of 13 truss distances from a principal component analysis on 201 *M. pacificus*, 11 *E. bathybius*, 94 *G. zachirus*, and 42 *P. vetulus*.

	Eigenvectors		Component correlations		Percent variation	
Truss Distance	PC1	PC2	PC1	PC2	PC1	PC2
1	0.28	-0.37	0.92	-0.23	84.4	5.3
2	0.25	-0.23	0.94	-0.16	87.9	2.5
3	0.28	0.15	0.96	0.10	91.8	1.0
4	0.25	0.26	0.96	0.18	91.8	3.3
5	0.30	-0.22	0.98	-0.13	96.2	1.7
6	0.31	-0.19	0.98	-0.12	96.1	1.3
7	0.26	0.12	0.97	0.09	93.0	0.7
8	0.28	0.56	0.93	0.35	85.9	12.3
9	0.26	-0.24	0.97	-0.17	93.6	2.7
10	0.26	0.34	0.97	0.24	93.1	5.6
11	0.27	0.26	0.98	0.18	95.9	3.2
12	0.30	-0.18	0.99	-0.11	97.0	1.2
13	0.30	-0.17	0.98	-0.10	96.8	1.1
Eigenvalues	2.30	0.08				
% variation	92.8	3.2				

Figure 1. Truss distances (numbers) between anatomical landmarks (letters) used to quantify body shape. Anatomical landmarks are described in Methods.

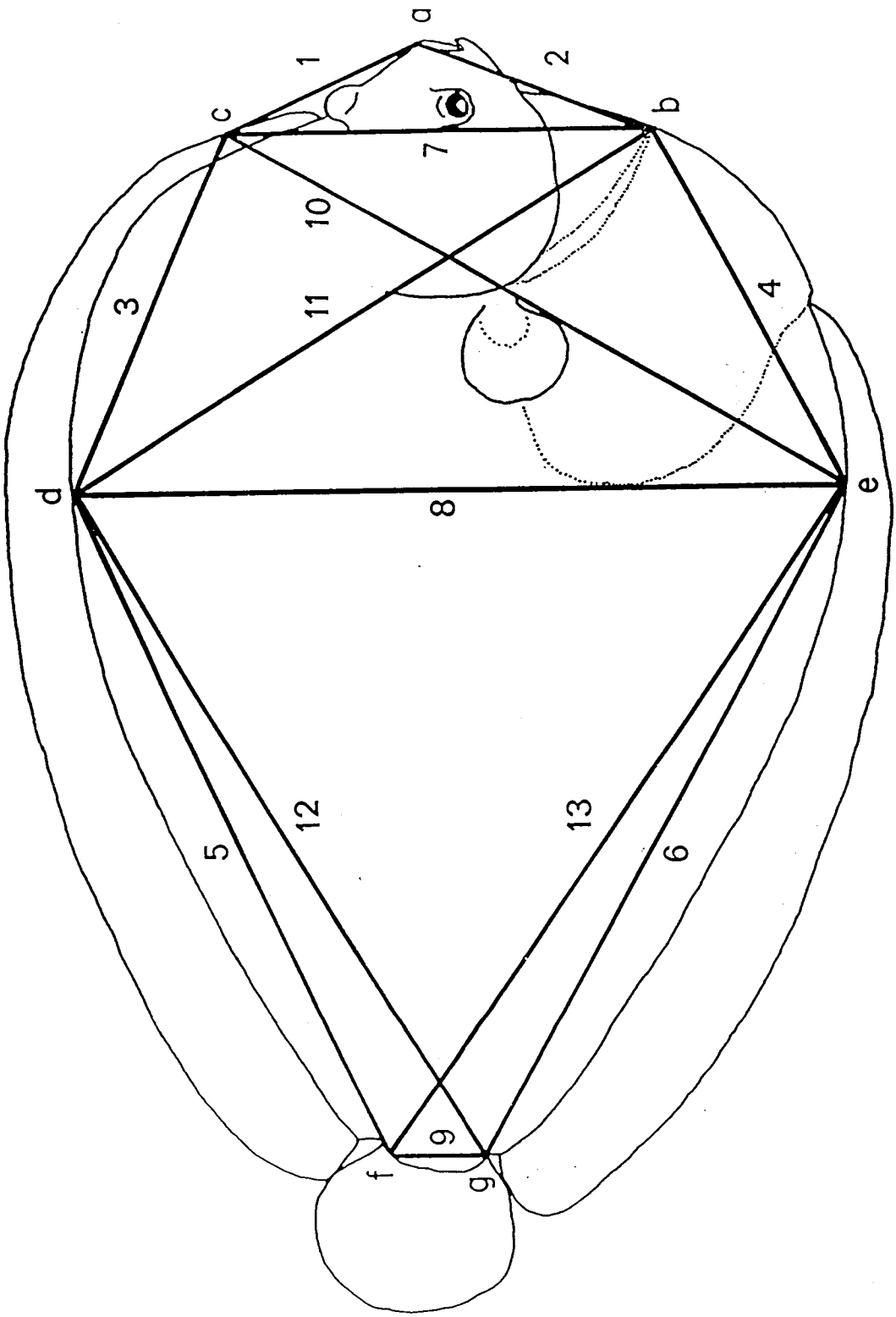


Figure 1

Figure 2. Head profiles illustrating position of the migrating left eye and dorsal fin on: A) Stage 1 larvae, OS12558, 45.7 mm SL; and B) Stage 4 larvae, OS12563, 50.2 mm SL.

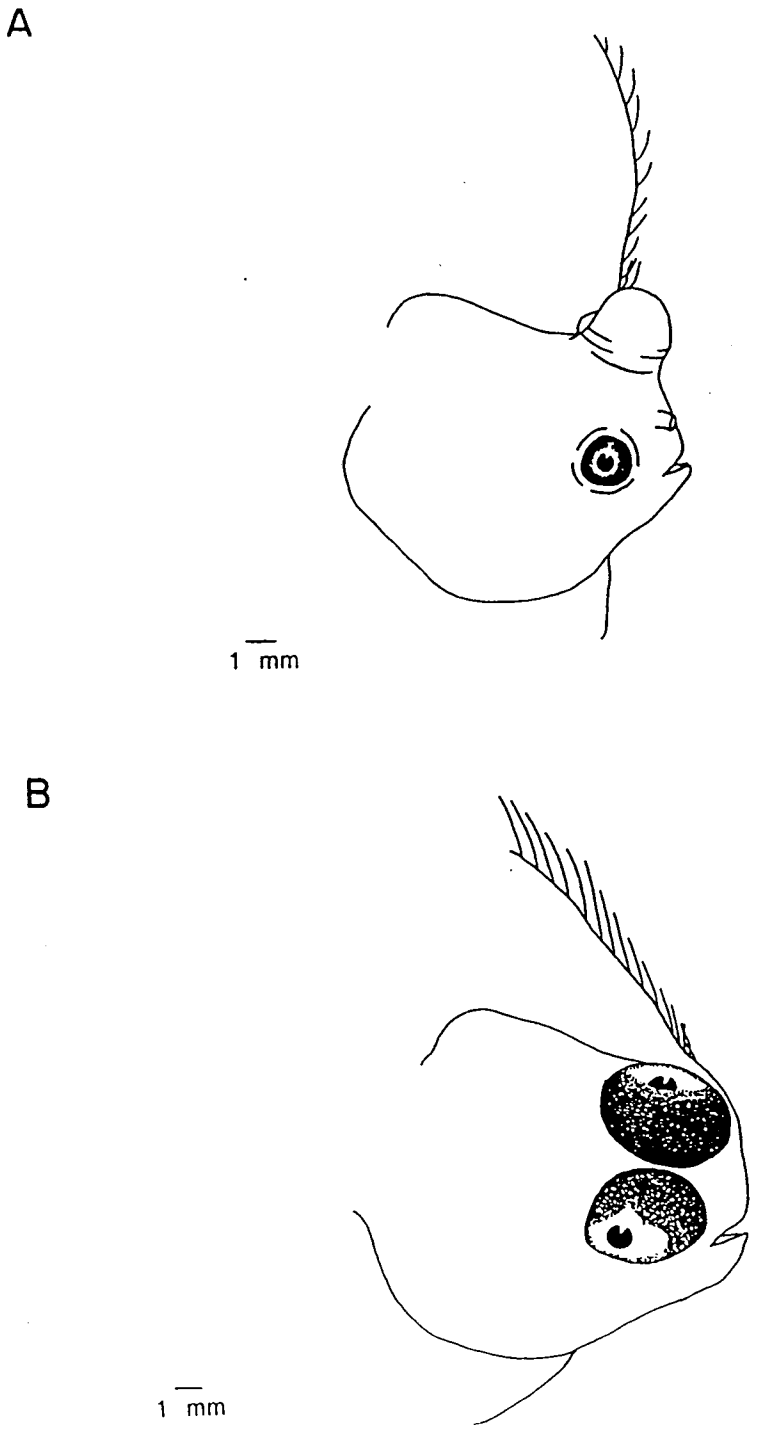


Figure 2

Figure 3. Lateral view of the right jaw apparatus of: A) Stage 1 larvae, OS12578, 55.6 mm SL; B) Stage 2 larvae, OS11377, 58.1 mm SL; C) Stage 5 juvenile, OS11288.

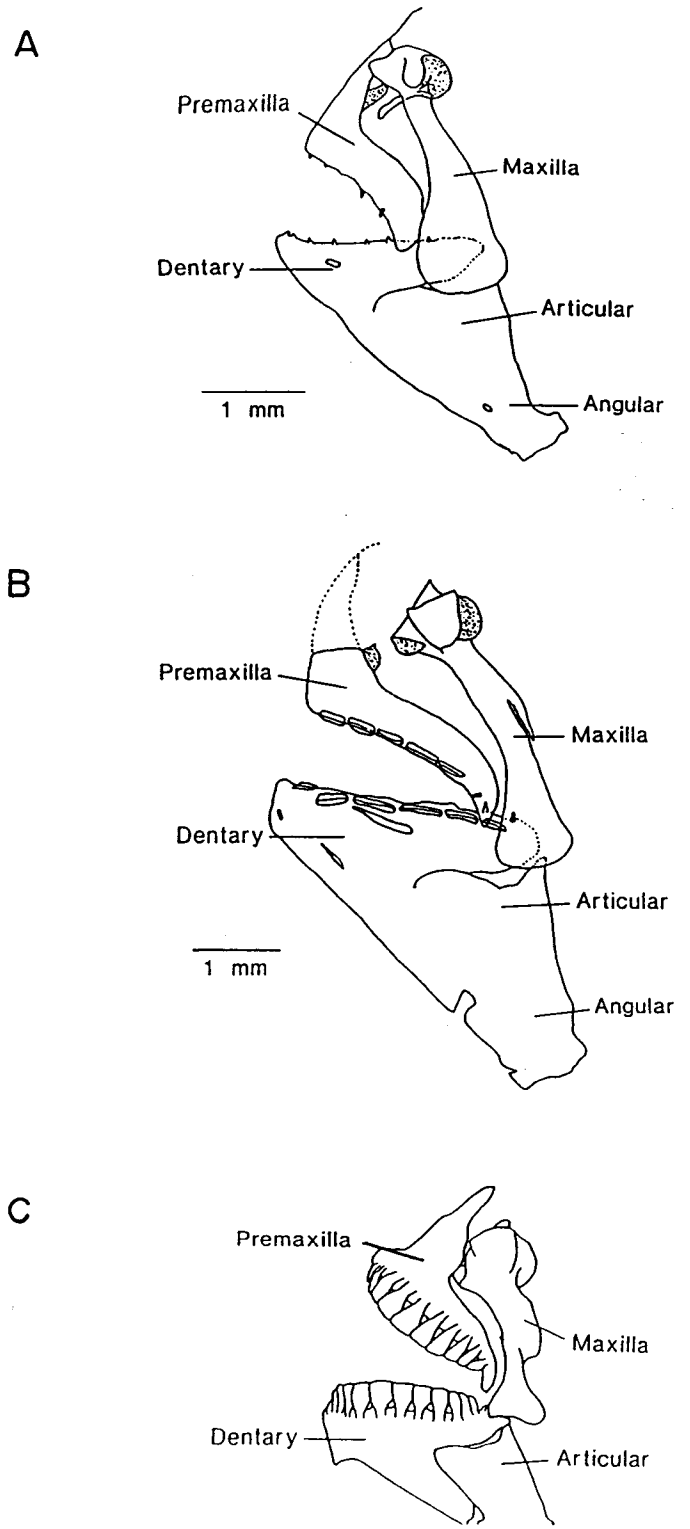
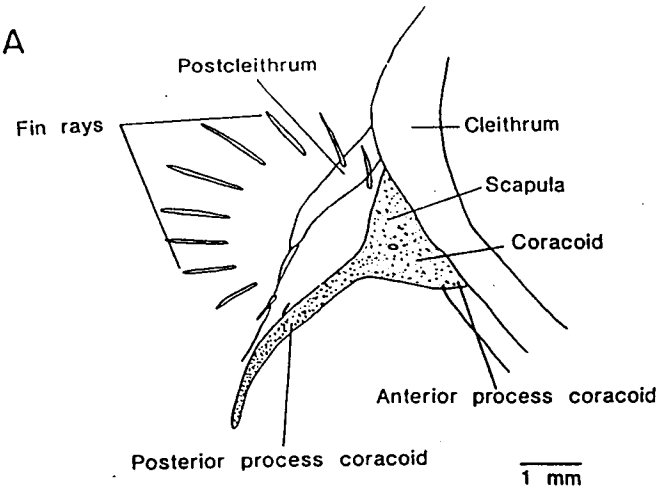
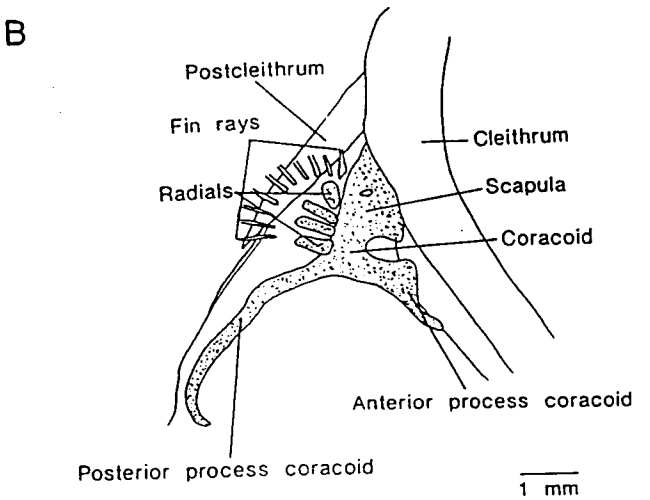

A
Premaxilla
Maxilla
Dentary
Articular
1 mm
Angular
B
Premaxilla
Maxilla
Dentary
Articular
1 mm
Angular
C
Premaxilla
Maxilla
Dentary
Articular

Figure 3

Figure 4. Lateral view of left pectoral girdle and pectoral fin of: A) Stage 1 larvae, OS12558, 45.7 mm SL; B) Stage 2 larvae, OS11377, 58.1 mm SL; C) Stage 4 larvae, OS12563, 50.2 mm SL.


