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Stages in the Early Development of *Parathemisto gaudichaudii* (Guér.) (Crustacea Amphipoda: Hyperiidea), the Development of Secondary Sexual Characters and of the Ovary

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Abstract

The female of the hyperiid amphipod *Parathemisto gaudichaudii* develops a very large brood-pouch at the last moult before the eggs are laid. Young animals in the pouch range in size from a diameter of 0.35mm (eggs and embryos) to a length of 2.8mm (free extended juveniles). It seems likely that two moults occur after the embryo frees itself from the egg-membrane and before it is able to leave the shelter of the pouch. Several post-brood-pouch stages are described. Later in life the sexes are readily distinguishable by secondary sexual characters which make it possible to assess their maturity. Developmental stages of these characters and of the ovary are described and figured to maturity.

Introduction

Parathemisto gaudichaudii is an oceanic swarming species of the Amphipoda Hyperiidea and is found mainly in surface waters. Although previous authors have recorded the presence or absence of the eggs and whether juvenile or adult animals were taken, the development and breeding cycle have not been studied in detail.

The young hatch as miniature adults in the brood-pouch of the mother and grow by a series of moults during which the pleopod rami become segmented and sensory filaments are added to the ventral surface of the first antenna. As the animals mature secondary sexual characters develop, making it possible to distinguish male and female animals. This paper is concerned with the development of first the brood-pouch and post-brood-pouch animals, and then with the secondary sexual characters and the ovary.

Material and Methods

Specimens were supplied by the New Zealand Oceanographic Institute from waters west and south of New Zealand (Kane, 1962). Material from the "Discovery" Collections has helped fill gaps in many of the stages described

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(Table I). Drawings were made with a camera lucida. In order to distinguish clearly the various developmental stages the animals were stained with lignin pink and permanent whole mounts made.

Table I.—STATION LIST.

Station No.	Latitude	Longitude
N.Z.O.I.		
A 313	46° 46′ S	164° 35′ E
B 30	59° 58′ S	169° 07′ E
B 33	52° 00′ S	167° 30′ E
B 35	48° 28′ S	167° 23′ E
B 113	60° 22′ S	170° 54′ E
B 114	59° 39′ S	171° 02′ E
B 118	55° 34.5′ S	170° 27′ E
B 120	53° 26.3′ S	170° 15′ E
"Discovery"		
960	58° 31.4′ S	150° 02.9′ W
2205	60° 49.1′ S	164° 07.6′ E
2449	51° 34.7′ S	20° 16.4′ E
2522	45° 38.9′ S	20° 15.2′ E
2623	47° 26.8′ S	19° 37.4′ E
2768	63° 00′ S	175° 05′ E
2873	54° 40′ S	56° 56′ E
2874	56° 50′ S	66° 12′ E

A. The Development of Brood-Pouch and Post-Brood-Pouch Stages

The juvenile animals examined include those from brood-pouches of ovigerous females in the N.Z.O.I. material and several from the "Discovery" Collections, and 860 post-brood-pouch stages up to 7mm in length. Data were obtained for total body lengths, the number of podomeres or segments on the outer ramus of the first pleopod, and the number of sensory filaments on the first antenna.

Measurements of the total length include the head (excluding the antennae) the peraeon and pleon segments and the urosome (including the uropods). Diameters are given for many of the brood-pouch animals because being circular in shape they are difficult to measure in any other way. There are three pairs of pleopods, each with an exopodite and an endopodite, which become segmented into what are here termed podomeres. (As the number of podomeres formed on the exopodite is usually one or two more than that on the endopodite only the former number is recorded here.) Sensory filaments develop on the ventral surface of the first antenna. In the early stages the number developed can be easily seen, but in post-brood-pouch stages counting becomes increasingly difficult and these data are not recorded here.

The data are recorded graphically in Fig. 4. This figure shows the increase in number of podomeres with increase in total body length, as well as the number of specimens and average total body length (marked by O) in each podomere group.

