PRELIMINARY REPORT ON THE BIOLOGY OF THE ENTEROPNEUST, *PTYCHODERA FLAVA* ESCHSCHOLTZ, IN THE VICINITY OF KUSHIMOTO, JAPAN[1]

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Contents

Introduction

So far the following six species of Enteropneusta have been recorded from the Japanese waters; *Saccoglossus sulcatus* (Spengel, 1893), *S. borealis* Okuda & Yamada, 1955, *Glandiceps hacksi* (Marion, 1855), *G. eximius* Spengel, 1893, *Balanoglossus misakiensis* Kuwano, 1902, and *B. carnosus* (Willey, 1899). In addition, another uncertain species of *Glandiceps*(?) was reported by Miyashita (1925) as the parent of *Tornaria*

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mortenseni Stiasny, 1921 and some unidentified enteropneusts were reported briefly by Yatsu (1890, 95, 97), Hirota (1894), Sunabe (1964) and Kuroshima (1970). Recently, in June 1973, several specimens of an enteropneust were found by Dr. Hiro'omi Uchida, one of the staff of the Sabiura Marine Park Research Station, on the rocky shore of Uematsu on the west coast of the town of Kushimato. They were rendered for identification by me and were clarified to be the tropical enteropneust, *Ptychodera flava* Eschscholtz, 1825, which was evidently new to the Japanese waters.

Many enteropneusts have been reported to regenerate lost parts of the body and a considerable number of detailed studies on the morphogenesis in regeneration have been done, whereas the significance of regeneration for sustaining the population, compared to that of sexual reproduction, has not been estimated on the basis of the numerical analysis up to now. Stimulated by the discovery of this worm in the vicinity of Kushimoto, I have continued to collect the sample once or twice a month in the same locality to study the population of this worm. Further, a large number of specimens thus gained made some morphological examinations possible. Here, I wish to report preliminarily the morphology and the biology of this tropical worm in its northern-most locality.

Geographical Distribution

Ptychodera flava had ever been split into several different species and varieties, but it became clear through the studies of Horst (1929, 32a, 39), Trewavas (1931), Rao (1952) and Björnberg (1955, 59) that the species previously described under the genus *Ptychodera* Eschscholtz, 1825 should be united into two species, the Indo-Pacific *flava* and the Atlantic *bahamensis,* though the two might be even regarded as con-specific (see Björnberg, 1959, p. 28). Admitting the validity of only these two species, the worldwide localities of adult *P. flava* so far recorded are as follows.

Central Pacific: Marshall Islands (Eschscholtz, 1825).
 Hawaiian Islands: Throughout the Islands (as *P. f. laysanica,* Edmondson, 1946) or Hawaii (Wollacott et al., 1972; Brandenburger et al., 1973); Laysan Is. (as *P. flava, P. caledoniensis* and *P. f. laysanica,* Spengel, 1903); at Hilo, Hawaii Is. (Horst, 1929).
 Funafuti Is. (Hill, 1897; as *P. f. funafutica,* Spengel, 1904a).
South Pacific: Great Barrier Reef of New Caledonia (as *P. f. caledoniensis,* Willey, 1898, 99); Low Isle, New Caledonia (Trewavas, 1931).
 Shell Harbour, New South Wales (Horst, 1929).
East Pacific: Galapagos (Trewavas, 1931).
Indian Ocean: Abrolhos, West Australia (as *P. pelsarti,* Dakin, 1916).
 Madras (?as *Glossobalanus minutus,* Menon, 1903).[1]
 Ceylon (as *Balanoglossus tricollaris,* Schmarda, 1871–72; as *P. ceylonica,* Spengel, 1893; see Spengel 1904b, pp. 53–4); Hikkaduwa, Ceylon (Welsch & Storch, 1970).
 Gulf of Manaar: Krusadai Is. (as *Chlamydothorax ceylonica,* Narayan Rao, 1936; as? *Ch. krusadiensis,* Narayan Rao, 1934; as *P. f. krusadiensis,* Rao, 1954a, 55c, 62; as *P. f. gigantica,* Rao, 1955c, 62); Shingle Is. (as *P. f. shinglensis* and *P. f. coralliformia,* Rao, 1954a, 55c, 62); Phallivausal Is. (as *P. f. phallivausalensis,* Rao, 1955c, 62).
 Maldive (as *P. f.* var. *saxicola,* var. *grandis,* var. *muscula,* var. *maldivensis,* var. *cooperi, P. viridis* and *P. asymmetrica,* Punnett, 1903).
 Laccadive (as *P. f.* var. *laccadivensis,* Punnett, 1903).
 Mauritius (Horst, 1932b).
 Inhaca Island, Delagoa Bay (Horst, 1939, 40; Macnae, 1958; Macnae & Kalk, 1962).
 Tanikely Is., Madagascar (Kirsteuer, 1965).
Red Sea: Tor (as *P. erythraea,* Spengel, 1893), Quseir (as *P. erythraea,* Klunzinger, 1902), Djibouti (as *P. erythraea;* Spengel 1893, Gravier 1905).

The other species, *Ptychodera bahamensis* Spengel, 1893, has been recorded from the following localities.

West Atlantic: Bahamas (Spengel, 1893), Curaçao (Horst, 1924), Bermudas (ibid.), Tortugas (ibid.; Hess, 1936; Cary, 1933), Tobago (Horst, 1929).
South Atlantic: St. Helena (Horst, 1939).

The present record extends the range of geographical distribution of *P. flava*

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[1] Spengel (1904a) suggested that *G. minutus* (Kowalevsky) of Menon, 1903, was identical with *P. flava,* but Rao (1962) objected to this.

to the western North Pacific and represents the northern-most locality for the genus *Ptychodera*.

On the other hand, two forms of tornaria of the "Typus II" have been reported from the Japanese waters; these are *Tornaria miyashitai* Stiasny, 1928 from Misaki and *Tornaria susakiensis* Tokioka, 1937 from Susaki near Shimoda. "Typus II" was thought to be derived from *P. flava* by Stiasny–Wijnhoff & Stiasny (1926, 27), though Rao (1954b, p. 152 and 55a, p. 273) objected against this. From the occurrence of *T. miyashitai*, Horst (1932, p. 100) ever presumed the third species of the genus in Japan as "... In den japanischen Gewässern muß also noch eine dritte *Ptychodera*-Art vorkommen, und vielleicht wird auch diese Art im erwachsenen Zustande kaum oder gar nicht von *Ptychodera flava* zu unterscheiden sein." As tornaria has neither been so far recorded from the field in the vicinity of Kushimoto nor gained in the laboratory from mature *P. flava* obtained from that locality, I cannot settle this problem at present.

Habitat

The occurrence of the species is seemingly limited to the district of Uematsu on the west coast of Kushimoto, which forms an open bight to the north of Cape Shiono-misaki and is affected directly by a branch of the warm Kuroshio current. According to the record at the Sabiura Marine Park Research Station in the vicinity of Kushi-moto (Irie and Tatsuki, 1976), the water temperature in the area fluctuated between 14.8°C and 28.5°C in the last three years and actually I found that the temperature in the tide pools in the locality fluctuated in the range from about 10°C at winter night to 35°C in the summer day time. Reflecting this, many kinds of reef corals are growing in the area, though any real reefs are not formed.

As seen from the topography of the area (fig. 4), the locality is well protected against the surges and swells from the south, but still exposed to significant surf. The most part of the coast is a sandy beach that continues westwards to the rocky shore of Cape Shionomisaki. *P. flava* dwells in the transitional zone between the sandy beach and the rocky shore. The foot of the rocky shore is more or less covered by sand before it reaches the gravel floor of the shallow sublittoral zone. The sand layer of a few to 20 cm is maintained between the rocky elevations and *P. flava* is found exclusively under or just near the stones lying on such sandy substratum around the low water mark, thus protected from being carried away together with sand at storms. The sand is composed mostly of coral sands, foraminiferan shells and molluscan shell fragments, and an example of particle size distribution is as follows; 9.9% of gravel and coarse sand, 81.1% of fine sand, 5.8% of very fine sand and 3.2% of silt and clay.