A
Postcleithrum
Fin rays
Cleithrum
Scapula
Coracoid
Anterior process coracoid
Posterior process coracoid
1 mm


B
Postcleithrum
Fin rays
Radials
Cleithrum
Scapula
Coracoid
Anterior process coracoid
Posterior process coracoid
1 mm

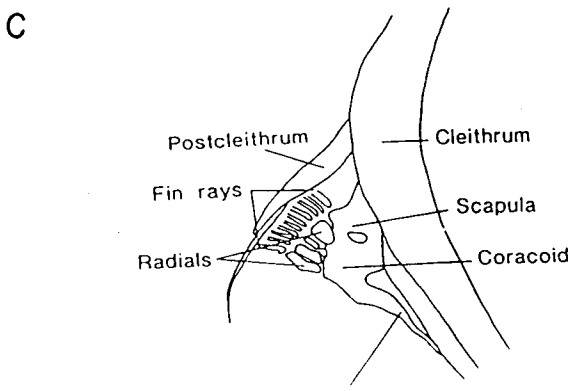

C
Postcleithrum
Fin rays
Radials
Cleithrum
Scapula
Coracoid

Figure 4

Figure 5. Right side, mid-lateral pigmentation patterns of: A) Stage 2 larvae, OS13115, larval pattern with no melanophores on myomeres; B) Stage 2 larvae, OS13118, transitional pattern with melanophores on myosepta; C) Stage 3 larvae, OS13117, aggregated, circular pattern of melanophores on caudal peduncle and anterior trunk.

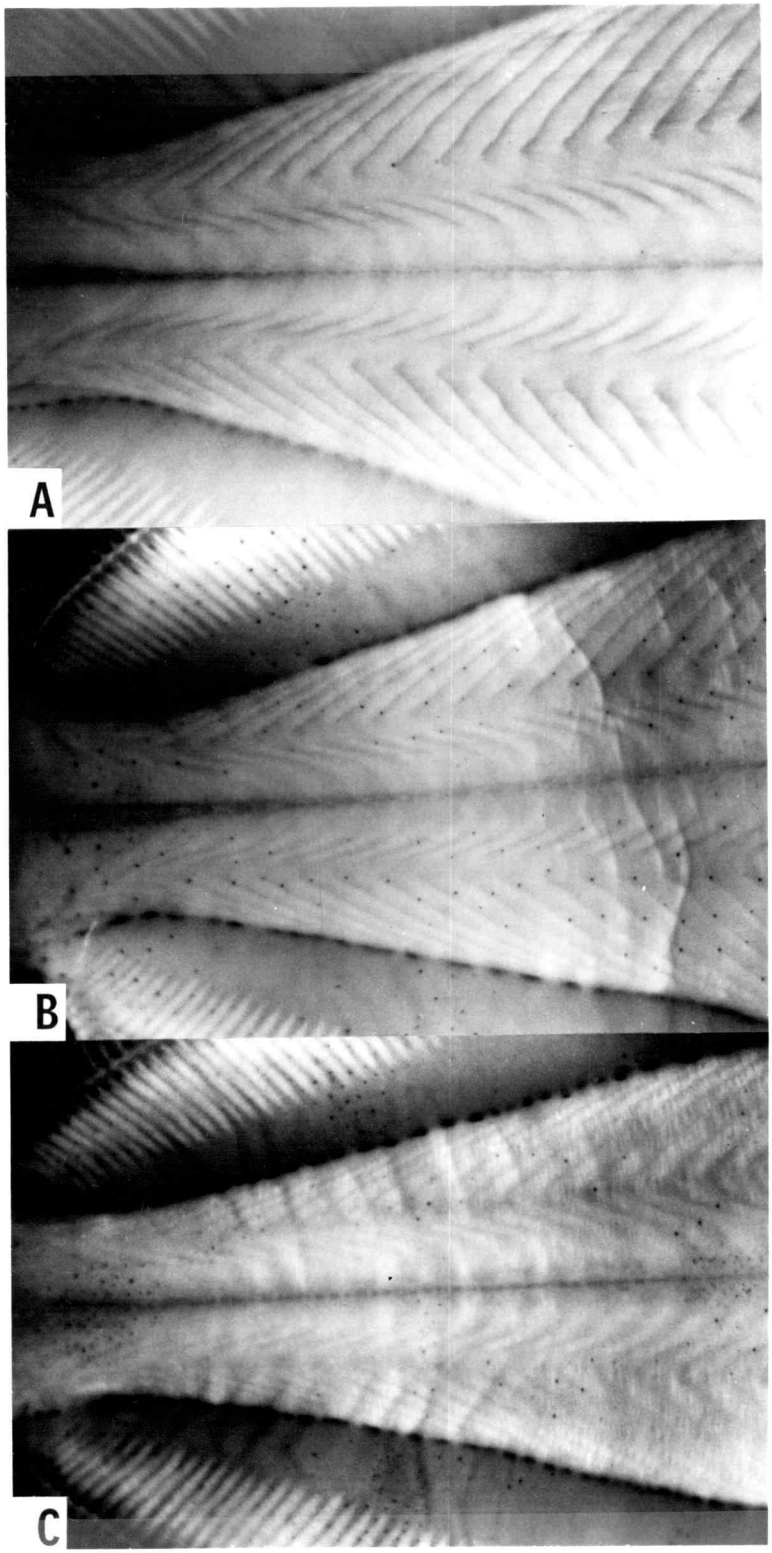


Figure 5

Figure 6. Right lateral view of 5 life history stages of Dover sole: A) Stage 1 larvae, OS13214, 20.4 mm SL; B) Stage 2 larvae, OS11377, 54.5 mm SL; C) Stage 3 larvae (benthic capture), OS13202, 52.4 mm SL; D) Stage 4 larvae, OS13202, 61.5 mm SL; E) Stage 4 larvae, OS13203, 58.4 mm SL; F) Stage 5 (juvenile), OS13204, 78.4 mm SL.

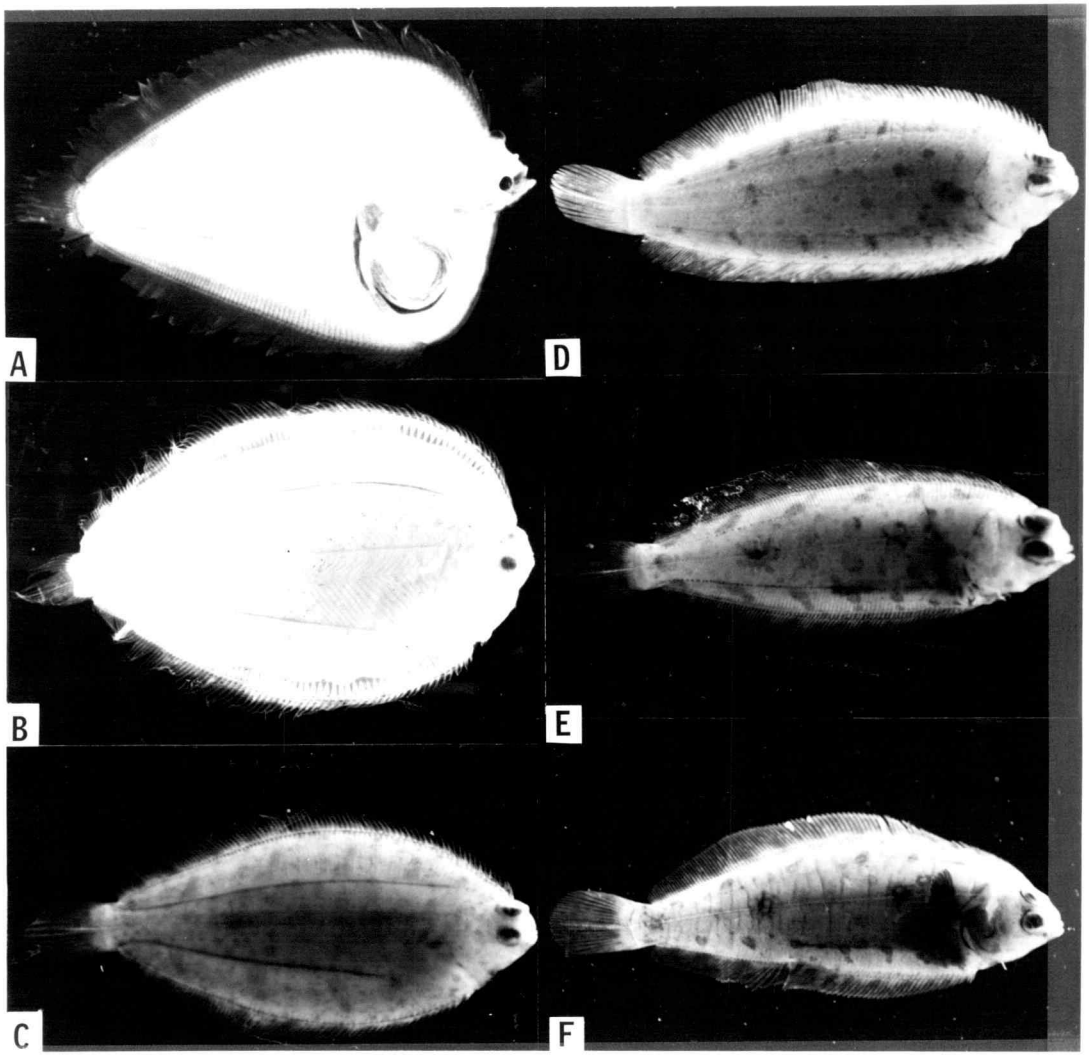


Figure 6

Figure 7. Plot of body depth against standard length for the five life history stages in Dover sole. Symbols represent: solid squares, Stage 1; open circles, Stage 2; solid triangles, Stage 3; solid circles, Stage 4; open squares, Stage 5.

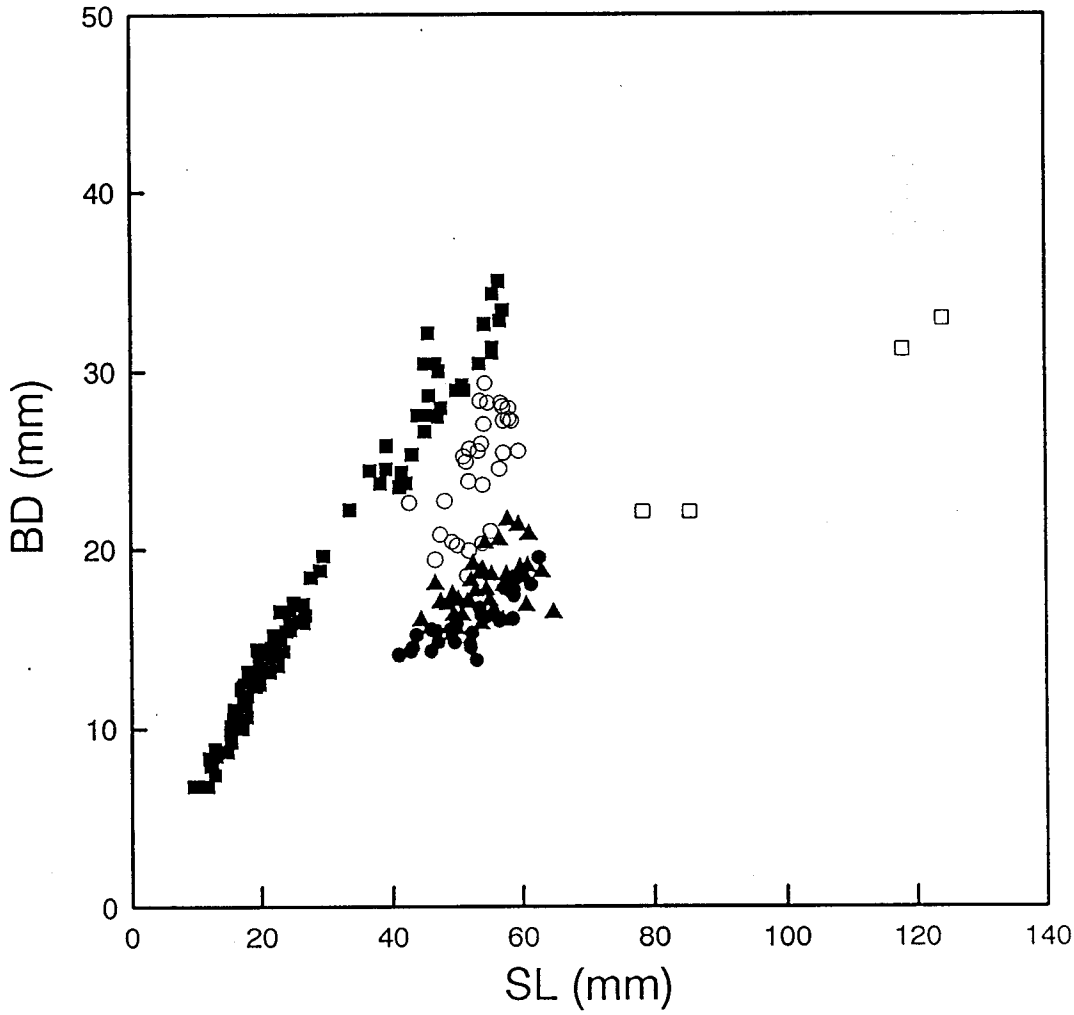


Figure 7

Figure 8. Plot of interorbital distance against standard length for the five life history stages in Dover sole. Symbols represent: solid squares, Stage 1; open circles, Stage 2; solid triangles, Stage 3; solid circles, Stage 4; open squares, Stage 5.