1. Brood-Pouch Animals

(a) Development Within the Egg-membrane

Thirty fertilised eggs were examined, ranging in diameter from 0.39mm to 0.5mm. These eggs are still contained within a tough egg-membrane and contain a large amount of yolk material, which seems to consist mainly of oil globules of varying sizes (Fig. 1a and 1c). There is an extensive development of peripheral cells and the formation of cephalic and posterior body rudiments (Fig. 1a). At a later stage these peripheral cells become arranged to form outlines of the

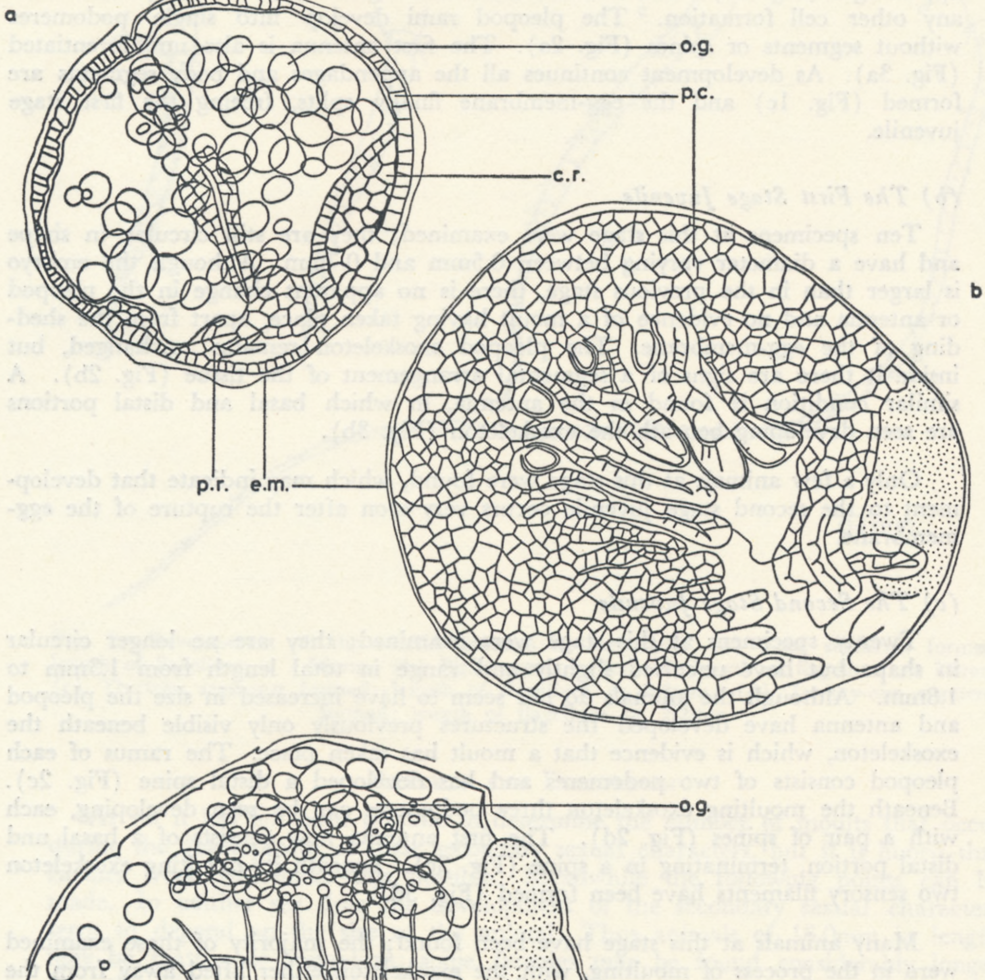


FIG. 1.—Development within the egg-membrane. (a) Internal view of 0.39mm x 0.39mm embryo. (b) Surface view of 0.52mm x 0.42mm embryo. (c) Lateral view of 0.52mm x 0.42mm embryo, which is breaking free from the egg-membrane. o.g. = oil globules; c.r. = cephalic rudiment; p.r. = posterior body rudiment; p.c. = peripheral cells; e.m. = egg-membrane; m.ex. × moulting exoskeleton.

appendages (Fig. 1b). The internal mass of oil globules at this stage obliterates any other cell formation. The pleopod rami develop into single podomeres without segments or spines (Fig. 2a). The first antenna is also undifferentiated (Fig. 3a). As development continues all the appendages and body segments are formed (Fig. 1c) and the egg-membrane finally splits, freeing the first stage juvenile.