Abundant animals in the locality are: echinids such as *Anthocidaris crassispina* (A. Agassiz), *Echinometoria mathaei* (Blainville), *Hemicentrotus pulcherrimus* (A. Agassiz), *Mespilia globulus* (Linné), etc., holothrians such as *Holothuria hilla* Lesson, *H. leucospilota* (Brandt) and some unidentified species, a cirratulid polychaete, *Cirriformia*

tentaculata (Montagu) and an ascidian, *Herdmania momus* Savigny. Following animals are also generally found in the habitats of *P. flava*.

Coelentrata: *Edwardsia* sp.

Nemertinea: *Lineus geniculatus* (Della Chiaje), *Amphiporus cervicalis* (Stimpson) and several unidentified species.

Polychaeta: *Psammolyce malayana* Horst, *Eurythöe complanata* (Pallas), *Perinereis cultrifera* (Grube), *Glycera onomichiensis* Izuka, *Lumbrinereis brevicirra* (Schmarda), *Nainereis laevigata* Grube, *Prionospio kurusadensis* Fauvel, *Arabella iricolor* (Montagu), *A. zonata* (Moore), *Oenoe fulgida* Savigny, *Cirriformia tentaculata* (Montagu), *Mesochaetopterus minuta* Potts, *Thelepus setosus* (Quatrefages) and *Armandia lanceolata* Willey.

Echiura: *Ochetostoma erythrogrammon* Leuckart & Rüppell.

Crustacea: *Calianassa japonica* Ortmann.

Echinodermata: *Fibularia* (*Fibulariella*) *acuta* (Yoshiwara).

When stones are removed, usually only the proboscis or the anal end of the worm is seen exposed above the surface of the sand, though sometimes the worm may be found among the nest tubes of *Pomatoleios kraussi* (Baird) attached to the undersurface of stones. The worm does not build any distinct burrows or mounds of castings. Such a mode of life is quite the same as what have been reported generally in this species. Rao (1954) reported other striking behaviors of this worm. According to him (p. 2), *P. flava krusadiensis* was "seen with the anterior half of their bodies on the surface of the sand" when the habitat was covered by water at high tide, while it was "confined to a U-shaped burrow" when exposed at low tide, and further, *P. f. coralliformia* inhabited crevices in the dead coral rocks, where there was no sandy element. I have, however, not observed any individuals living in such ways as mentioned above at any of about forty visits to Kushimoto in the last three years.

External Features of the Worm

The external features (fig. 1A) conform in detail to the descriptions made by Willey (1899).

The proboscis is well developed, longer than the collar and exposed almost completely. The racemose organ is present on the ventral surface at the base of the proboscis. The collar may be divided into three zones by distinct transverse furrows; the anterior zone occupies anterior two-thirds of the collar while the posterior zone represents the annular swelling at the posterior end of the collar. The furrow between the middle and posterior zones is more remarkable than that between the anterior and middle ones. The anterior end of the collar sometimes forms a funnel opened anteriorly. The genital wings containing gonadal masses originate at almost the same level as the ventrum, entirely envelop the branchial region, and gradually diminish the width posteriorly till they disappear in the anterior part of the hepatic region. Gill slits open directly to the exterior; synapticula are present between tongue bars. The hepatic region begins as a pair of longitudinal rows of

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indistinct external swellings of the dorsal wall; these swellings are elongated transversely and gradually grow larger posteriorly to form elliptical blind sacs (hepatic saccules), the anterior and posterior faces of which are distinctly ribbed in larger specimens. The hepatic region and the following caudal region are not divisible so clearly
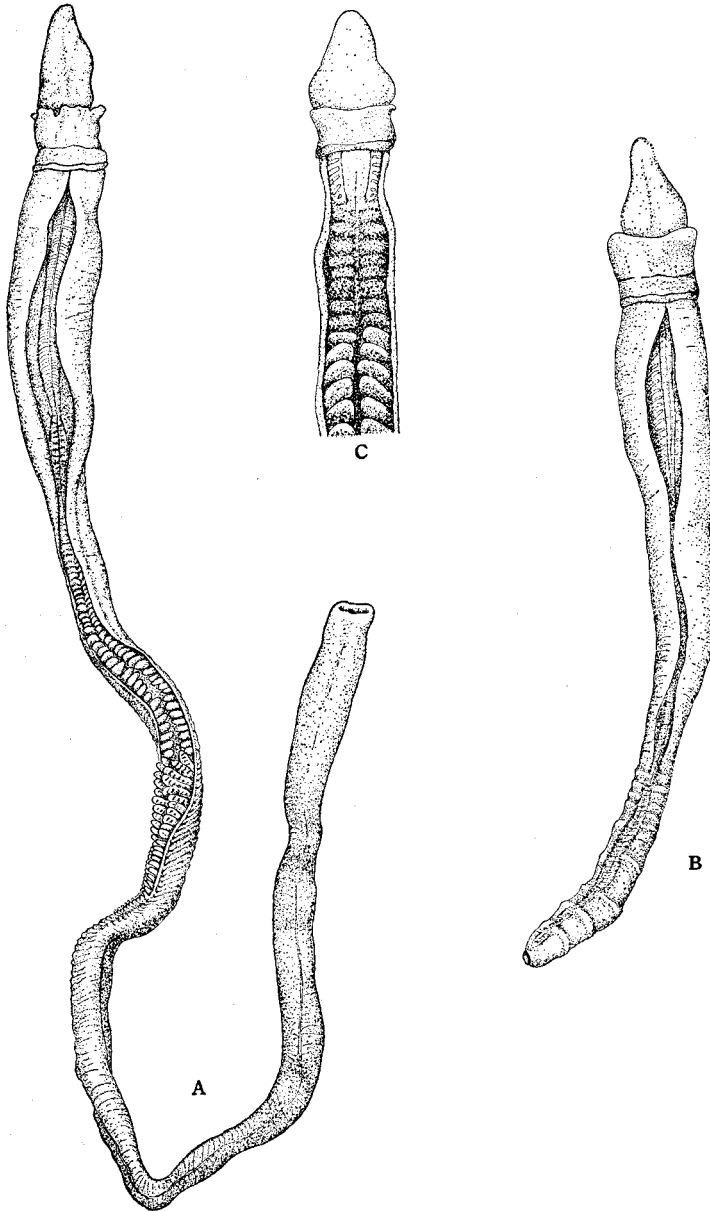

Fig. 1. *Ptychodera flava* Eschscholtz from the vicinity of Kushimoto; A. "Normal" individual, ×1.2, B. "Branchiogenital" individual, ×4. C. Anterior part of "Hepatic" individual, ×3.3.

because of the occurrence of paired vestigial saccules between the two regions. Anus terminal. The epidermis of the trunk, especially of the caudal region, is annulated almost regularly, while on the integument covering the inner surface of genital wings such regular annulations are replaced by elliptical patches of various sizes arranged transversely. This species is hardly distinguishable from the other species, *P. bahamensis*, except by its thinner limitating membrane in the collar coelomoduct as compared with that of the latter.

Size: The total length of probably normal individuals varies from about 40 mm to 200 mm, as shown by Willey (1899), Punnett (1903), Horst (1929) and so on. The measurements of the largest preserved specimen collected on April 28, 1975 are as follows; total body 235 mm, proboscis 11 mm, collar 6.5 mm, branchial region 22 mm, transitional region 30 mm, hepatic region ca. 50 mm and caudal region ca. 110 mm in length, and maximum width ca. 5 mm. None of the gigantic individuals, that were reported from the Hawaii Islands by Horst (1929, p. 196) as with the 63 mm long branchial region, from the Hawaii Islands by Edmondson (1946, p. 318) as with 18 inch long total body or from the Gulf of Manaar by Rao (1962, p. 229) as with the 8.5 cm long branchial region and 46 cm long total body, have been found in the present locality.