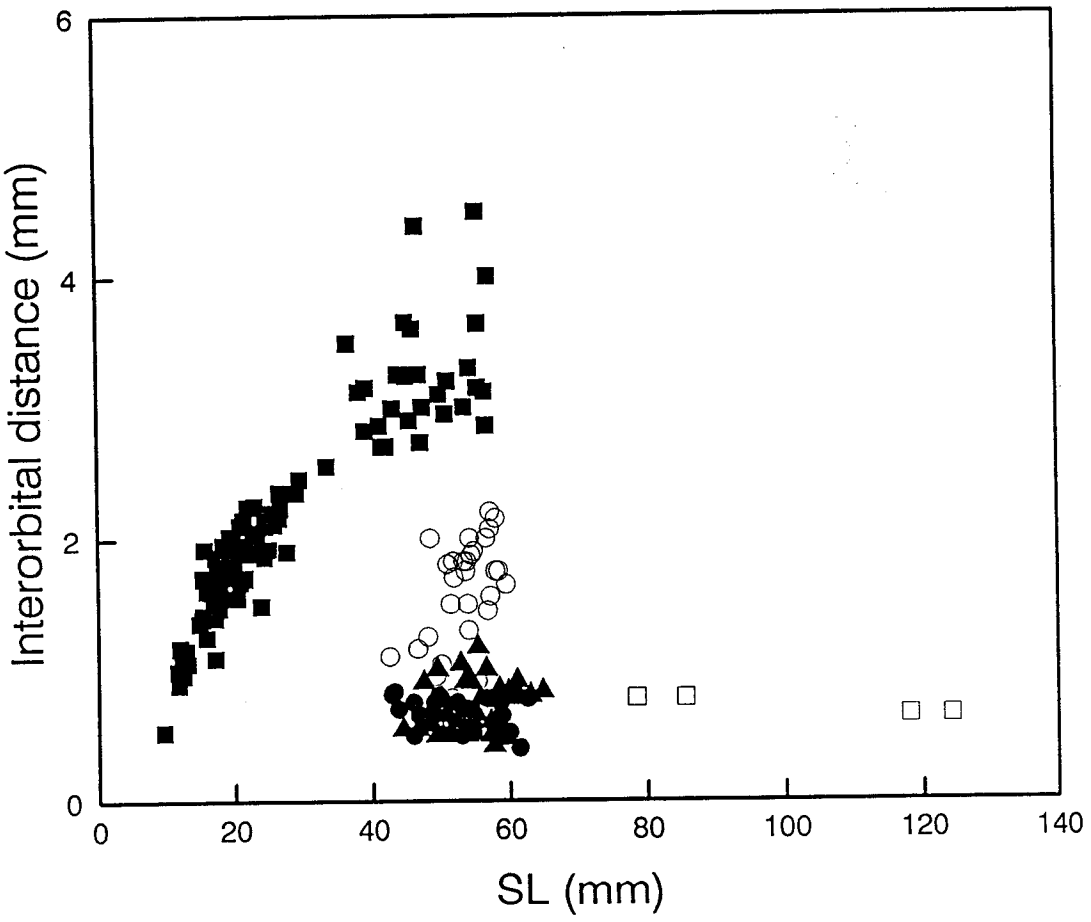


Figure 8

Figure 9. Plot of right eye diameter against standard length for the five life history stages in Dover sole. Symbols represent: solid squares, Stage 1; open circles, Stage 2; solid triangles, Stage 3; solid circles, Stage 4; open squares, Stage 5.

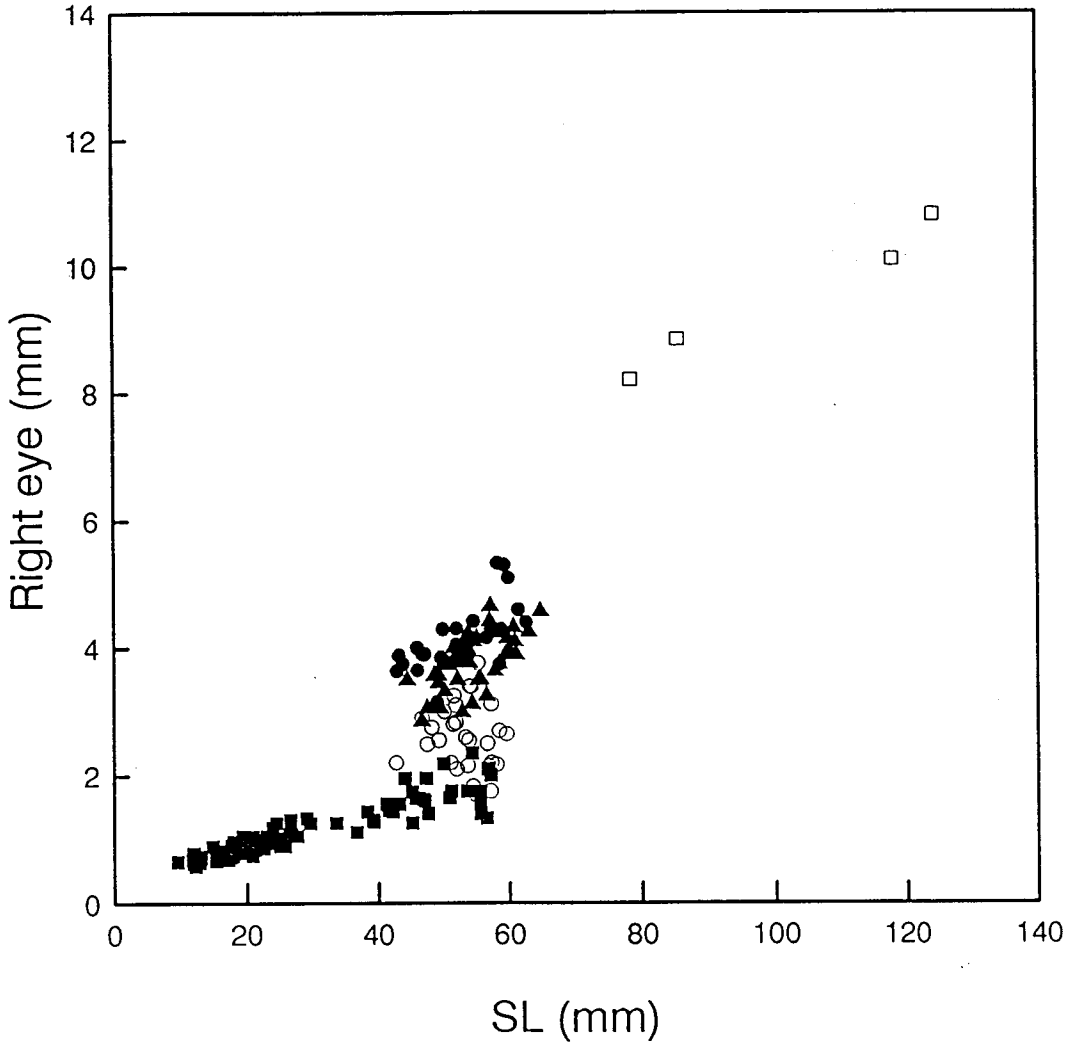


Figure 9

Figure 10. Plot of right premaxilla length against standard length for the five life history stages in Dover sole. Symbols represent: solid squares, Stage 1; open circles, Stage 2; solid triangles, Stage 3; solid circles, Stage 4; open squares, Stage 5.

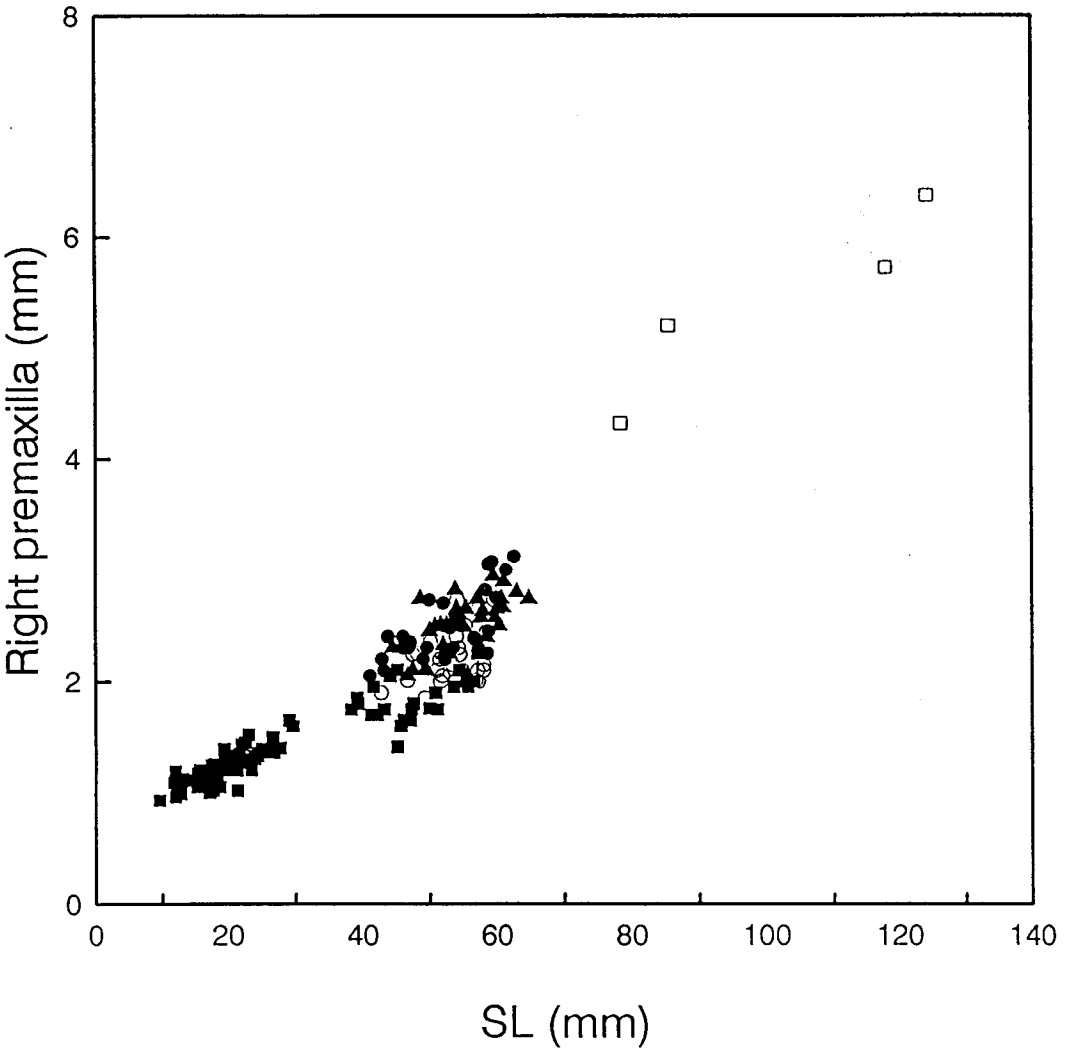


Figure 10

Figure 11. Scatterplot of discriminant functions scores on the first two discriminant functions axes for the five life history stages of Dover sole. Symbols represent: solid squares, Stage 1; open circles, Stage 2; solid triangles, Stage 3; solid circles, Stage 4; open squares, Stage 5.