(b) The First Stage Juvenile

Ten specimens at this stage were examined; they are still circular in shape and have a diameter varying between 0.5mm and 0.7mm. Although the embryo is larger than in the previous stage, there is no apparent change in the pleopod or antenna and no evidence of a moult having taken place, apart from the shedding of the egg-membrane. The pleopod exoskeleton remains unchanged, but inside it there are signs of a segmental arrangement of the tissue (Fig. 2b). A similar condition is found in the antenna, in which basal and distal portions are seen developing beneath the exoskeleton (Fig. 3b).

Only a few animals at this stage were found, which may indicate that development to the second stage juvenile occurs very soon after the rupture of the egg-membrane.

(c) The Second Stage Juvenile

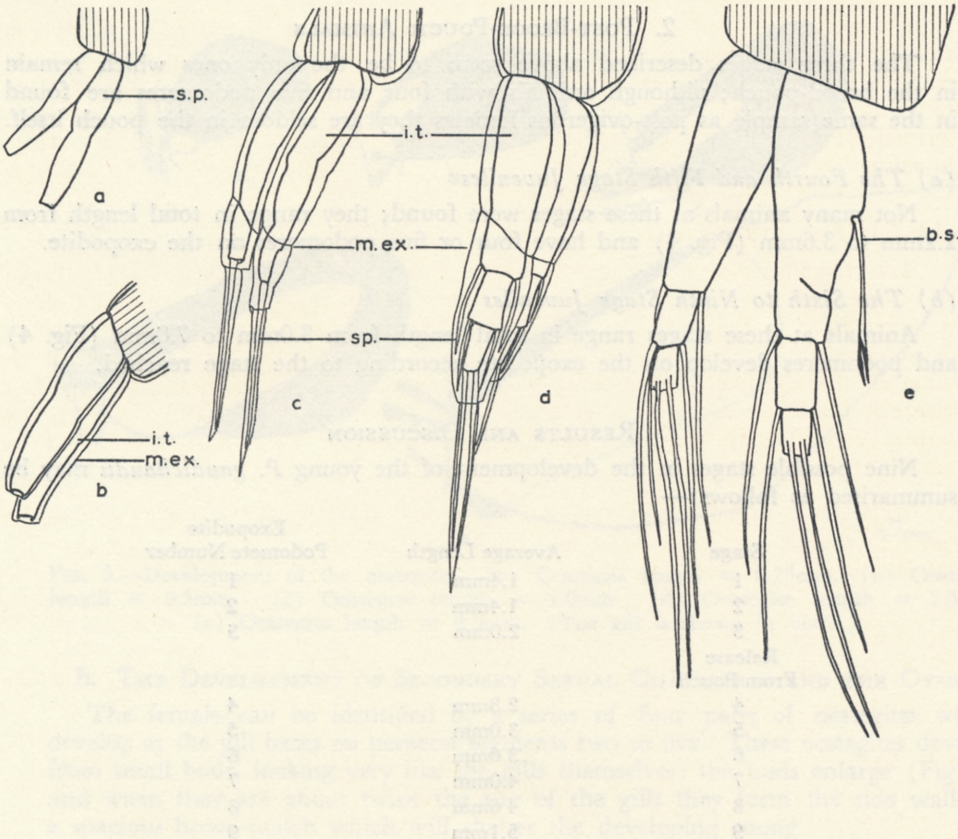
Twenty specimens at this stage were examined; they are no longer circular in shape but have uncurled slightly and range in total length from 1.3mm to 1.8mm. Although the animals do not seem to have increased in size the pleopod and antenna have developed the structures previously only visible beneath the exoskeleton, which is evidence that a moult has taken place. The ramus of each pleopod consists of two podomeres and has developed a distal spine (Fig. 2c). Beneath the moulting exoskeleton three podomeres can be seen developing, each with a pair of spines (Fig. 2d). The first antenna now consists of a basal and distal portion, terminating in a spine (Fig. 3c). Beneath its moulting exoskeleton two sensory filaments have been formed (Fig. 3d).