Coloration of Live Specimen: The proboscis is yellowish white, the collar yellowish or orange, the gonad and genital wings yellow, and the caudal region is faintly yellowish, sometimes with a greenish tint. Hepatic saccules in the anterior third of the hepatic region are dark brown or sometimes yellowish, while those in the other part are always yellowish. The collar is generally divisible into three color zones independent of the three morphological ones; the anterior and posterior zones pale yellow and containing numerous minute glistening granules, while the middle zone which may be indistinguishable in some specimens is orange and containing only a few glistening granules.

External Morphology: There were found always two proboscis pores in the specimens examined, though the worms with only a single pore were reported by Rao (1952, p. 343 and 1962, p. 227). Genital wings seem to develop generally with the growth of the branchial basket. The protuberances of the racemose organ are generally more numerous in the larger specimens, and needless to mention, much fewer in the "hepatic" individuals of different thickness with the regenerating proboscis.

Variations in External Features: Punnett (1903) ever tried to find out any effective character in distinguishing different varieties of *P. flava* from one another and concluded as follows: "...Assuming them, and the assumption seems a fair one, that growth has little or no effect on the relative proportions of the body in *Ptychodera* we have before us an easily applied criterion..." (p. 665). Thus, he took the branchial ratio as the criterion that was thought by him effective to define five varieties in *P. flava* (Table 8 and Figure 121 on pages 665 and 666 respectively). But his idea has never been accepted generally and, on the contrary, *P. flava* has been thought to be very variable in the relative length of different body parts because of a great individual variation, the change with the growth (Horst, 1929, p. 196; 1932a, p. 99;

1939, p. 721 and Trewavas, 1931, p. 45, et al.) and rather frequent regeneration (Crozier, 1920b, p. 186 and Packard, 1968, p. 272). However, seemingly this has not yet been actually demonstrated, therefore I want to make clear in the following lines whether or not the branchial ratio changes with the growth in both the normal and regenerating individuals.

   As the soft body of the worm shrank very differently at fixation even after anes-thetization, I selected out the samples which contained less shrunk specimens as numer-ously as possible. Measurement was made with an occular micrometer set in a stereo-
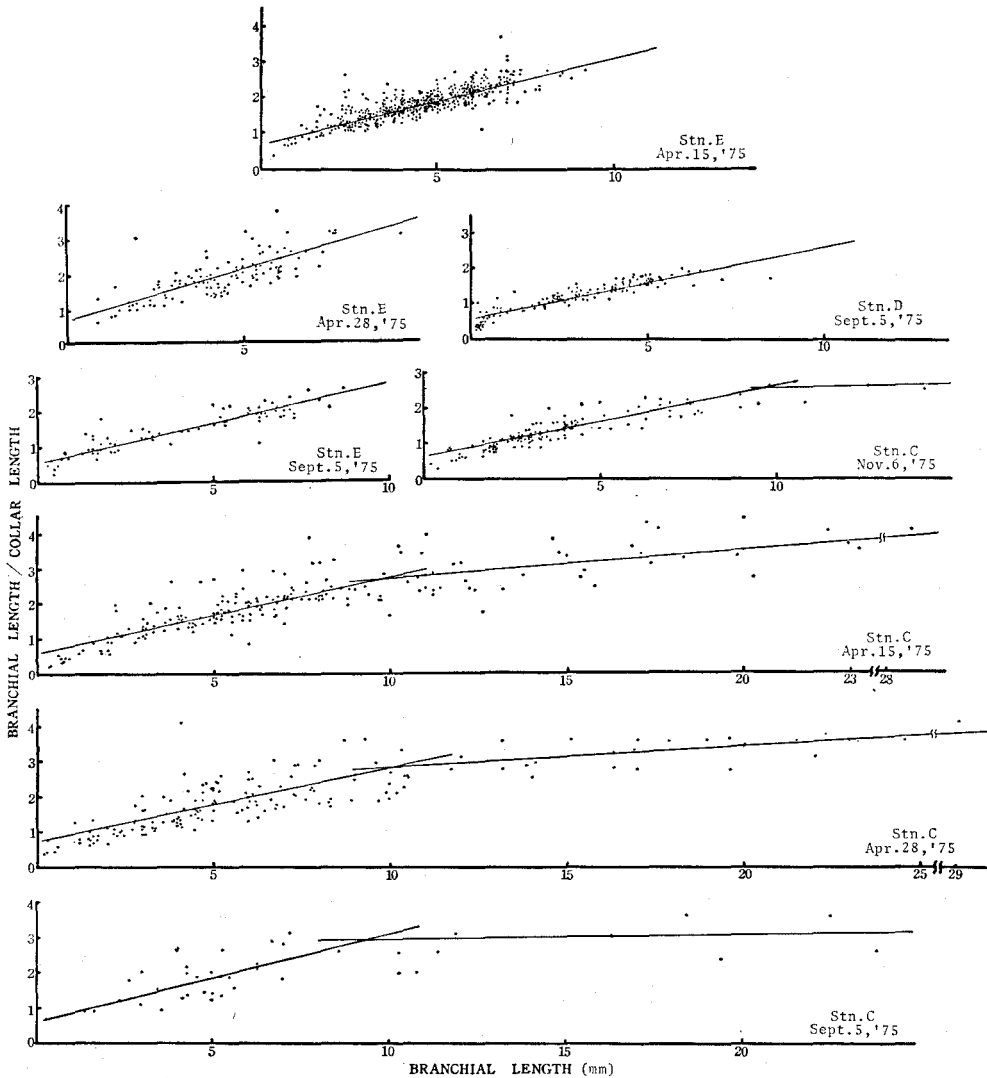

Fig. 2. Ratio of the branchial length to branchial length/collar length in the materials of *P. flava* from different stations in different seasons in the vicinity of Kushimoto. Calculated regression lines are shown.

microscope. Figure 2 shows the ratios of the branchial length to branchial length/ collar length in the materials from different stations in different seasons in Kushimoto. From this the following tendencies may be suggestible:

1) Together with a marked individual variation, the ratio changes with the growth at least in the individuals in later normal development or in a considerably advanced stage of regeneration, and seemingly remains around 3 in the individuals with about 10 mm long branchial region.

2) Throughout all the patterns of the ratio presented here no essential differences are seen between different stations or seasons.

Figure 3A shows the pattern of the same ratio in the material collected on Kohamajima Island, Okinawa Prefecture and Figure 3B shows that plotted on the basis of the measurements given in tables 11 to 13 in the paper of Punnett (1903) and those given in the two tables on pages 42 and 45 in the paper of Trewavas (1931). These two patterns may safely be regarded to be roughly similar to those in the materials from Kushimoto shown in Fig. 1.

Thus it may be concluded that the idea of Punnett is quite unacceptable and that the ratio of the branchial length to the collar length shows no distinct geographical differences so far as the information available at present is concerned.

Fig. 3. Ratio of the branchial length to branchial length/collar length in *P. flava*. A: in the materials collected by Dr. Nishihira on Mar. 30, 1975 on Kohamajima Island. Calculated regression lines are shown. B: plotted on the basis of the measurements given by Trewavas (1931) as to *P. flava* from Low Isles (solid triangle) and Galapagos (solid square) and by Punnett (1903) as to *P. flava muscula* (open square), *P. flava maldivensis* (open triangle), *P. flava gracilis* (inverted solid triangle) and *P. flava laccadivensis* (solid circle).

Excretion of Mucus: The whole worm body is always covered with the clear colorless mucus. When stimulated, both normal and regenerating worms secrete not only the above-mentioned colorless mucus, but also the clear yellow mucus that has not been reported so far.

Bioluminescence: When the worm was stimulated with a pin or forceps in the darkness in the laboratory, the bioluminescence was observed as reported by Edmondson (1946, p. 318) and Rao (1954a, p. 3) in the same species and by Crozier (1915, p. 471, 1917, p. 215 and 1920a, p. 186) and Harvey (1952, p. 204) in the closely related species *P. bahamensis*.

Smell: The worms from Kushimoto are always free from emitting an odor of iodoform, though this smell is reported by Edmondson (1946) in *P. flava* from the Hawaii Islands.