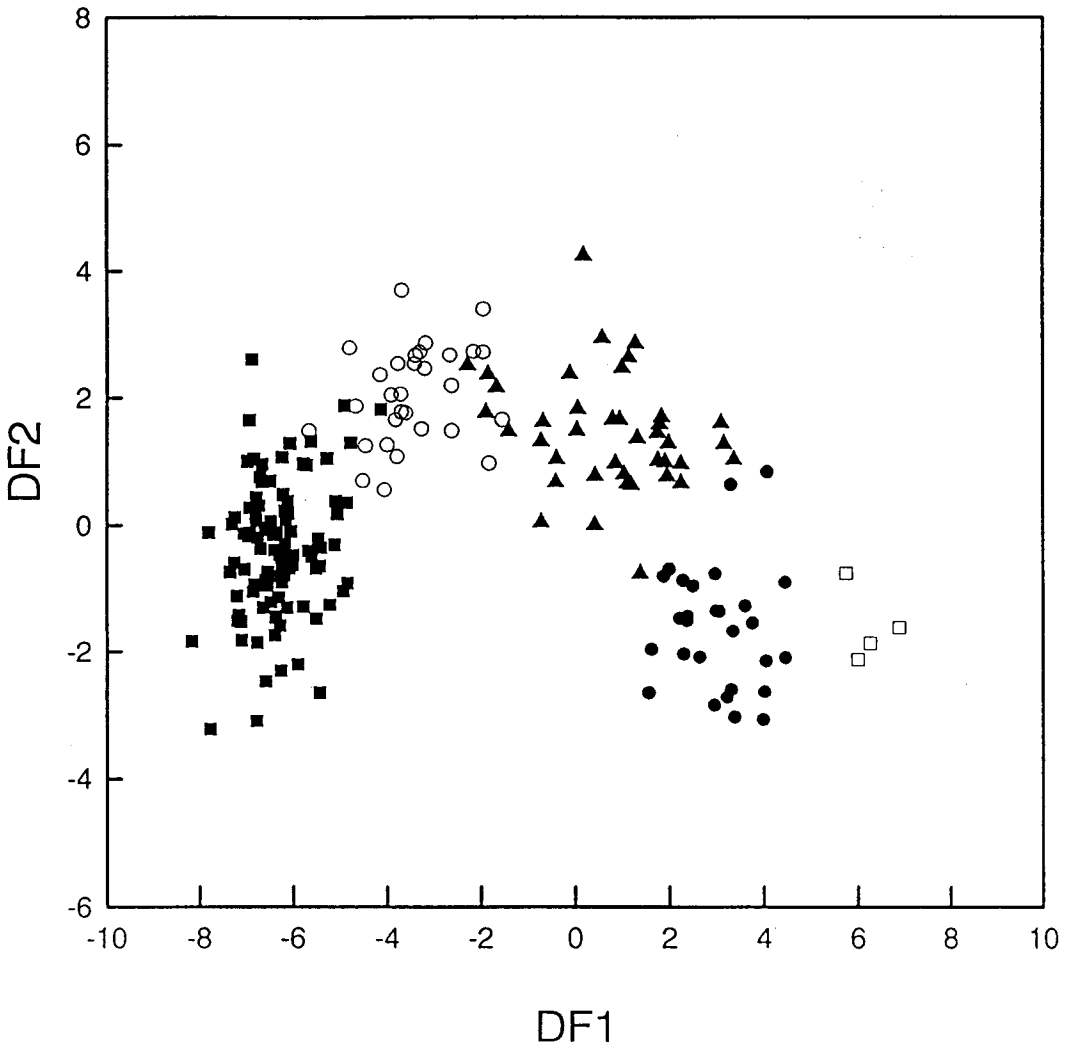


Figure 11

Figure 12. Scatterplot of principal component scores on the first two principal component axes for the five life history stages in Dover sole. Symbols represent: solid squares, Stage 1; open circles, Stage 2; solid triangles, Stage 3; solid circles, Stage 4; open squares, Stage 5.

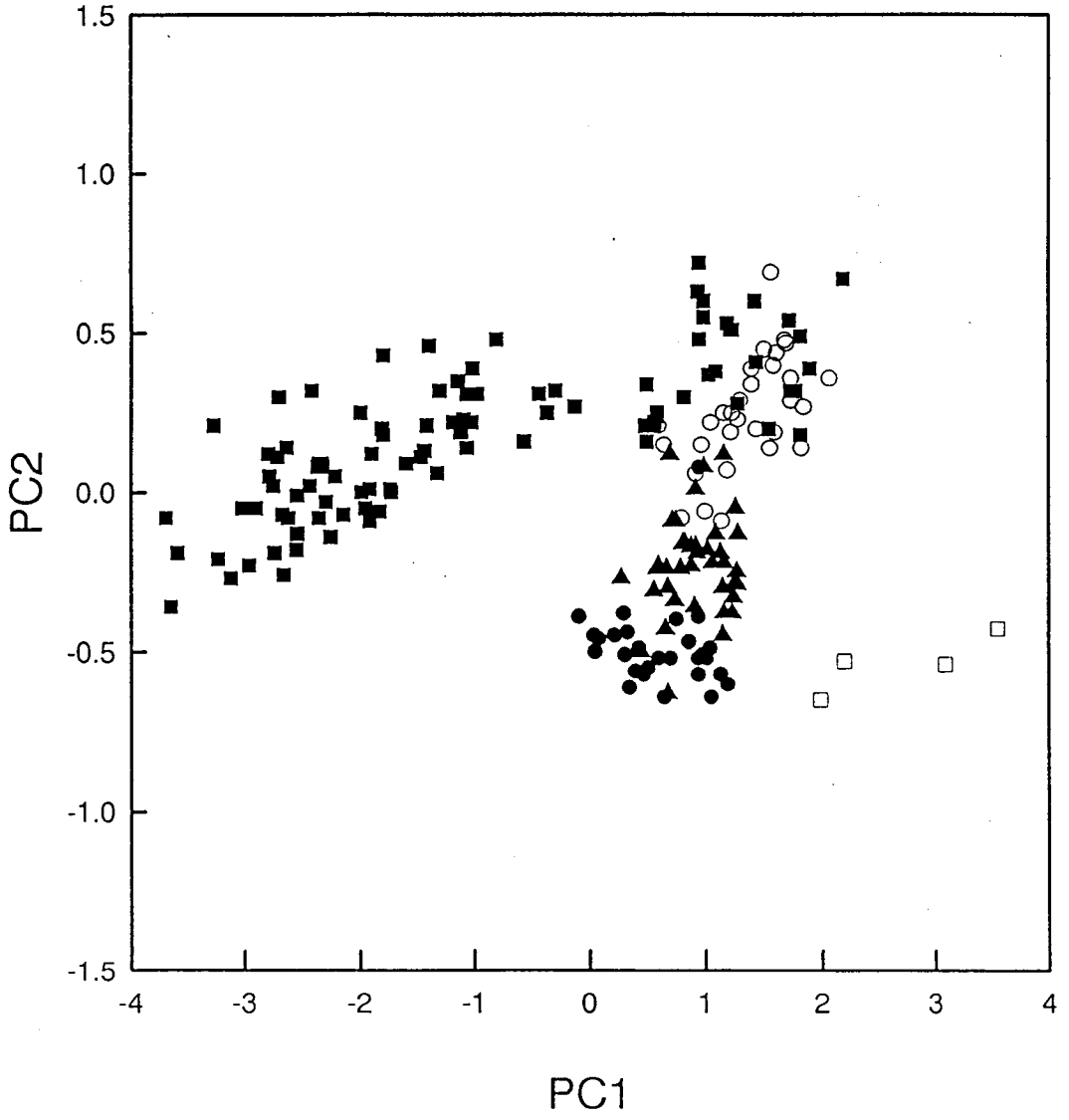


Figure 12

Figure 13. Plots of mean length of: A) first caudal-neural spine (CNS), B) immediate anterior pterygiophore (ACNS), and C) immediate posterior pterygiophore (PCNS) against SL size classes for the five life history stages in Dover sole. Symbols represent: solid squares, Stage 1; open circles, Stage 2; solid triangles, Stage 3; solid circles, Stage 4; open squares, Stage 5.

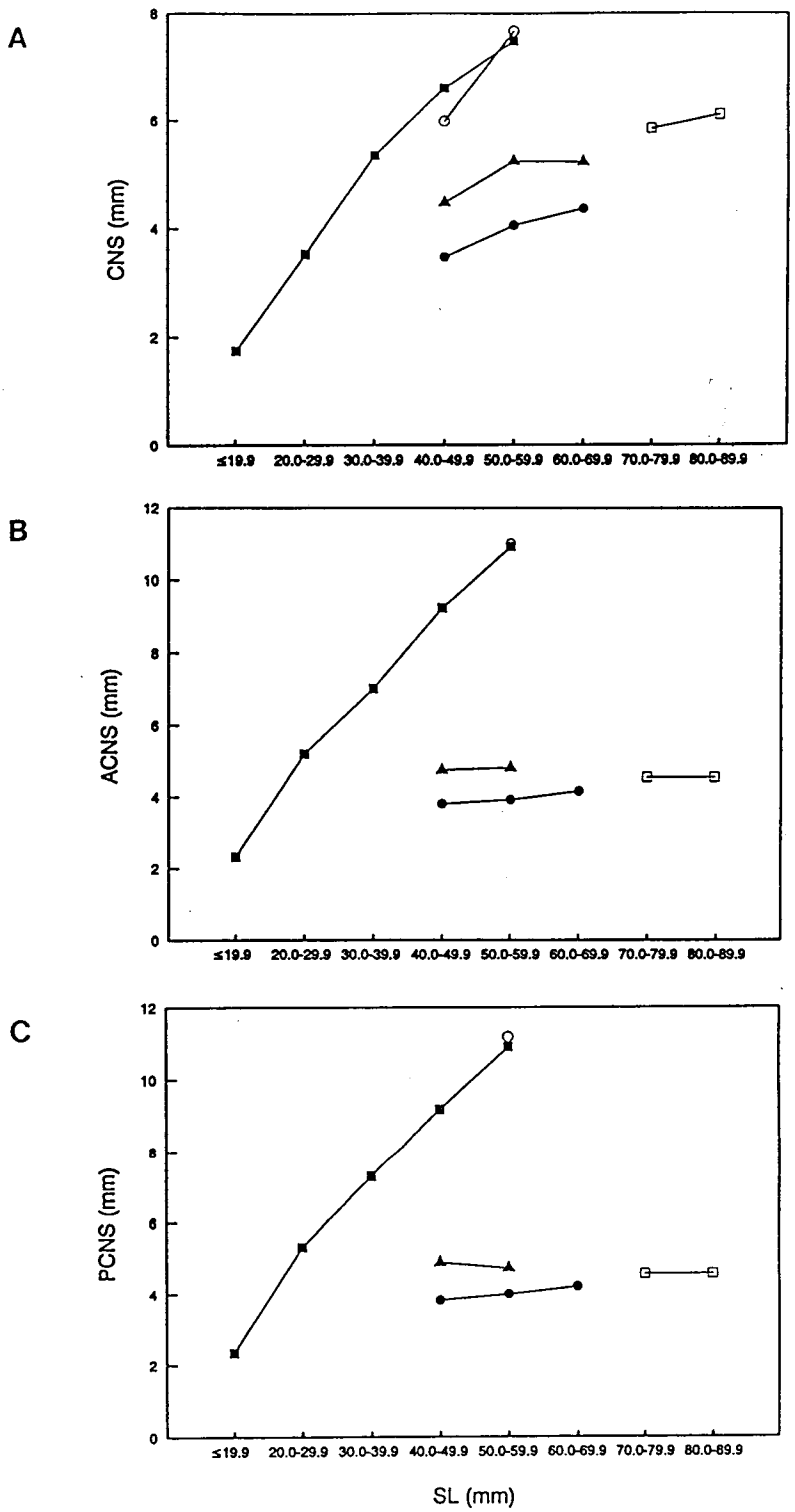

A
CNS (mm)
0
2
4
6
8
≤19.9
20.0-29.9
30.0-39.9
40.0-49.9
50.0-59.9
60.0-69.9
70.0-79.9
80.0-89.9
B
ACNS (mm)
0
2
4
6
8
10
12
≤19.9
20.0-29.9
30.0-39.9
40.0-49.9
50.0-59.9
60.0-69.9
70.0-79.9
80.0-89.9
C
PCNS (mm)
0
2
4
6
8
10
12
≤19.9
20.0-29.9
30.0-39.9
40.0-49.9
50.0-59.9
60.0-69.9
70.0-79.9
80.0-89.9
SL (mm)

Figure 13

Figure 14. Plots of mean length of: A) first hemal spine (CHS), B) immediate anterior pterygiophore (ACNS), and C) immediate posterior pterygiophore (PCNS) against SL size classes for the five life history stages in Dover sole. Symbols represent: solid squares, Stage 1; open circles, Stage 2; solid triangles, Stage 3; solid circles, Stage 4; open squares, Stage 5.