Many animals at this stage have been found; the majority of those examined were in the process of moulting, with the exoskeleton either lifted away from the underlying tissue or already being sloughed off.

(d) The Third Stage Juvenile

Fifty specimens at this stage were examined; they are uncurled, fully extended and range in total length from 1.5mm to 2.6mm. Besides a considerable size increase features that were previously visible developing beneath the exoskeleton have now been fully formed and few moulting animals are found. The pleopod rami have three well developed podomeres, each with a pair of distal spines (Fig. 2e), and the endopodite develops the bifid spine (Fig. 2e) first noticed by Stebbing (1888). The antenna has basal and distal portions, the peduncle and flagellum, with distal spines and two sensory filaments (Fig. 3e).

There is still a large quantity of yolk in the gut region, and it seems likely that the young animal still uses this as its main source of food. Most of the mouthparts were formed, but the mandibular palp is still only a small, unsegmented structure. Many third stage juveniles were found, the majority of which appear to have just moulted.



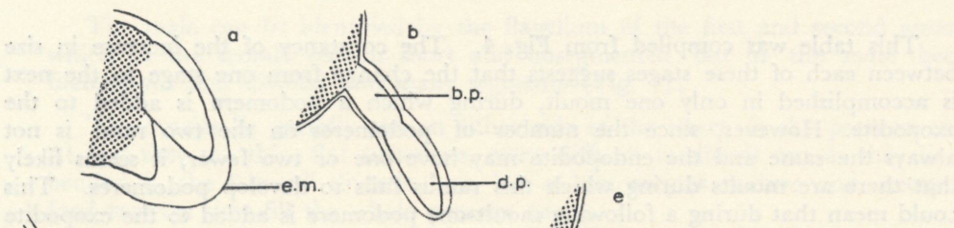


FIG. 2.—Early development of the first pleopod. (a) Stage within the egg-membrane. (b) Stage 1. (c) Stage 2. (d) Stage 2. (e) Stage 3. s.p. = single podomere; m.ex. = moulting exoskeleton; i.t. = internal tissue; sp. = spines; b.s. = bifid spine.

FIG. 3.—Early development of first antenna. (a) Stage within egg-membrane. (b) Stage 1. (c) Stage 2. (d) Stage 2. (e) Stage 3. e.m. = egg-membrane; b.p. = basal portion; d.p. = distal portion; m.ex. = moulting exoskeleton; sp. = spine; s.f. = sensory filament; p. = peduncle; f = flagellum.

2. Post-Brood-Pouch Animals

The three stages described above seem to be the only ones which remain in the brood-pouch; although animals with four and five podomeres are found in the same sample as post-ovigerous females they are seldom in the pouch itself.

(a) The Fourth and Fifth Stage Juveniles

Not many animals at these stages were found; they range in total length from 2.2mm to 3.6mm (Fig. 4) and have four or five podomeres on the exopodite.

(b) The Sixth to Ninth Stage Juveniles

Animals at these stages range in total length from 3.0mm to 7.0mm (Fig. 4) and podomeres develop on the exopodite according to the stage reached.

Results and Discussion

Nine possible stages in the development of the young *P. gaudichaudii* may be summarised as follows:—

Stage	Average Length	Exopodite Podomere Number
1	1.4mm	1
2	1.4mm	2
3	2.0mm	3
Release From Pouch		
4	2.8mm	4
5	3.0mm	5
6	3.6mm	6
7	4.0mm	7
8	4 6mm	8
9	5.1mm	9

This table was compiled from Fig. 4. The constancy of the increase in size between each of these stages suggests that the change from one stage to the next is accomplished in only one moult, during which a podomere is added to the exopodite. However, since the number of podomeres on the two rami is not always the same and the endopodite may have one or two fewer, it seems likely that there are moults during which this ramus fails to develop podomeres. This could mean that during a following moult one podomere is added to the exopodite and two to the endopodite, so that once again both rami have the same number of podomeres. Alternatively it could indicate that there are moults between the stages I have described above, and during these the exopodite remains unchanged, but the endopodite develops new podomeres. If these intermediate stages do exist they will not become apparent until endopodite data have been examined.