Parasitic Copepods: An individual collected in Kushimoto was found carrying a gall on the genital wing, which contained a parasitic copepod quite similar to *Ive balanoglossi* Paul Mayer, 1879 found in the anterior, mainly branchial, part of the "Leibeshöhle" of *Glossobalanus minutus* (Kowlevsky) and "Zum wenigsten traf ich einige darin an, ..." (Mayer, 1879, p. 515).   This was so far the only case of infestation in the specimens from Kushimoto.   To my surprise, however, 21.2% of the 104 individuals of *P. flava* collected by Dr. M. Nishihira, on March 30, 1975 from a square meter of the beach on Kohamajima Island, the Yaeyama Islands, were infested by the parasitic copepods closely related to *I. balanoglossi*, living in a single or two galls formed on either free edge of the genital wing of respective hosts.   Each gall contained a large ovigerous female together with a small male, numerous eggs and nauplii, as reported by Hill (1897, pp. 7–8) in a large proportion of *Balanoglossus australiensis* Hill observed in New South Wales.   On the other hand, Willey (1898, p. 168) reported that "only two individuals out of many" *P. flava* from New Caledonia were infested with probably the same copepod (also see Willey, 1899, p. 244).   I wonder why such great differences are seen in the percentage of infestation among the different localities.

Regeneration

The regeneration has been referred to in various enteropneusts by many authors; Spengel (1893, p. 684, pl. 26, figs. 14–18) in *Glossobalanus minutus* in the Bay of Naples, Hill (1895, p. 6) in *Balanoglossus australiensis* in New South Wales, Cori (1902, p. 364) in *Bal. clavigerus* in the Bay of Trieste, Kuwano (1902, p. 78, presumption only) in *Bal. misakiensis* in Moroiso Bay, Assheton (1908, p. 519) in *Saccoglossus ruber* (as *Dolichoglossus serpentinus*, a synonymy of *S. ruber* by Burdon–Jones & Patil, 1960) on the west coast of Scotland, Willey (1898, p. 168) in *Ptychodera flava* in the Great Barrier Reef of New Caledonia, and Edmondson (1946, p. 318) in the same species in Hawaii. For instance, Cori recorded as "... in Aquarien gehaltene *Balanoglossus*, welch in der Geschlechtsregion zerrissen waren, regenerierten innerhalb 3 Monaten sowohl die Leber-, als auch die Darmregion, ..."; Assheton stated, "Small pieces on the surface of the sand began to regenerate lost parts and burrowed into the sand ...", and Edmondson wrote, "... if the body is severed transversely into several parts, each segment will, in course of time, develop into a new animal."   On the other hand, detailed studies of regeneration, especially of change in the internal morphology or organogenesis in regeneration, have been made by the following researchers; Willey (1899,

pp. 245–247, pl. XXVI, fig. 5, and pl. XXVII, figs. 66–68) on *Ptychodera flava* from the West Indies, Dawydoff (1902, 1907a, b, 1908 and 1909) on *Glossobalanus minutus*, and Rao (1955b) on *P. flava* from the Gulf of Manaar.

Gilchrist (1923) reported from False Bay, South Africa that the hepatic saccules appeared on the trunk of *Balanoglossus proliferans* Gilchrist kept in the aquarium towards the end of summer and, thus, this species was found to be quite identical with *B. capensis* Gilchrist, and that some pieces torn off from "the extremity of the tail" of *B. proliferans* developed into typical *B. capensis* in summer. And he speculated that *B. capensis* had two types of reproduction, asexual in the "*proliferans*" stage and sexual in the "*capensis*" stage, the two alternating annually, and that individuals of "*proliferans*" type arose "from *P. capensis* by a division of the latter (=*B. capensis*) in front of or at the hepatic region, and subsequent prolongation and proliferation of the genital region, followed finally by regeneration of the lost hepatic and caudal region" (p. 397). Lately Packard (1968) observed the asexual reproduction of a burrowing enteropneust, *Balanoglossus australiensis* of Morton (1950), not *B. australiensis* (Hill), consisting of vegetative division in the transitional region and regeneration of fragments ("regenerant") thus produced as in the "*proliferans*" phase of *B. capensis* reported by Gilchrist, in its natural population on the sheltered Pacific sandy shore on the east to north coasts of Aukland, New Zealand. He also reported the occurrences of "branchiogenital" and "hepatic" individuals of the same species and at the same time confirmed the occurrences of "hepatic individuals, branchiogenital individuals and regenerants" (p. 262) of *Glossobalanus minutus* in its natural population in the Bay of Naples.

The "branchiogenital" and "hepatic" individuals of *P. flava* have been generally collected in the vicinity of Kushimoto, too, but no "regenerants" produced by vegetative division.

In order to clarify the regenerative manner in different body parts, regenerative sequence and the time needed, some simple experiments were tried twice in the laboratory in the period from June to August, 1974. In the present experiments, the fragments were made by cutting the normal immature animals or the "branchiogenital" individuals of various sizes inclusive of "adult" (see p. 407) at various positions and were kept in glass containers without sand on the bottom; all these containers were then set in a large vessel supplied with the running sea water of about 22°C to 28°C, but no food. The fragments, discriminated by the general body features together with the position of containers where they were kept in a vessel, were observed and sketched under a stereo-microscope once or twice a day. Many fragments disappeared during the experiments, leaving only a dozen of fragments traced throughout the experiments. The results of the experiments may be summarized as follows;

1) Isolated proboscis, a fragment with the proboscis, the collar and a small remnant of the wing, or such a fragment with the branchial part in addition never regenerated any of lost posterior parts, only the cut end was closed and in the last fragment the branchial basket was reduced, some of gill bars being fused one another.

2) Individuals lost the posterior part of the hepatic region and the caudal part

never regenerated the lost parts, only the posterior end was closed and some of gill bars were fused one another.

3)   An individual lost most part of the hepatic region and the caudal region produced two fragments (3.5 mm and 4.0 mm long respectively) by autotomy occurred in the posterior terminal portion including the transitional part and the anterior part of the hepatic region after the closure of the cut wound; each fragment firstly closed the cut surface at both ends, and then regenerated anteriorly the proboscis, the collar and a few pairs of gill bars, as observed by Packard (1968).

4)   The fragment with only the genital wing and the transitional region never regenerated the lost anterior and posterior parts, but only the closure of the cut wound took place at both ends.

5)   Fragments consisting of the posterior end of the genital wing and the following body parts to the anus regenerated anteriorly the proboscis, the collar and gill bars.

6)   Fragments with only the posterior part of the branchial region and the anterior half of the hepatic region regenerated anteriorly the proboscis, the collar and gill bars, but the hepatic saccules were somewhat reduced.

7)   Fragments with only the posterior half of the hepatic region and the anterior half of the caudal region regenerated anteriorly the proboscis, the collar and gill bars, but the posterior cut surface was closed or remained open.

8)   Pieces consisting of the posterior part of the caudal region regenerated anteriorly the proboscis and the collar, but unfortunately the regeneration of gill bars was not confirmed, for the pieces were accidentally lost.

The above-mentioned features but 3) were common to the fragments coming from individuals of different sizes.

The fusion of some gill bars observed in the present experiments seems unusual, for any individuals with such fused gill bars have never been collected in the field.

From these observations, it is generally confirmed, as already observed by Rao (1955, p. 1), that this animal can regenerate lost anterior body parts, though it remains still unknown whether the lost posterior body parts, the genital wing or hepatic saccules are actually regenerated even in the "branchiogenital" individuals as observed by Gilchrist, and whether the fragments of sexually matured individuals regenerate lost body parts.   Field observations seem to suggest that the natural breakage occurs exclusively in the transitional region as noted by Packard (1968, p. 264 and pp. 270–71).

The sequence and the time needed for regeneration were as follows;

a)   Closure of cut wound within 1 to 3 days.

b)   Appearance of proboscis as a minute projection within 2 to 5 days.

c)   Opening of mouth within 2 to 6 days.

d)   Appearance of collar within 6 days.

e)   Appearance of gill bars within 14 to 15 days.