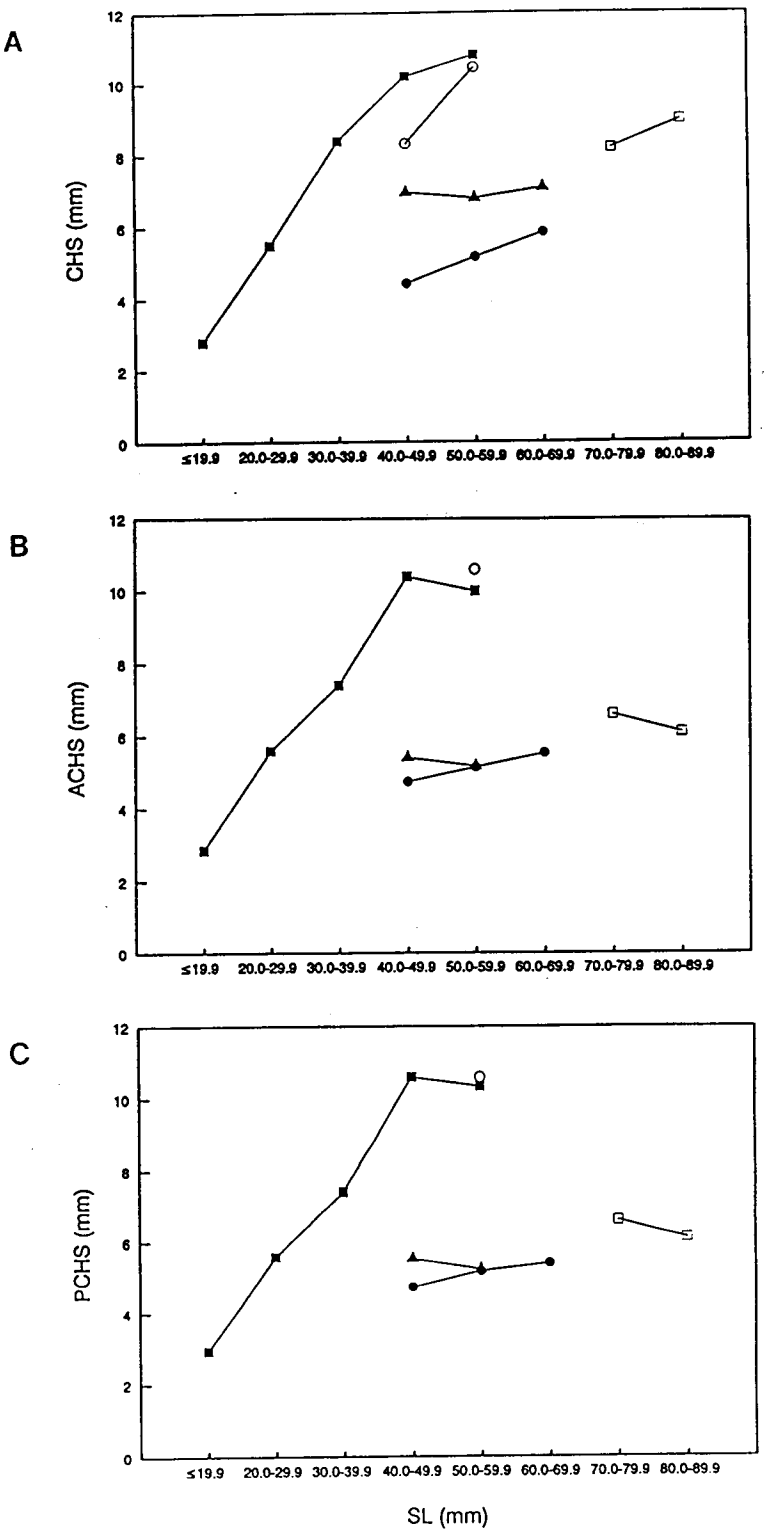

A
CHS (mm)
B
ACHS (mm)
C
PCHS (mm)
0
2
4
6
8
10
12
≤19.9
20.0-29.9
30.0-39.9
40.0-49.9
50.0-59.9
60.0-69.9
70.0-79.9
80.0-89.9
SL (mm)

Figure 14

Figure 15. Bar chart of the relative abundance of planktonic larvae in Stages 1-3 collected in midwater trawls, 1961-1978. Symbols represent: solid bars, Stage 1; cross-hatched bars, Stage 2; gray bars, planktonic Stage 3.

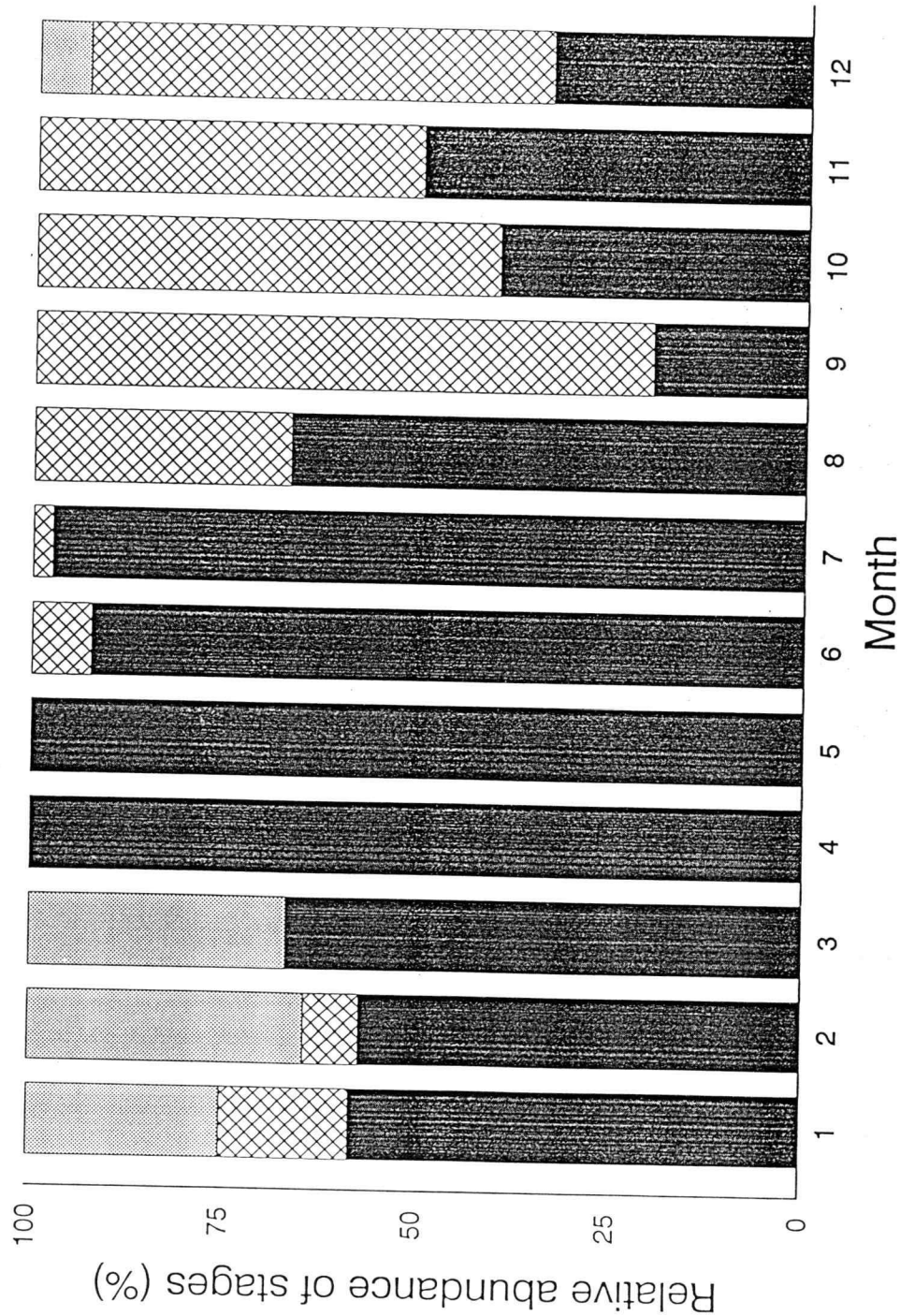

Relative abundance of stages (%)
100
75
50
25
0
1
2
3
4
5
6
7
8
9
10
11
12
Month

Figure 15

Figure 16. Bar chart of the relative abundance of planktonic larvae in Stages 1-3 collected in midwater trawls, 1961-1978, along the Newport Hydroline transect (Pearcy et al., 1977a) at 44°40′ N latitude. Symbols represent: solid bars, Stage 1; cross-hatched bars, Stage 2; gray bars, planktonic Stage 3.

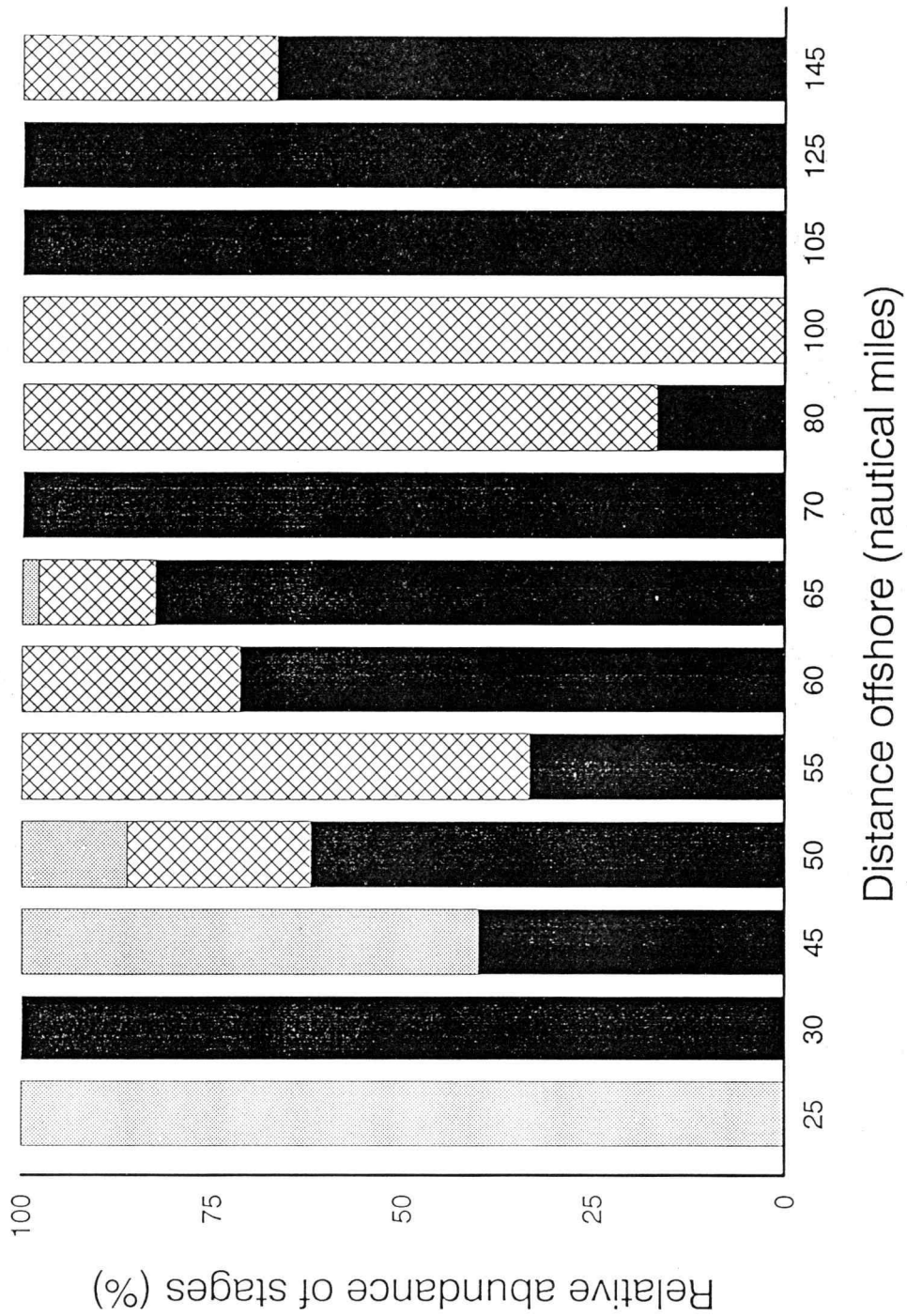

Relative abundance of stages (%)
100
75
50
25
0
25
30
45
50
55
60
65
70
80
100
105
125
145
Distance offshore (nautical miles)

Figure 16

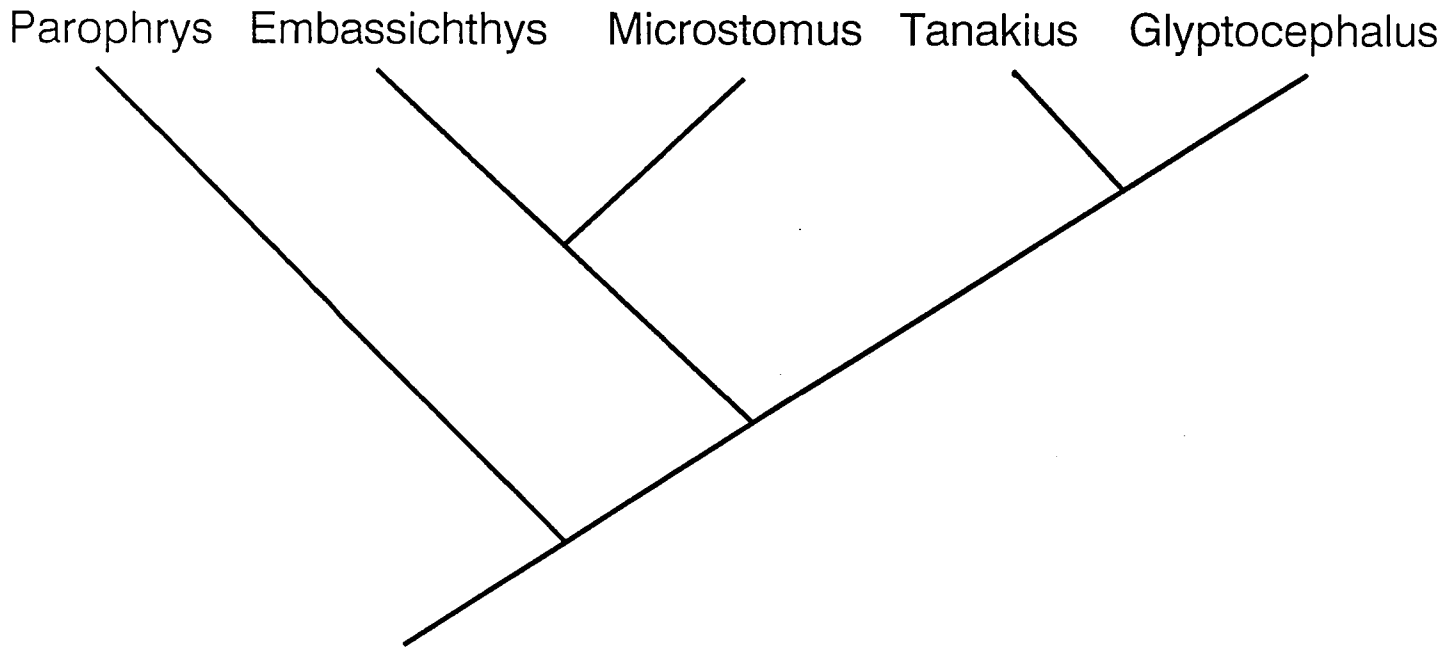
Figure 17. Bar chart of the relative abundance of planktonic larvae in Stages 1-4 collected in midwater trawls, 1961-1978, from 0-2,000 m depth. Symbols represent: solid bars, Stage 1; cross-hatched bars, Stage 2; gray bars, planktonic Stage 3.