As the average body lengths of the first two stages are the same there is not obviously a moult between them; however, if the pleopods and antennae of the two stages are compared it is clear that the difference between the two exoskeletons can only be accounted for by moulting. Also the average lengths of the fourth and fifth stages are very similar; these animals have just escaped from the brood-pouch and are beginning to use their pleopods for swimming. Thus an increase in podomere number would probably be advantageous, and if this were so moulting from the fourth to fifth stage may take place so rapidly that an increase in total length is almost negligible.

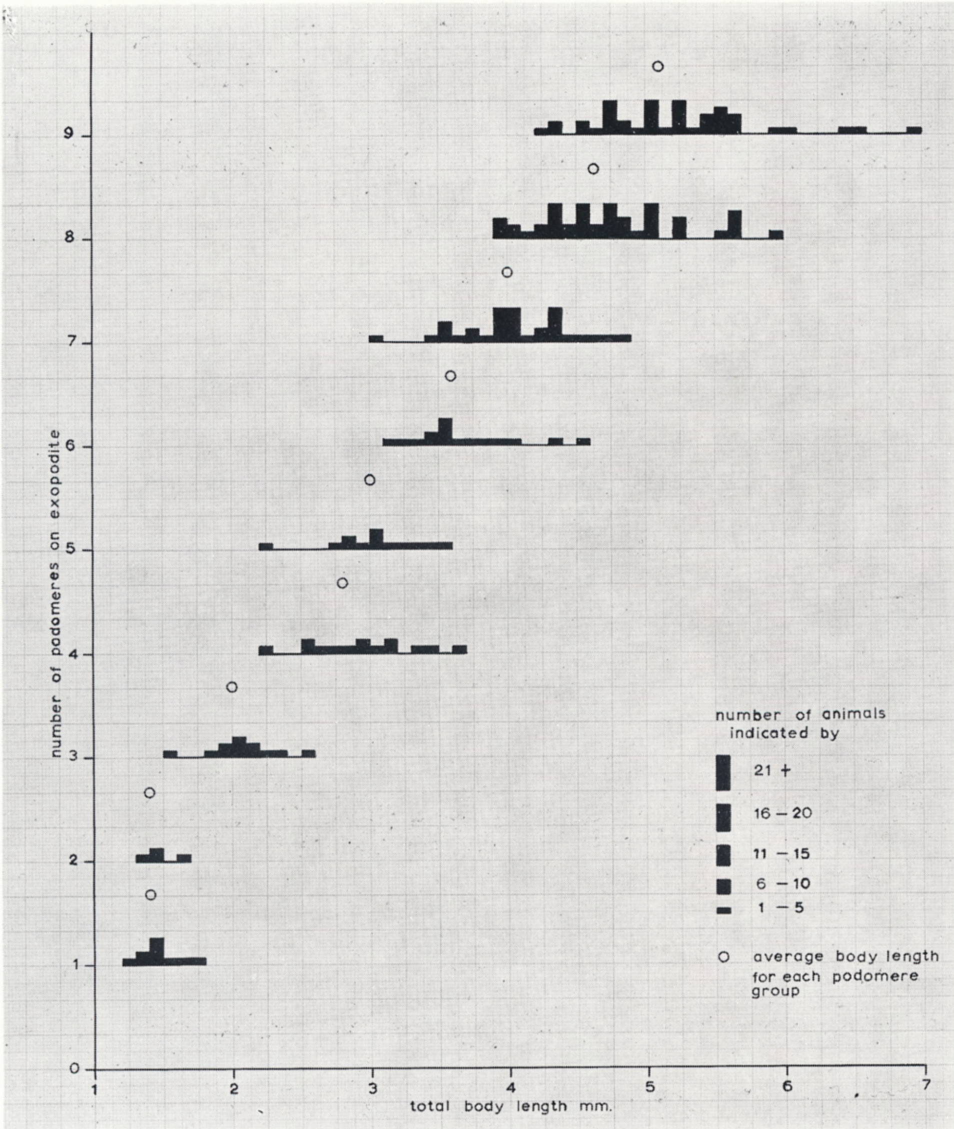


Fig. 4.—Addition of pleopod segments in relation to growth. O = average total body length for each podomere group.