Generally speaking, it takes seemingly about three weeks in laboratory conditions to reach the stage (e) in cut pieces or in small pieces produced by autotomy.

The epidermis of the proboscis of the normal individuals contains numerous minute glistening granules, but the granules are distributed very sparsely in the initial stage of regeneration. The proboscis and collar newly regenerated are very weak and almost transparent, but they become indistinguishable from those of normal individuals in about two months. Regenerating proboscis may easily drop off from the body and it was observed once that the regenerating collar was reduced after the proboscis dropped. Cut fragments will become active when the proboscis is completed functionally. Sometimes regenerating fragments were observed creeping around on their dorsal side.

Notes on the Population of *P. flava* in the Locality

Stations and Method of Observation: As seen in the map of Fig. 4, five stations were selected to cover the various habitats within the locality. At Stn. C, a much thicker layer of fine sand is deposited upon the rocky substratum and is furnished sparsely with larger boulders, while at the other stations there are only smaller boulders or pebbles over the surface of the poorly developed sand layer, especially at Stn. E where the stones are much smaller. At Stns. A, B, and D narrow sandy patches are formed here and there on an exposed rocky flat. Two or three collecting points were chosen at each station.

Regular sampling of the population was made at the lowest water of every spring tide, when the habitats of the worm were more or less exposed. Therefore, collections were made at midnight in winter. Sampling was sometimes disturbed by higher low tide, surfs and waves and night darkness. All individuals found under one or a few stones covering an area of about 400 cm² were collected at each collecting point. The area of collection at respective points were roughly measured, except at Stn. E where a quadrat of 20 cm × 20 cm could be used, because there only smaller stones were dispersed evenly.

A sieve was used several times to collect minute materials, that are, settled larvae, juveniles or "regenerants" (see p. 403) of *P. flava*, but this was not successful. Instead, all the individuals collected with a sieve were more or less broken off, so sieving was abandoned and the materials were picked up from the sand. The samples of the sand from the locality were also examined under a stereo-microscope, but neither larvae nor juveniles were found.

The collected specimens were anaesthetized with menthol after the coloration and the state of gonads were checked, and then fixed with the Bouin's fluid for about a day before they were preserved in 70% alcohol.

The specimens were divided into the following 4 groups according to their external appearances:

I) "Normal" — having a complete set of body parts: proboscis, completely formed collar, longer branchial region and distinct hepatic saccules.

II) "Branchiogenital" — having the complete anterior body parts, with either the long branchial region and well developed genital wings or the short branchial

region and less developed genital wings, and posteriorly including no or less developed hepatic saccules (see Fig. 1B).

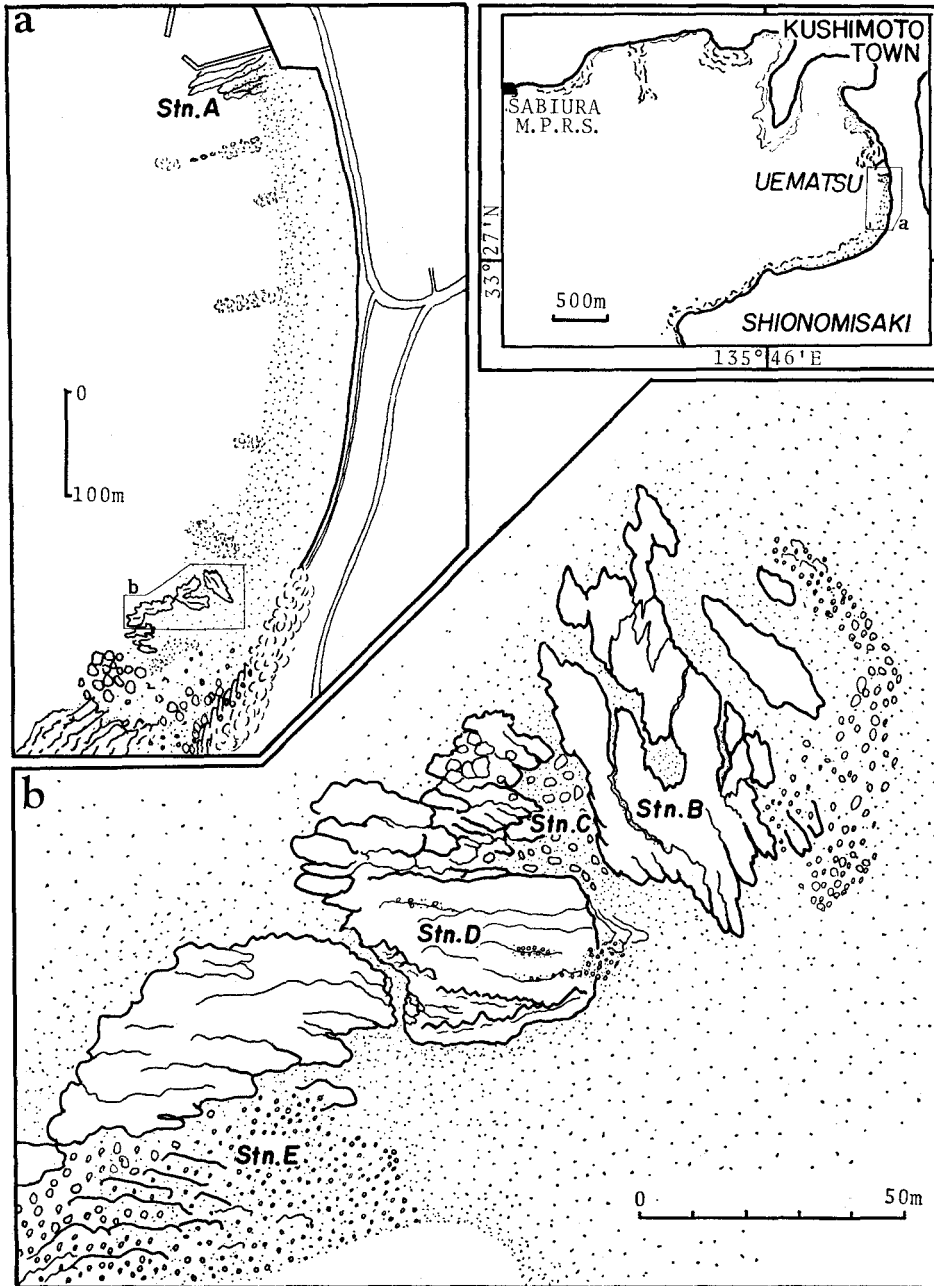III) "Hepatic" — having the well-developed hepatic region and the subse-

Fig. 4.   Map showing the observation stations A to E in the vicinity of Kushimoto.

quent caudal region and anteriorly including the less developed collar and a few or none of gill bars. This may be regarded as representing a state regenerating the lost parts (see Fig. 1C).

IV) "Juvenile(?)" — being thinner, with less developed branchial and hepatic regions. As the whole life cycle has not yet been confirmed by myself, I fear if the name of this group is inadequate.

Mature Season: As shown in Figs. 6 and 7, a small number of ovigerous females occurred so far exclusively at Stn. C in January, March, July, September, October and December. They are longer and thicker and the branchial length of ovigerous worms is usually over 10 mm as seen in Fig. 6. And as shown in Fig. 2, the point of inflection in the pattern of the ratio of the branchial length to branchial length/collar length is situated at about 10 mm in the branchial length. From this coincidence, it is proposed here to define provisionally the individuals with the branchial region longer than 10 mm as adult worms.

Ovarian eggs are pale yellow and 140 to 230 μ in diameter in the worms from the locality, while the matured(?) eggs from the same species in the Gulf of Manaar are described by Rao (1954b, p. 147) to be purplish orange and about 140 μ in diameter. Matured males have not been collected yet, though a few individuals with a brownish tint reminiscent of the "brown pigment in the integument covering the testes" (Willey, 1899, p. 228; also see, Willey, 1899, p. 240 and Rao, 1954, p. 3) have ever been collected in winter in the vicinity of Kushimoto. This means an extremely low sex-ratio of male to female in the present species, as reported by Rao (1954a) who recorded the ratio of one male to about 65 females in the Gulf of Manaar (p. 3).