Relative abundance of stages (%)
100
75
50
25
0
≤99
100
200
300
400
500
600
900
1000
1100
1200
2000
Depth (m)

Figure 17

Figure 18. Cladogram of relationships between the glyptocephaline flounders (sensu Chiu, 1987) Embassichthys, Microstomus, Tanakius, Glyptocephalus, and the out-group Parophrys.

Figure 18



From Chiu (1987).

Figure 19. Right lateral view of Embassichthys, Microstomus, and Glyptocephalus benthic larvae and juveniles illustrating the larval-like appearance to the eyes of recently settled Embassichthys and Microstomus: A) Embassichthys, top - benthic juvenile, OS12295, 59.7 mm SL; bottom - benthic juvenile, OS12295, 65.0 mm SL; B) Microstomus, top - benthic larvae (Stage 3), OS13468, 58.0 mm SL; bottom - benthic juvenile (Stage 5), OS13467, 55.5 mm SL; C) Glyptocephalus, top - planktonic metamorphic larvae (score = 6), OS12310, 56.7 mm SL; bottom - benthic metamorphic larvae (score = 9), OS12683, 61.4 mm SL.

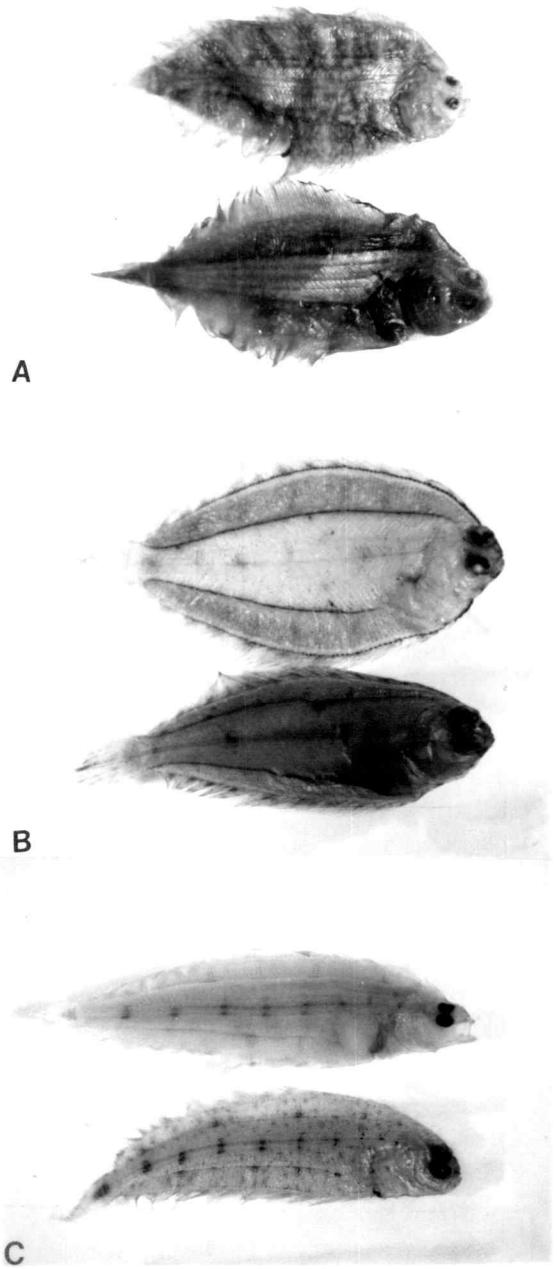


Figure 19

Figure 20. Scatterplot of principal component scores on the first two principal component axes for 201 M. pacificus (M - solid line), 94 G. zachirus (G - solid line), 12 E. bathybius (E - dotted line), and 42 P. vetulus (P - dashed line).

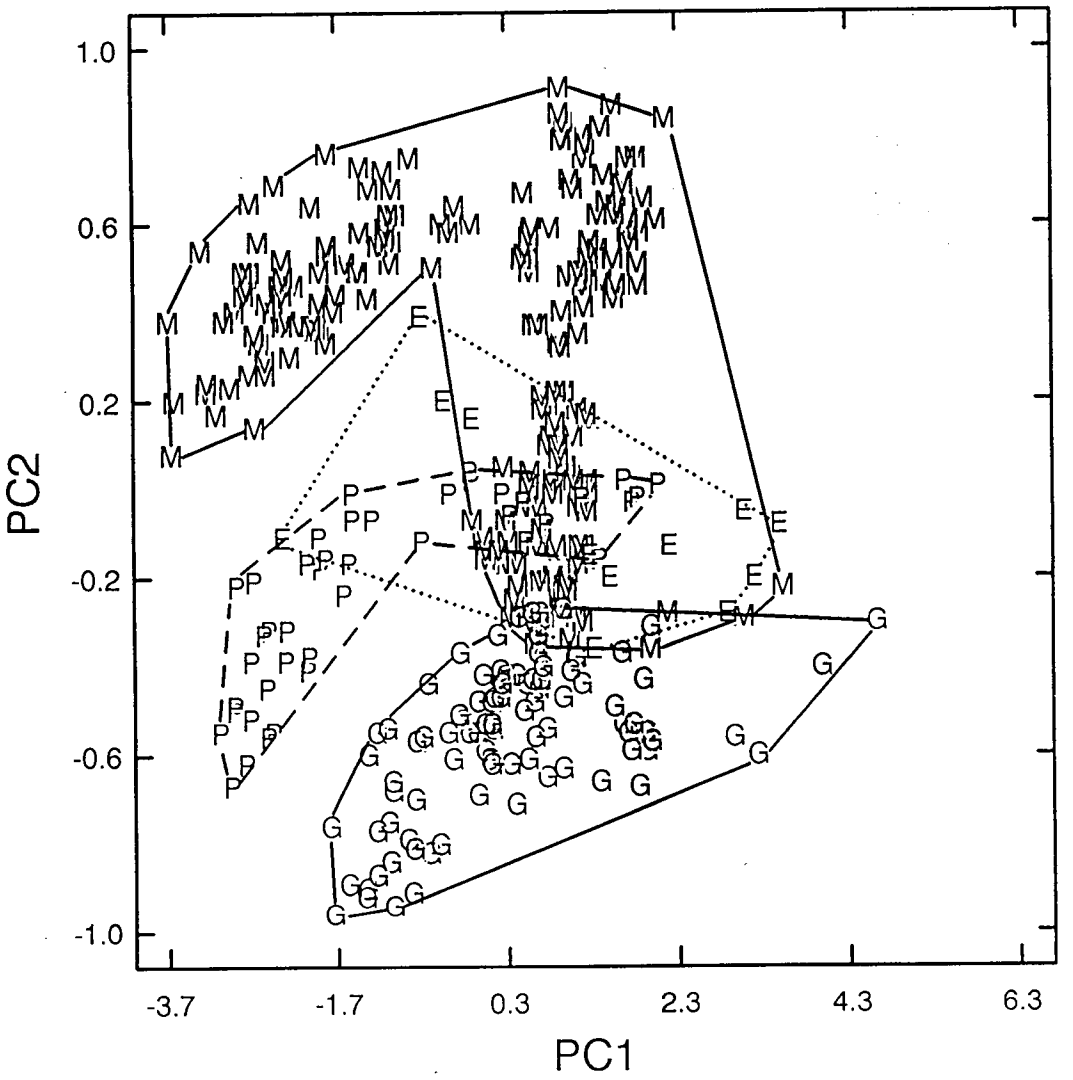


Figure 20

Figure 21. Scatterplot of principal component scores on the first two principal component axes for 201 M. pacificus (solid line), 94 G. zachirus (solid line), 12 E. bathybius (dotted line), and 42 P. vetulus (dashed line). Specimens are represented by metamorphic score

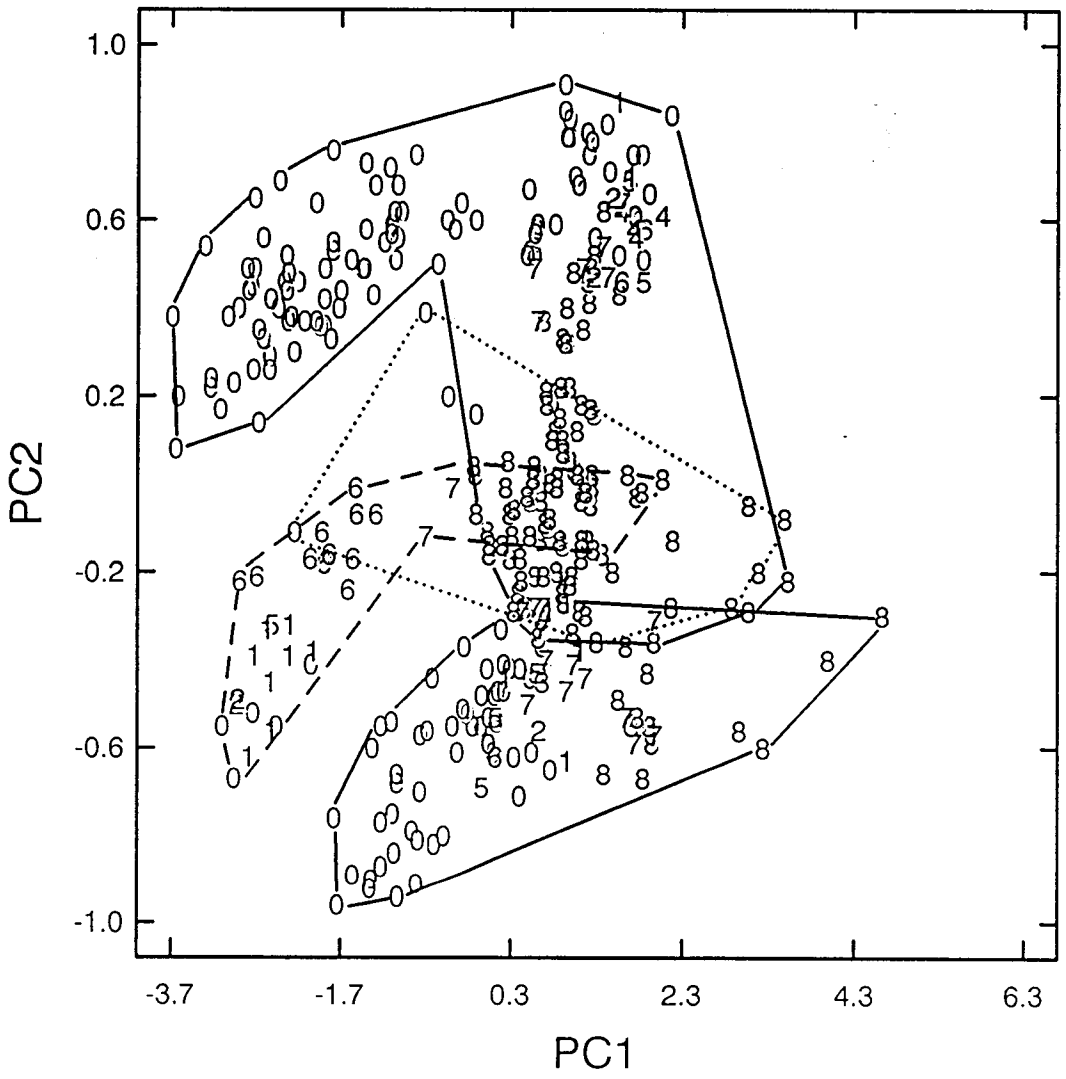


Figure 21

Figure 22. Illustrations of larval body shape: A) G. zachirus, OSUO MT274, 68 mm SL. B) M. pacificus, OSUO MT2437, 53.9 mm SL. C) E. bathybius, OS 13522, 35.6 mm SL.