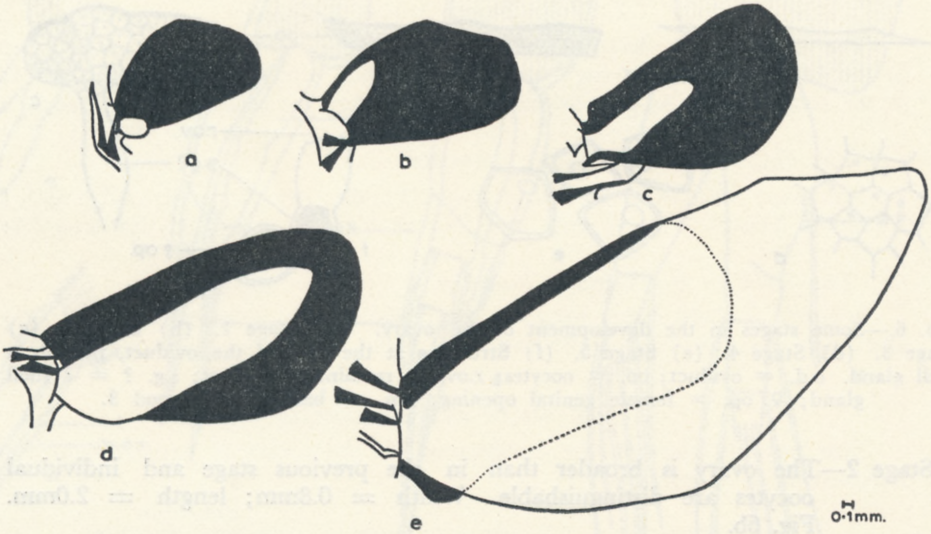


FIG. 5.—Development of the oostegites. (a) Oostegite length = 0.25mm. (b) Oostegite length = 0.5mm. (c) Oostegite length = 1.0mm. (d) Oostegite length = 2.5mm. (e) Oostegite length = 4.0mm. (The gill is shown in black.)

B. THE DEVELOPMENT OF SECONDARY SEXUAL CHARACTERS AND THE OVARY

The female can be identified by a series of four pairs of oostegites which develop at the gill bases on peraeon segments two to five. These oostegites develop from small buds, looking very like the gills themselves; the buds enlarge (Fig. 5) and when they are about twice the size of the gills they form the side walls of a spacious brood-pouch which will shelter the developing young.

The male can be identified by the flagellum of the first and second antennae which in the female remain short and unsegmented but in the male become filamentous and divided into many segments (Fig. 7).

The ovaries lie dorsally one on either side of the heart, in the peraeon cavity. At first they are thin, flat structures, often difficult to dissect out as they lie embedded in the "liver" diverticulum, but as the oocytes mature and incorporate food reserves they fill the whole peraeon cavity.

1. FEMALE

(a) Oostegites

Oostegites of 272 females, ranging in total length from 12.0mm to 27.0mm, were examined and their lengths found to vary from 1.0mm to 5.0mm. I have been unable to detect any correlation between oostegite length and total body length. Five stages in the development of the oostegite are shown in Fig. 5.

(b) Ovary

Ovaries from the above animals have been dissected out, and can be divided into five stages according to the development of the oocytes. These are illustrated in Fig. 6; the drawings are not to scale and stage numbers are given for reference only.

Stage 1—The ovary is a long, thin structure with a small oviduct. Width = 0.2mm; length = 1.5mm. Fig. 6a.