As to the mature season of *P. flava,* Devansen and Varadarajan (1940, cited from Rao, 1954, p. 4 and 1955, p. 275) reported the breeding season of this species to extend from December to February in the Gulf of Manaar(?), and Rao (1954, p. 4) stated that "... the percentage of mature individuals was considerably high during March" and then he suggested, judging from the occurrence of Tornaria larva off Madras, as "two or more breeding periods occur in a year." So far as the above-mentioned field data in the vicinity of Kushimoto are concerned, it can not be judged conclusively whether *P. flava* in this locality is sexually matured throughout the year, or it has some definite breeding periods in a year.

Density: The density, calculated as the number of individuals per 100 cm^2, is shown in Fig. 5 for each time of collection at each station, though it is only a rough estimation for Stns. B, C and D. From this figure, no distinct signs of seasonal change can be seen, although much greater fluctuation may be noticed at Stns. D and E than at Stns. B and C, the causes of which are unknown. The lower density at Stn. C may be related to the larger body size of individuals occurred there.

Population Composition and Discussions: The relative composition of four groups (see pp. 405–7) in each sample collected at Stns. B to E is given in Fig. 6. Significantly lower rate of "juvenile(?)" individuals throughout the stations and the year might at least partly be attributable to the unsatisfactory way of collection as stated

elsewhere, though it cannot be explained exactly at present. Nevertheless, the follo-
wing features appear to be noticeable from this figure:

    1)   The population composition varies irregularly throughout the stations.

    2)   The rate of "branchiogenital" individuals is generally 10 to 20%, while that
of "hepatic" individuals is roughly 10 to 25%, considerably higher than the rate,
"some 8–10%", of regenerating individuals recorded by Punnett (1903, p. 604) who,
however, seems to have included only the "hepatic" individuals defined in the present
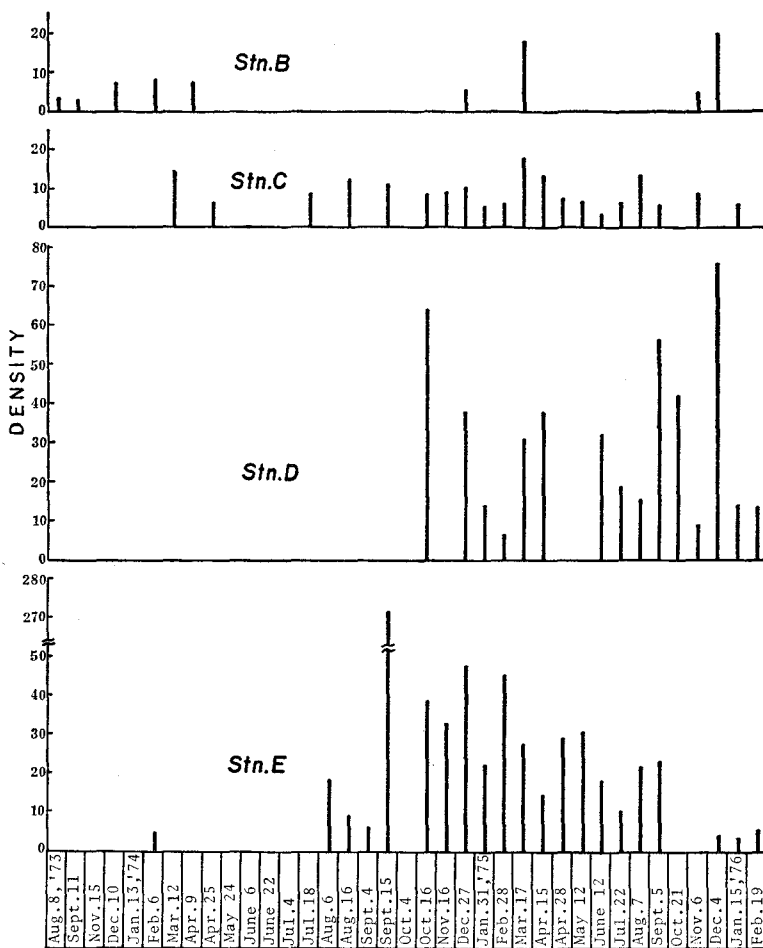paper in his regenerating individuals and not those of "branchiogenital".

Fig. 5.   Density of *P. flava* at each station on respective days of collection.

    The branchial length of the worm may be thought to be related closely with
the time needed for regeneration or normal development, except in the case of
"branchiogenital" individuals, and morphologically the individuals with longer bran-
chial region are generally larger, except in the case of "hepatic" individuals, and are
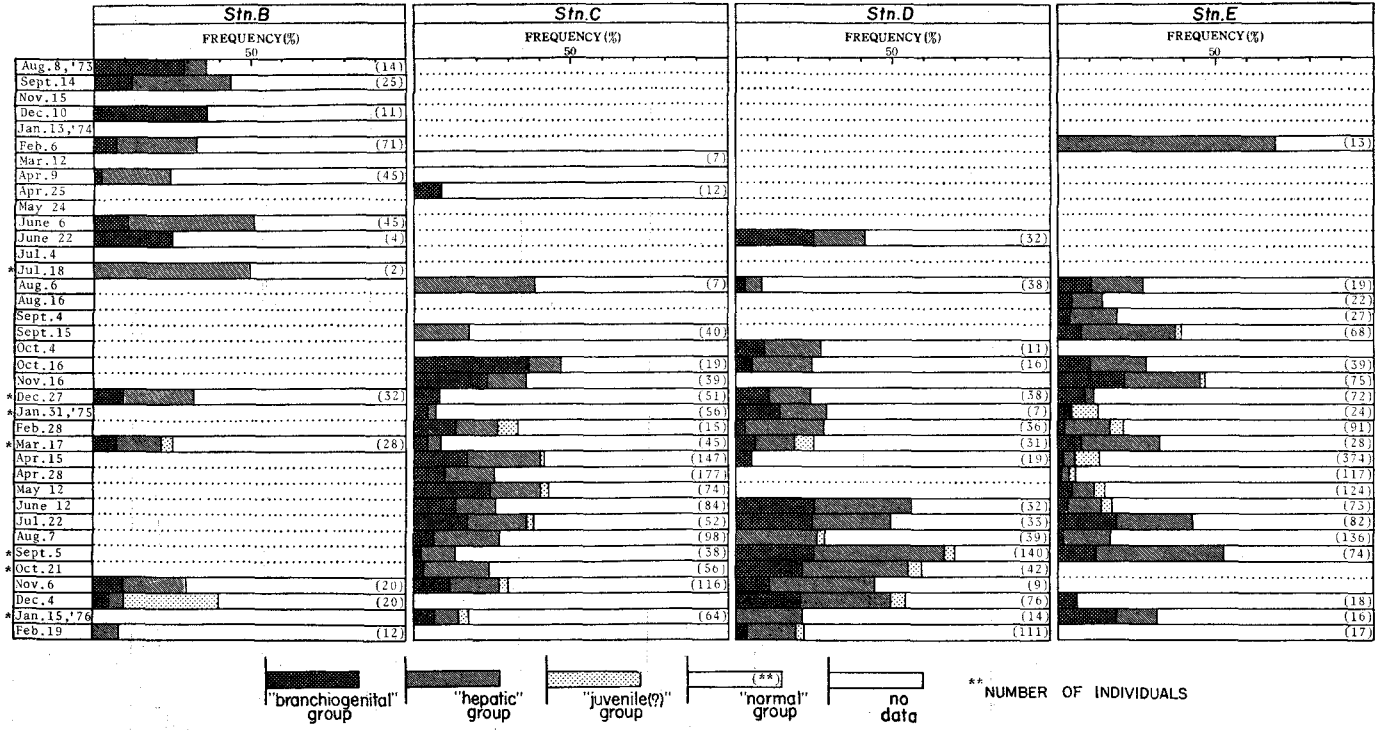provided with well developed genital wings.