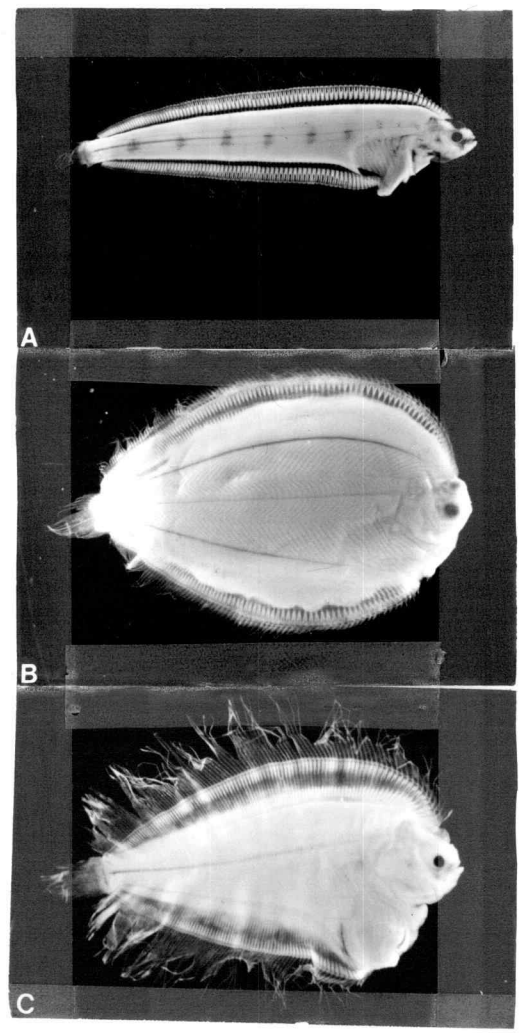


Figure 22

Figure 23. Simple linear regression of the relationship between BD and SL for G. zachirus (G) and P. vetulus (P).

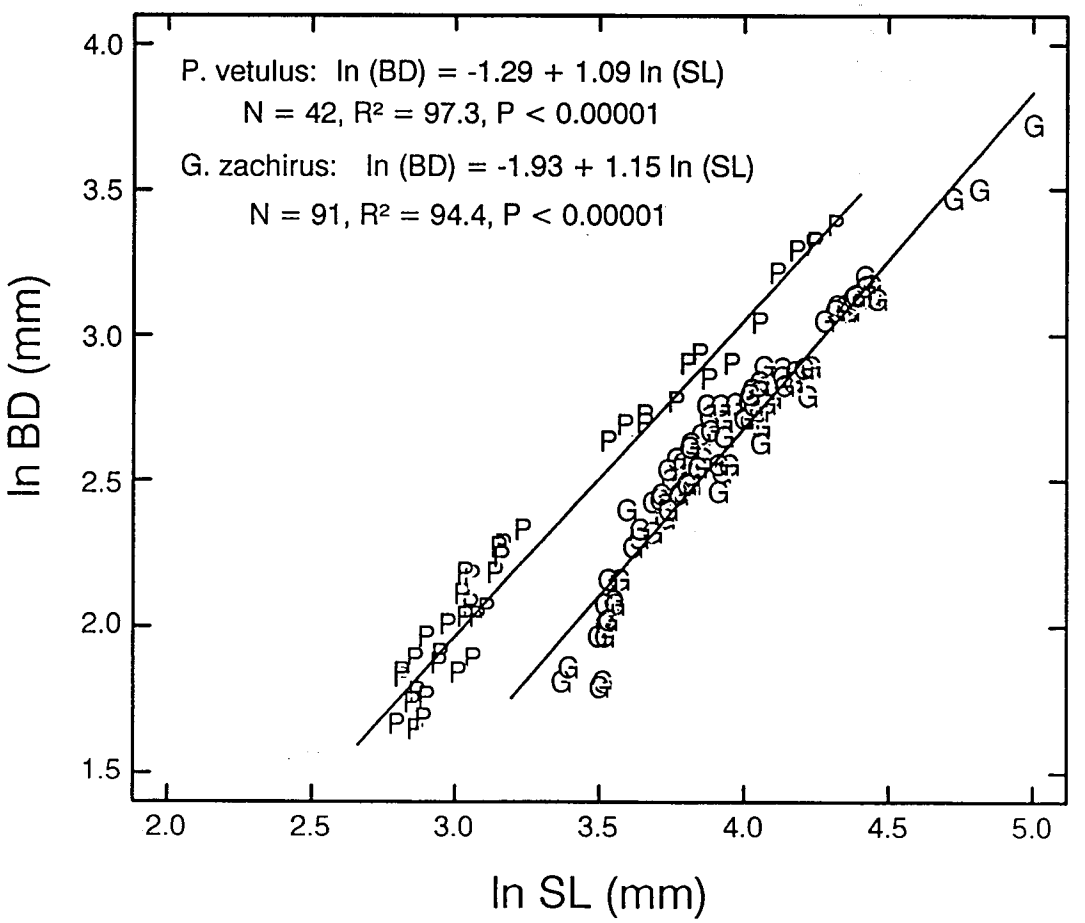

P. vetulus: ln (BD) = -1.29 + 1.09 ln (SL)
N = 42, R² = 97.3, P < 0.00001
G. zachirus: ln (BD) = -1.93 + 1.15 ln (SL)
N = 91, R² = 94.4, P < 0.00001
ln BD (mm)
ln SL (mm)
4.0
3.5
3.0
2.5
2.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0

Figure 23

Figure 24. Simple linear regression of the relationship between BD and SL for premetamorphic larvae (0) and juveniles (8) of M. pacificus represented by metamorphic score. The position of metamorphic M. pacificus larvae (1-7) and all E. bathybius (E) are also illustrated.

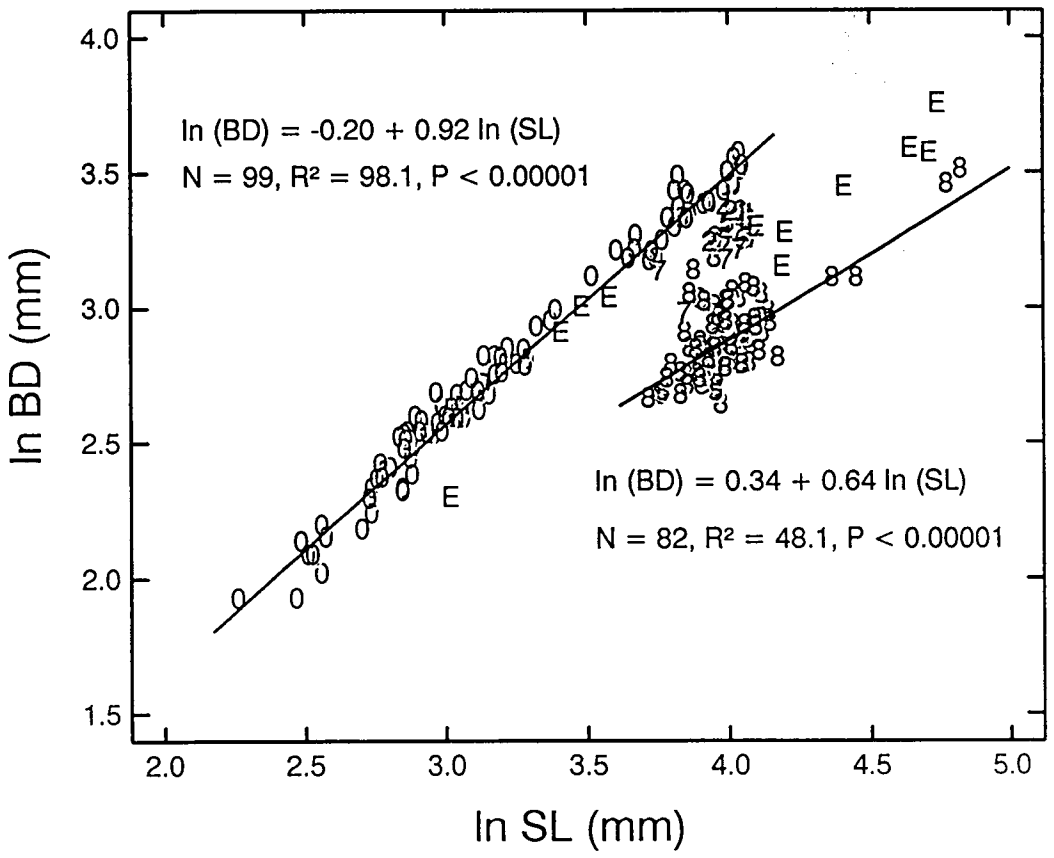

ln (BD) = -0.20 + 0.92 ln (SL)
N = 99, R² = 98.1, P < 0.00001
ln (BD) = 0.34 + 0.64 ln (SL)
N = 82, R² = 48.1, P < 0.00001
ln BD (mm)
ln SL (mm)
4.0
3.5
3.0
2.5
2.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0

Figure 24

Figure 25. Illustration of E. bathybius premetamorphic larvae, OS 11455, 32.0 mm SL.