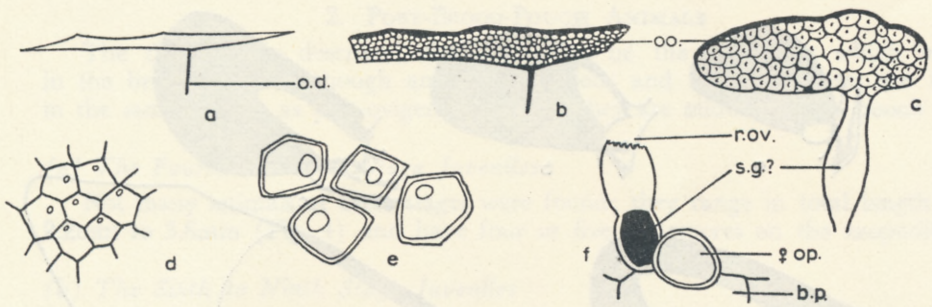


Fig. 6.—Some stages in the development of the ovary. (a) Stage 1. (b) Stage 2. (c) Stage 3. (d) Stage 4. (e) Stage 5. (f) Structure at the base of the oviduct, possibly a shell gland. o.d. = oviduct; oo. = oocytes; r.ov. = remains of oviduct; s.g. ? = ? shell gland; ♀ op. = female genital opening; b.p. = base of peraeopod 3.

Stage 2—The ovary is broader than in the previous stage and individual oocytes are distinguishable. Width = 0.8mm; length = 2.0mm. Fig. 6b.

Stage 3—The oocytes have expanded slightly and the ovary is no longer a flat structure; the oviduct is thicker and has a median swollen region which may represent a "shell gland". The dimensions have increased slightly from the previous stage. Fig. 6c.

Stage 4—The oocytes have expanded considerably and are globular in shape. They adhere to one another and seem to be incorporating oil globules at the same time as the "liver" is becoming empty of its reserves. Diameter of oocytes = 0.3mm x 0.4mm. Fig. 6d.

Stage 5—The oocytes are much more expanded and are free from one another, filling the peraeon cavity. Diameter of oocytes 0.5mm. Fig 6e. There is a distinct swollen structure which seems to be part of the oviduct and is attached at the base of the third peraeopod, near the female genital opening (Fig. 6f); this may represent the "shell gland" seen in Stage 3.

2. Male

(a) Flagellum

Flagella of the second antennae of 234 males, ranging in total length from 13.0mm to 28.0mm, were examined and the number of segments developed varied from two to twenty-two. I have been unable to detect any correlation between increasing number of segments and total body length. (Only undamaged antennae were examined.) Four stages in the development of the second antennal flagellum are shown in Fig. 7.

(b) The "Excavate Organ"

Another structure develops in the male at the same time as the flagellum; this takes the form of a pair of "excavations" developing opposite one another on the inner margins of the rami of the first pair of uropods. At first these are shallow, hollowed out regions, but gradually they deepen and become lined with a row of strong bristles and filled with many fine hairs.