Fig. 6. Composition of collected samples from Stns. B, C, D and E. *Ovigerous individuals were found only in the sample from Stn. C.

The frequency (%) distributions of the branchial length in respective samples collected at Stns. C, D and E in the period from October 1974 to February 1976 are shown in Fig. 7. From this figure the following features may be deducible:

1) The pattern of the frequency distribution is, on the whole, almost the same throughout the period of observations and the samples from the three stations, with a peak of frequency usually between 3 to 6 mm of the branchial length and without
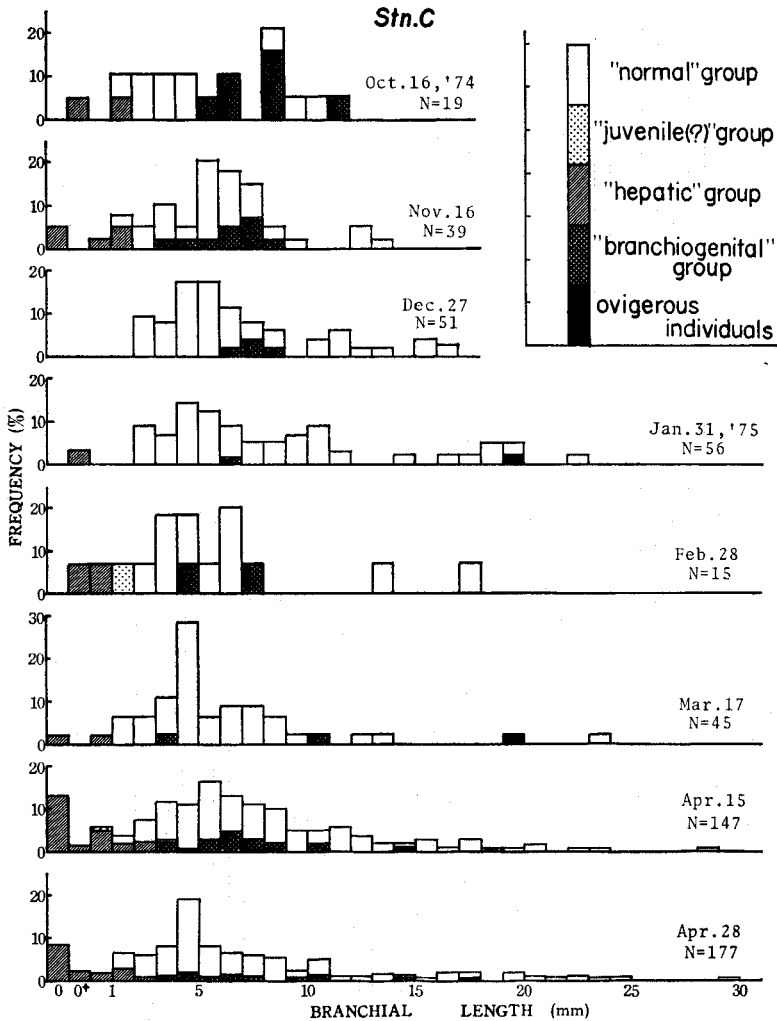

Fig. 7. Frequency (%) distribution of branchial length in respective collected samples from Stns. C, D and E during the observation from October 1974 to February 1976. Zeros on the abscissa represent the individuals without any trace of branchial region, 0 shows such individuals with only the proboscis and 0+ concerns those with both the proboscis and collar.

any tendency of regular shifting of the peak. This clearly suggests an incessant occurrence of some kinds of recruitment.

    2) The recruitment of young individuals and changes duly caused by it in the pattern of frequency distribution were indistinct.

    3) Adults, as defined in the present paper, were found throughout the year mainly at Stn. C. This may probably be related to the larger thickness of the sand layer deposited on the rocky substratum at this station.

    The occurrence of comparatively higher percentages of regenerating individuals in respective samples, as seen in Figs. 5 and 6, can not be considered as being brought about by artificial breakage at repeated monthly collection, for most of the samples

Fig. 7. (continued).

Fig. 7. (continued).

collected at initial sampling at respective stations or the samples collected at intervals
of more than two months in any seasons and at any stations comprised significant
number of "hepatic" or "branchiogenital" individuals and especially the latter al-
most always included the individuals without any gills or with a few pairs of gill bars,
that had evidently been produced by the breakage in the transitional region within

Fig. 7. (continued).

Fig. 8. Frequency (%) distribution of body width in collected samples from Stn. C
in 1975, for measurement of body width see the text (p. 415).

three weeks, as suggested from the results of laboratory experiments (p. 404).