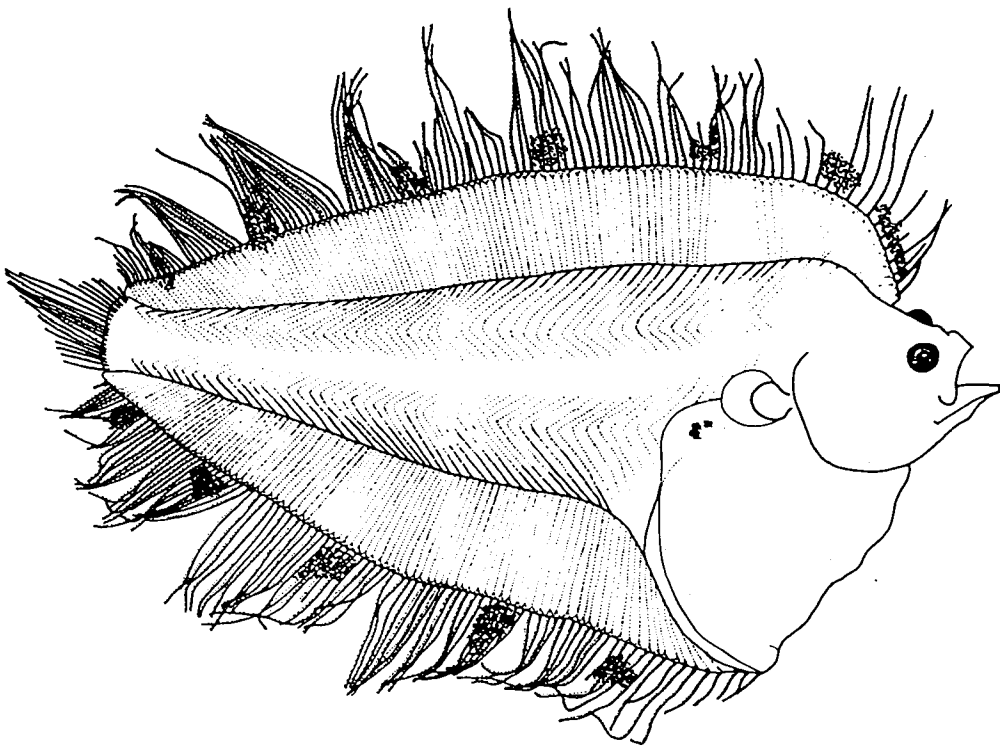


Figure 25

APPENDIX

Specimens Examined

Microstomus pacificus (Lockington, 1878-79): OS11377 (XR, CS), 44°40′00" N, 125°55′00" W, five specimens, 53.7-58.1 mm. OS12558 (CS), 44°40′00"-39′05" N, 125°49′08"-54′05" W, two specimens, 24.5-45.7 mm. OS12559 (CS), 45°04′00" N, 124°15′01" W, one specimen, 26.5 mm. OS12560 (XR, CS), 46°14′04"-15′00" N, 125°14′07"-12′06" W, one specimen, 53.0 mm. OS12561 (CS), 44°32′04-34′07" N, 125°17′05"-17′06" W, one specimen, 50.0 mm. OS12562 (CS), 44°15′09"-17′04" N, 125°15′00" W, one specimen, 12.3 mm. OS12563 (XR, CS), 44°31′06"-29′08" N, 125°24′05"-27′00" W, one specimen, 50.2 mm. OS12564 (XR, CS), 44°37′07"-15′09" N, 125°16′06"-15′00" W, one specimen, 47.6 mm. OS12565 (CS), 44°39′01"-39′00" N, 127°23′00"-26′01" W, one specimen, 17.2 mm. OS12566 (XR, CS), 43°20′05"-20′03" N, 125°37′05"-41′06" W, one specimen, 60.4 mm. OS12567 (CS), 44°40′05"-26′09"N, 125°19′06"-16′00" W, one specimen, 20.9 mm. OS12568 (XR,CS), 44°40′00" N, 125°40′00" W, twenty-one specimens, 15.9-51.2 mm. OS12569 (XR, CS), 44°35′05"-29′02" N, 125°19′02"-18′07" W, one specimen, 56.6 mm. OS12570 (CS), 44°17′08"-24′07" N, 125°18′00"-16′02" W, one specimen, 22.5 mm. OS12571 (CS), 44°31′03"-21′06" N, 125°17′02"-16′07" W, one specimen, 21.4 mm. OS12572 (XR, CS), 44°27′00"-30′03" N, 125°17′07"-15′09" W, one specimen, 46.7 mm. OS12573 (XR, CS), 44°30′03"-21′02" N, 125°15′09"-16′09" W, one specimen, 52.9 mm. OS12574 (XR, CS), 46°14′08"-14′05" N, 125°38′00"-33′00" W, two specimens, 19.4 and 47.5 mm. OS12575 (CS), 46°14′04"-14′05" N, 125°15′00"-10′07" W, one specimen, 26.8 mm. OS12576 (XR, CS), 43°20′07"-20′08" N, 124°53′07"-58′05" W, one specimen, 49.5 mm. OS12577 (CS), 44°39′00"-39′01" N, 126°01′00"-04′01" W, two specimens, 46.8 and 55.6 mm. OS12578 (CS), 44°39′01" N, 125°35′00"-29′06" W, one specimen, 55.6 mm. OS12579 (CS), 43°19′06"-19′04" N, 124°44′01"-47′05" W, one specimen, 25.0 mm. OS12580 (XR, CS), 43°59′09" N, 125°08′01"-03′05" W, one specimen, 49.3 mm. OS12581 (XR, CS), 44°39′01"-40′01" N, 124°36′05"-38′09" W, one specimen, 55.7 mm. OS12582 (XR, CS), 44°35′05"-38′05" N, 125°15′00"-14′09" W, two specimens, 46.7 and 51.9 mm. OS12583 (XR, CS), 44°33′09"-29′08" N, 125°17′04"-17′00" W, one specimen, 21.3 mm. OS12584 (XR, CS), 44°39′01"-40′00" N, 125°28′09"-35′00" W, one specimen, 50.0 mm. OS12585 (CS), 44°34′08"-36′06" N, 125°17′02"-19′04" W, one specimen, 18.3 mm. OS12631 (XR, CS), 44°39′02"-39′01" N, 125°02′00"-06′07" W, one specimen, 49.3 mm. OS12634 (CS), 44°40′00"-41′08" N, 125°15′00" W, one specimen, 23.0 mm. OS12635 (CS), 44°38′05"-42′06" N, 125°18′00" W, one specimen, 20.5 mm. OS12636 (XR, CS), 44°38′07"-37′02" N, 125°14′09"-15′04" W, one specimen, 56.8 mm. OS12637 (CS), 44°33′04"-40′05" N, 125°14′09"-16′02" W, three specimens, 19.5-22.8 mm. OS12638 (CS), 43°23′05"-20′08" N, 125°17′07"-12′03" W, one specimen, 17.8 mm. OS12639 (CS) 44°42′01"-37′05" N, 125°16′02"-14′03" W, two specimens, 22.6 and 25.8 mm. OS12640 (CS), 43°20′07"-20′05" N, 124°55′05"-49′05" W, one specimen, 26.6 mm. OS12641 (CS), 46°14′00"-14′04" N, 126°36′06"-33′00" W, two specimens, 17.2 and 20.9 mm. OS12643 (CS), 44°32′01"-31′03" N, 125°18′01"-20′00" W, one specimen, 17.3 mm. OS12644 (CS), 46°14′08"-05′08" N, 124°44′05"-55′05" W, one specimen, 20.3 mm. OS12645 (CS), 46°05′08"-10′00" N, 125°55′05"-49′05" W, one specimen, 19.8 mm. OS12646 (CS), 44°34′07"-37′00" N, 125°17′05"-16′05" W, one specimen, 27.7 mm. OS12647 (CS),

44°23′02"-43′02" N, 125°30′00"-05′00" W, one specimen, 21.6 mm. OS12648 (CS), 44°28′08"-26′00" N, 125°29′00"-32′00" W, one specimen, 23.4 mm. OS12649 (CS), 44°39′01"-15′05" N, 125°19′08"-43′02" W, one specimen, 42.6 mm. OS12650 (CS), 44°35′05" N, 125°07′01"-11′00" W, one specimen, 29.1 mm. OS12651 (CS), 43°14′02"-16′05" N, 130°34′05"-32′00" W, one specimen, 23.0 mm. OS12652 (CS), 43°20′06" N, 127°40′05"-40′09" W, one specimen, 22.0 mm. OS12653 (CS), 43°19′00"-24′00" N, 125°44′02"-42′00" W, two specimens, 23.9 and 24.4 mm. OS12654 (CS), 44°40′01"-39′05" N, 125°32′09"-47′05" W, two specimens, 22.5 and 24.5 mm. OS12655 (CS), 44°40′02"-39′08" N, 125°34′04"-29′03" W, one specimen, 26.5 mm. OS12656 (CS), 44°39′02"-38′08" N, 125°24′07"-21′02" W, one specimen, 41.6 mm. OS12657 (XR, CS), 44°57′01"-54′01" N, 125°58′07"-56′03" W, one specimen, 51.5 mm. OS12658 (CS), 44°39′02" N, 125°33′01"-26′06" W, four specimens, 12.9-17.4 mm. OS12569 (CS), 44°46′08"-49′03" N, 125°44′01"-44′00" W, seven specimens, 9.6-13.1 mm. OS12666 (XR), 44°58′08"-58′09" N, 124°13′04"-13′05" W, fifteen specimens, 48.6-64.8 mm. OS12667 (XR), 44°46′04"-65′05" N, 124°21′01"-21′03" W, four specimens, 78.5-124.3 mm. OS12668 (XR), 44°54′08"-54′06" N, 124°12′04"-11′09" W, five specimens, 49.2-61.1 mm. OS12669 (XR), 44°56′06"-56′04" N, 124°15′02"-14′08" W, three specimens, 53.5-60.0 mm. OS12670 (XR, CS), 36°50′05"-51′05" N, 121°55′01"-56′05" W, thirty-one specimens, 41.1-62.6 mm. OS13225 (XR), 44°18′00" N, 125°30′00" W, one specimen, 57.2 mm. OS13226 (XR), 43°22′05"-22′06" W, 127°09′06"-11′02" N, one specimen, 59.6 mm. OS13227 (XR), 45°30′00"-30′01" N, one specimen, 57.3 mm. OS13228 (XR), 44°37′04"-37′03" N, 125°25′04"-22′02" W, one specimen, 58.5 mm. OS13229 (XR), 45°34′04" N, 125°40′06" W, one specimen, 52.0 mm. OS13230 (XR), 44°31′04"-28′00" N, 125°19′02"-17′04" W, two specimens, 54.0 and 54.1 mm. OS13231 (XR), 44°00′00"-28′03" N, 126°39′00"-28′00" W, one specimen, 51.1 mm. OS13232 (XR), 44°50′02"-51′00" N, 124°56′02"-56′04" W, one specimen, 58.1 mm. OS13233 (XR), 44°36′02"-35′02" N, 125°34′04"-35′02" W, one specimen, 48.6 mm. OS13234 (XR), 44°42′01"-40′01" N, 125°39′03"-39′02" W, one specimen, 51.9 mm. OS13235 (XR), 44°39′06"-39′01" N, 125°07′01"-10′03" W, one specimen, 55.4 mm. OS13236 (XR), 44°39′02" N, 125°09′06" N, one specimen, 54.4 mm. OS13237 (XR), 44°34′06"-40′02" N, 125°34′05"-39′01" W, one specimen, 48.2 mm. OS13238 (XR), 44°44′01"-41′02" N, 125°17′06"-15′04" W, one specimen, 56.7 mm.

Glyptocephalus zachirus Lockington, 1878-79: OS2138 (XR), 46°10′00" N, 124°05′00" W, one specimen, 169.1 mm. OS11203 (XR), 46°59′18" N, 124°53′13" W, one specimen, 82.1 mm. OS12289 (XR), 45°55′01" N, 124°24′01" W, three specimens, 111.4-147.2 mm. OS12707 (CS), 44°40′00" N, 125°40′00" W, three specimens, 33.0-40.4 mm. OS12708 (CS), 44°59′01"-45′00" N, 125°43′04"-43′09" W, one specimen, 33.8 mm. OS12709 (CS) 44°39′01"-40′01" N, 124°36′05"-38′09" W, one specimen, 50.0 mm. OS12710 (CS), 44°40′00" N, 125°40′00" W, twenty-three specimens, 36.1-61.7 mm. OS12711 (CS), 44°37′02"-35′02" N, 125°16′07"-19′01" W, one specimen, 51.3 mm. OS12712 (CS), 44°39′01"-39′02" N, 125°01′08"-04′03", two specimens, 49.5 and 49.5 mm. OS12713 (CS), 44°43′04"-38′02" N, 125°55′05"-52′09" W, one specimen, 37.0 mm. OS12714 (CS), 43°23′05"-20′08" N, 125°17′07"-12′03" W, six specimens, 19.5-59.4 mm. OS12715 (CS), 44°48′04"-46′01" N, 125°41.5′ W, one specimen, 33.4 mm. OS12716 (CS) 44°40′00" N, 125°40′00" W, two specimens, 33.4-34.5 mm. OS12717 (CS), 44°40′00" N, 125°45′00" W, one

specimen, 36.8 mm. OS12718 (CS), 44°43′02"-48′03" N, 125°51′03"-52′05" W, one specimen, 64.5 mm. OS12719 (CS), 44°49′00"-50′00" N, 125°44′00" W, one specimen, 34.6 mm. OS12720 (cs), 44°46′05"-48′06"′N, 125°20′05"-20′00" W, one specimen, 28.8 mm. OS12721 (CS), 44°39′03"-39′02" N, 125°06′04"-11′06" W, one specimen, 67.3 mm. OS12722 (CS) 44°40′00" N, 125°45′00" W, three specimens, 29.5-37.7 mm. OS12723 (CS) 44°39′02"-39′01" N, 125°33′01"-26′06" W, one specimen, 32.8 mm. OS12724 (CS), 44°27′00"-30′03" N, 125°17′07"-15′09" W, one specimen, 68.1 mm. OS12725 (CS), 44°21′02"-24′04" N, 125°32′04"-33′02" W, one specimen, 57.0 mm. OS12726 (CS), 44°38′00"-38′01" N, 126°40′00"-36′02" W, four specimens, 32.6-33.8 mm. OS12727 (CS), 45°34′06" N, 125°40′01"-44′00" W, one specimen, 40.6 mm. OS12728 (CS), 44°40′03"-40′07" N, 125°42′00"-24′02" W, two specimens, 50.4-57.8 mm. OS12729 (CS), 44°39′02"-57′08" N, 125°28′06"-29′08" W, one specimen, 57.2 mm. OS12730 (CS), 44°39′03" N, 125°09′00"-04′00" W, one specimen, 57.2 mm. OS12731 (CS), 44°36′00"-52′05" N, 125°29′06"-29′04" W, one specimen, 55.5 mm. OS12732 (CS), 44°39′00" N, 124°30′00" W, one specimen, 43.5 mm. OS12733 (CS), 44°39′00" N, 124°45′00" W, one specimen, 43.1 mm. OS12734 (CS), 44°39′00"N, 125°24′00" W, one specimen, 31.7 mm. OS12735 (CS), 44°39′00" N, 125°26′00" W, one specimen, 41.6 mm. OS12736 (CS), 44°39′00" N, 125°30′00" W, one specimen, 44.3 mm. OS12737 (CS), 44°24′04"-27′09" N, 125°33′02"-33′08" W, two specimens, 56.2 and 60.1 mm. OS13532 (XR), 46°34′00" N, 124°26′02" W, fourteen specimens, 71.4-85.6 mm.

Embassichthys bathybius (Gilbert, 1891): OS11455 (XR), 44°51′05"-49′02" N, 125°40′00"-41′00"W, one specimen, 130.0 mm. OS12295 (XR), 44°28′00" N, 125°03′00" W, three specimens, 59.7-81.5 mm. OS12298 (XR), 44°26′03" N, 125°03′00" W, two specimens, 66.1 and 103.1 mm. OS12301 (XR), 45°52′00" N, 125°53′00" W, one specimen, 110.4 mm. OS12450 (XR), 44°39′01" N, 125°06′02" W, one specimen, 113.9 mm. OS12872 (XR), 45°53′03" N, 124°54′00" W, one specimen, 99.7 mm. OS13522 (XR), 44°40′00" N, 125°45′00" W, two specimens, 32.3 and 35.6 mm. OS13658, 44°39′00" N, 125°30′00" W, one specimen, 21.0 mm SL.

Parophrys vetulus Girard, 1856: OS5173 (XR), 45°24′00" N, 123°57′04" W, five specimens, 44.7-69.3 mm. OS5904 (XR), 45°24′00" N, 123°57′04" W, eight specimens, 38.5-74.4 mm. OS11234 (CS), 47°35′00" N, 122°30′00" W, three specimens, 16.6-18.1 mm. OS11236 (CS), 44°34′00" N, 124°02′00" W, four specimens, 17.5-23.6 mm. OS11239 (CS), 47°35′00" N, 122°30′00" W, two specimens, 20.7 and 21.2 mm. OS11241 (CS), 44°34′00" N, 124°02′00" W, four specimens, 16.6-23.5 mm. OS11242 (CS), 44°39′00" N, 124°04′00" W, one specimen, 25.3 mm. OS11244 (CS), 47°35′00" N, 122°30′00" W, two specimens, 21.1 and 23.0 mm. OS13523 (CS), 44°39′01" N, 124°08′00" W, one specimen, 21.3 mm. OS13524 (CS), 44°39′01" N, 124°17′07" W, one specimen, 18.1 mm. OS13525 (CS), 44°39′01" N, 124°06′00" W, two specimens, 16.3 and 18.9 mm. OS13526 (CS), 44°39′01" N, 124°17′07" W, one specimen, 17.4 mm. OS13527 (CS), 44°39′01" N, 124°08′00" W, one specimen, 22.3 mm. OS13528 (CS), 44°39′01" N, 124°08′00" W, three specimens, 17.9-20.7 mm. OS13529 (CS), 44°39′01" N, 124°12′00" W, one specimen, 21.6 mm. OS13530 (CS), 44°39′01" N, 124°17′07" W, one specimen, 19.0 mm. Uncataloged (XR), IPMN XXII-1 C1, 9VI82, 2204-0004, one specimen, 34.0. Uncataloged (XR), IPMN3 C1 BTM, 24VI82, 2225-0135, one specimen, 36.0 mm.