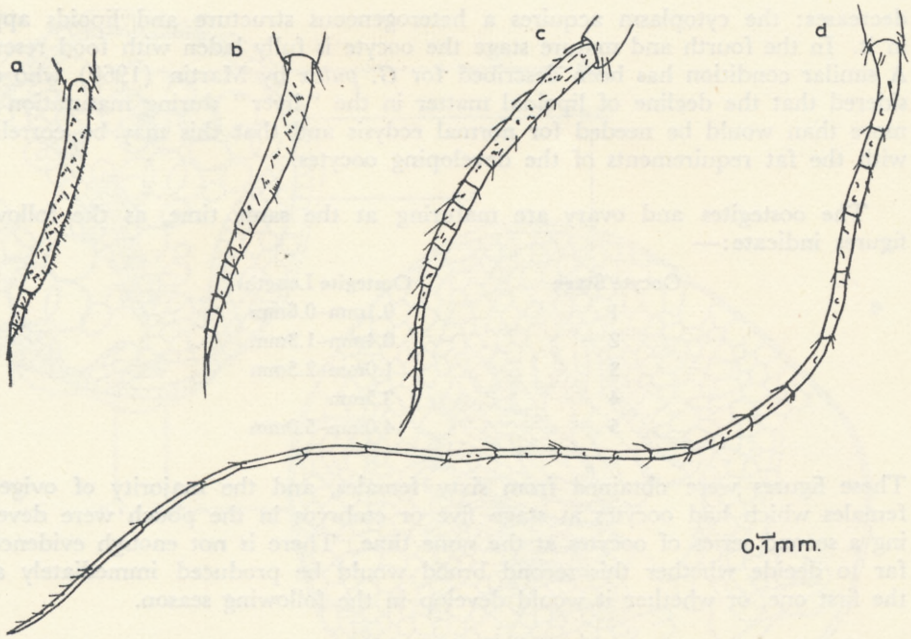


FIG. 7.—Development of the second antenna in the male. (a) Four segments formed; length of flagellum = 1.56mm. (b) Eight segments formed; length of flagellum = 1.8mm. (c) Thirteen segments formed; length of flagellum = 2.4mm. (d) Twenty-one segments formed; length of flagellum = 7.2mm.

RESULTS AND DISCUSSION

As yet it has not been possible to determine the number of moults that occur during the development of these secondary sexual characters, but it is hoped that further work will make this possible. Meanwhile the following points can be made. In neither sex does the development of the secondary sexual characters seem to depend on the size of the animal. Thus animals of 16.0mm in length may be mature or immature; some females may be found considerably longer than this with oostegites of only 0.5mm long. From the data available for females there seem to be two ways of explaining why immature animals may be much larger than mature ones. The simple explanation would be that maturation can take place over a wide size range and is influenced by environmental factors such as temperature or amount of food available. However, many mature animals which have a full brood-pouch also have a second series of oocytes (see below), and this suggests that these animals will become immature for a period after shedding their first brood. During this period they will increase in size, develop another pouch, and produce another brood of young before dying.

During maturation of the oocytes there is a sudden increase in their volume, which is due to an uptake of lipoidal matter. Bisson (1950) working on *Gammarus pulex* L. also mentions this increase in volume of the oocytes. He has divided the development of the ovary into four stages according to the oocyte diameters 20μ, 60μ, 100μ–150μ, and 200μ–320μ. In the first stage the nucleus is large and the cytoplasm strongly basophilic; in the second stage the nucleus is still large and there is abundant cytoplasm. It is in the third stage that the volume of cytoplasm increases considerably and the ratio of nucleus to cytoplasm decreases; the cytoplasm acquires a heterogeneous structure and lipoids appear in it. In the fourth and mature stage the oocyte is fully laden with food reserves. A similar condition has been described for *G. pulex* by Martin (1960) who considered that the decline of lipoidal matter in the "liver" during maturation was more than would be needed for normal ecdysis and that this may be correlated with the fat requirements of the developing oocytes.

The oostegites and ovary are maturing at the same time, as the following figures indicate:—

Oocyte Stage	Oostegite Lengths
1	0.1mm–0.6mm
2	0.4mm–1.3mm
3	1.0mm–2.5mm
4	3.5mm
5	4.0mm–5.0mm

These figures were obtained from sixty females, and the majority of ovigerous females which had oocytes at stage five or embryos in the pouch were developing a second series of oocytes at the same time. There is not enough evidence so far to decide whether this second brood would be produced immediately after the first one, or whether it would develop in the following season.

The function of the "excavate organ" is unknown; Bovallius (1889) suggested that it might have a glandular or sensory function, but I have been unable to detect ducts or nerves supplying it or any obvious glandular tissue. The mature "excavate organ" is in the form of a circular hair lined groove, and it would be interesting to know if this is used to transfer sperm during mating.

Summary

1. Developing embryos remain in the brood-pouch of the mother and pass through two moults during which they become miniature adults with functional appendages. Immediately after leaving the pouch, moulting to the next two stages (namely, 4 and 5), seems to be particularly rapid. There is probably only one moult between each of the stages described, and it is hoped that further work will confirm this.

2. Development of the oostegites of the female and the antennal flagellum of the male are described and figured. The development corresponds in outline with that described by Bowman (1960, p. 362) for *P. pacifica* Stebbing and by Dunbar (1946) for *P. libellula* Litchtenstein (an Arctic species).

3. The development of the ovary is described, and it is suggested that female *P. gaudichaudii* produce at least two broods during their life, but more data are needed to establish whether both broods are produced in the same year.

Acknowledgments

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