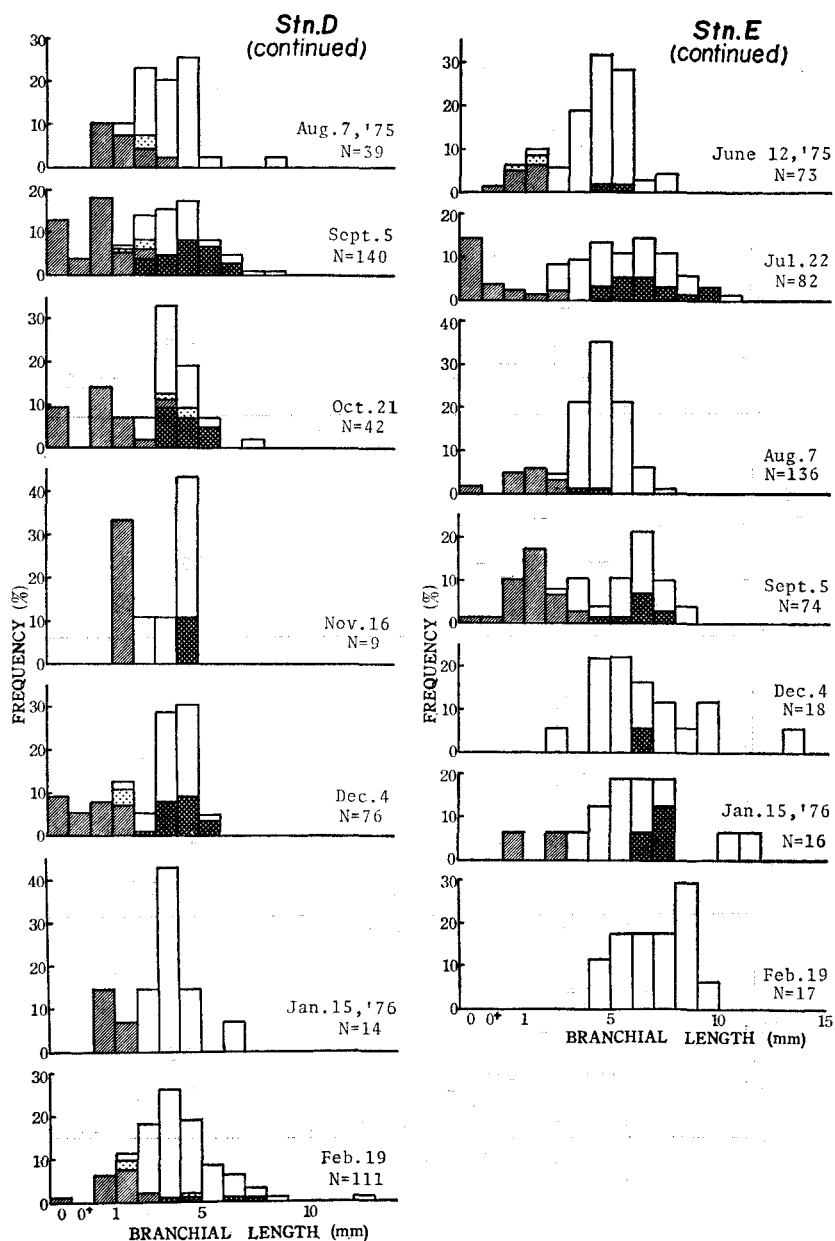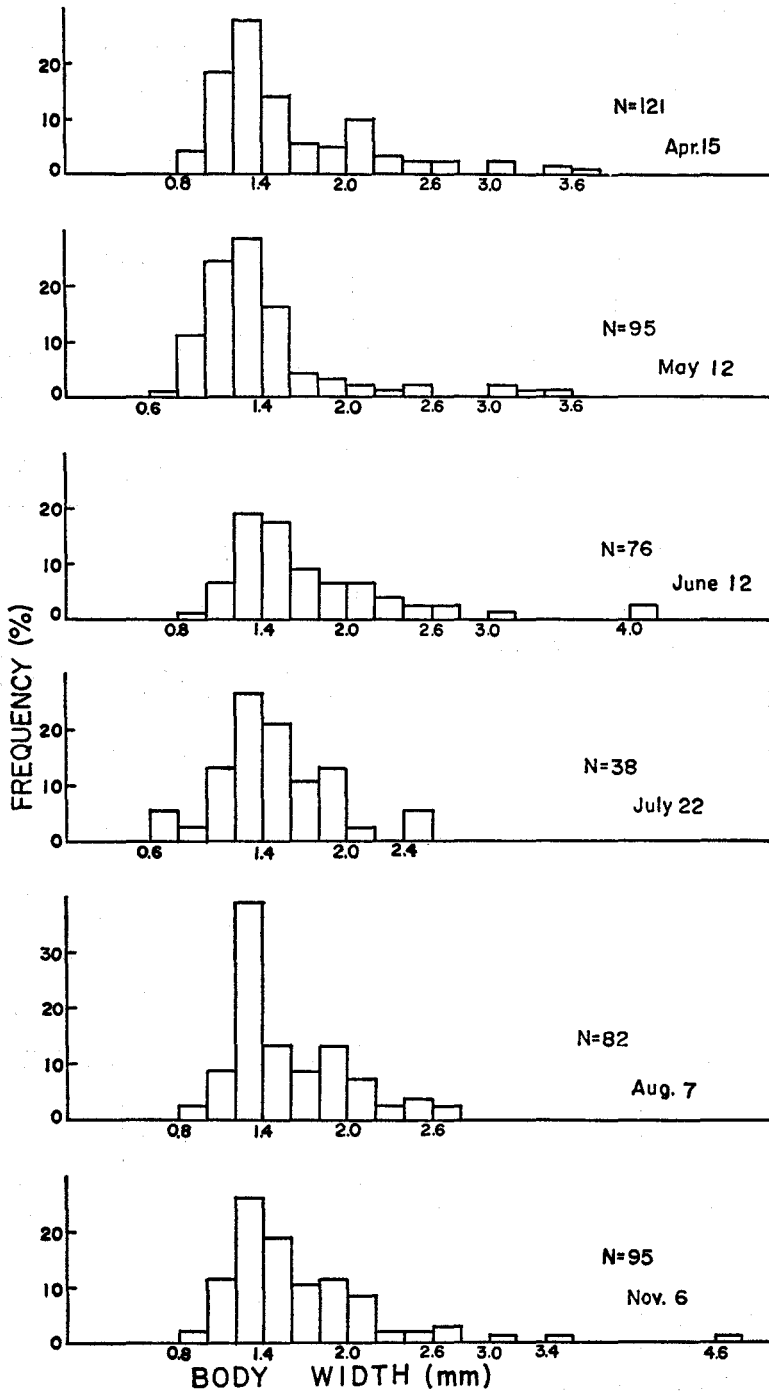The width of the body, especially that of the posterior part of the trunk, may safely be accepted as the best feature suggesting the age of the worm or of the oldest part of individuals in the course of regeneration, though unfortunately the body width in the living state can hardly be retained satisfactorily in preserved specimens because of irregular contraction or shrinkage at fixation. Figure 8 is an example of frequency (%) distribution of the trunk width, measured between the swellings at the posterior end of genital wings where the anterior-most pair of hepatic saccules are inserted, in the samples collected at Stn. C. The patterns in different seasons show an amazing similarity, that can be regarded as the result of the continued recruitment of young (=thinner) individuals produced sexually or, if present, asexually. The absence or very lower percentages of young individuals, as seen in this figure, can not be explained clearly at present, though it may happen that the habitat of settling larvae is different from that of grown worms.

The similarity or relative stableness of the patterns of frequency distribution of branchial length throughout the year, indicated elsewhere, is a matter of consideration. Although no or few settling larvae or "juveniles" have been collected, the recruitment of young individuals to the population is certainly occurring, seemingly with no respect to season. On the other hand, regenerating individuals are also naturally appearing quite frequently in all seasons. There is no reason to suppose that these two processes are not related to the similarity of frequency distribution concerned. Further, it is likely that the regeneration plays some role in maintaining the population and that it should be very significant, if the recruitment of young individuals does not take place successfully under natural conditions in the locality. Much lower percentages of ovigerous individuals which produce planktonic larvae, may cause an objection to attributing the primary role to sexual reproduction in the population. On the contrary, if successive budding, too, is to occur in the transitional region of *P. flava,* as in *Balanoglossus australiensis* of Morton and *B. capensis* (see p. 403), then "hepatic" or "branchiogenital" individuals may be only the by-products of the asexual reproduction achieved by regeneration of fragments produced by this budding. Anyhow, in order to reveal the relative role of regeneration, sexual reproduction and asexual budding in the population, the life cycle of *P. flava* in the vicinity of Kushimoto must be traced more closely in the future.

Summary

1. The tropical enteropneust *Ptychodera flava* was found for the first time in Japan in the vicinity of Kushimoto, Wakayama Prefecture, so far the northern-most locality for the species.

2. The worms were found almost exclusively under stones lying on the thin sandy substratum deposited between the rocky elevations on the shore.

3. The ratio of the branchial length to the collar length seems to change with growth, but the pattern of change seemingly remains invariable between stations

in the locality or between localities as well as throughout the year.

4. The experiments to learn the regenerative manners in the various parts of body showed that this animal could regenerate lost anterior body parts, as reported by Rao (1955), and that newly separated pieces were furnished with gill bars about three weeks later.

5. The ovigerous individuals were collected in January, March, July, September, October and December.

6. Adults, provisionally defined as the individuals with the branchial region longer than 10 mm, were found mainly at Station C in the locality; this might be related to the much thicker sand layer at that station.

7. Comparatively high percentages of regenerating individuals throughout the year depend on the frequent natural breakage in the transitional region of the worm.

8. The change in the population at respective observation stations is seemingly quite irregular, while the pattern of the frequency distribution of the branchial length seemingly quite invariable throughout the collected samples.

9. The pattern of the frequency distribution of the body width which may show directly the age of the worm seems quite invariable among the samples collected in different seasons at Station C. This may suggest a steady recruitment of young individuals to the population.

10. The possible outline of maintaining the population and the role of regeneration as compared with that of sexual reproduction and asexual budding in the population in the vicinity of Kushimoto were discussed briefly.

Appendix

Further localities of *P. flava* in Japan

The other finds of *Ptychodera flava* so far confirmed in Japan are located in the so-called Nansei Islands stretched between Kyûshû and Formosa and including the Islands of Tokara, Amami and Okinawa. In these localities the worms are always found in the superficial layer of clean sand, but rarely under stones as in the vicinity of Kushimoto. Sometimes their indistinct mounds of casts may be observed.

The Tokara Islands; Kuchinoshima Is. Nishinohama (May 6, 1976). Takarajima Is. Akagizaki (May 2, 1976), south-western coast facing Muudachi (May 4, 1976).

The Amami Islands; Tokunoshima Is. Kametoku (Apr. 20, 1976). Okinoerabu Is. Wadomari (Apr. 19, 1976). Yoron Is. Akazaki (May 12, 1975 by Y. Nakajima and Apr. 16, 1976), Tijizaki (May 2, 1976 by Y. Nakajima), Terazaki (Apr. 18, 1976).

The Okinawa Islands; Okinawa Is. Heianzajima (Mar. 26, 1975 by M. Nishihira), Awase (Mar. 21, 1975). Ishigaki Is. Kabira (Apr. 1 and June 24, 1975). Kohamajima Is. (Mar. 30, 1975 by M. Nishihira).

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