Sexual phases in the prawn, *Pandalus kessleri* Czerniavski, with special reference to the reversal of sex.[1]

By

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(With 3 Text-figures and 1 Plate)

Natural reversal of sex has been known in some types of animals. In 1929 Miss A. Berkeley, working on the rate of growth in the Canadian Pacific prawn, *Pandalus danae*, found that the animal is protandric and later becomes a true female. Since then similar cases have been noted by several authors in a few species of Pandalidae ; such as *P. borealis* by Leopoldseder ('34), Jägersten ('36), Hjort and Ruud ('38) and Rasmussen ('42) ; *P. montagui* by Leloup ('36) ; *P. nipponensis* by Tamura ('50) ; *P. hypsinotus* by Igarashi ('51) ; *P. kessleri* by Kubo ('51). These authors are in agreement that the prawns are protandrously hermaphrodite and that they change their sex from male to female as they grow. So far as the writer is aware, the exact mechanism of their sexual conversion has not yet been ascertained, therefore it seems to be of some interest to study the problem histologically in detail and compare the writer's result with those obtained by the previous authors.

This paper deals with the differentiation and development of sex in the prawn, *Pandalus kessleri* Czerniavski, with special reference to the sex-reversal during its life-history. For the purpose of clarifying the correlation between the sexual phase and the age of the animals, the embryonic development and the rate of growth of the animal have also been briefly referred to.

Before going further, the writer wishes to express his most cordial thanks to Professor Tohru Uchida at whose kind suggestion this work has been undertaken. The writer is also indebted to Messrs. Kiichiro Yamamoto, Mitsuharu Miyawaki and Fumio Iwata of the Akkeshi Marine Biological Station, for their unstinted coöperation in collecting the material which made the present study possible.

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Material and Methods

Most of the animals employed as the material in the present study were collected by a drag-net in Akkeshi Bay, on the eastern coast of Hokkaido, over a period of one year from September 1950 excepting the four winter months of December to March. The animals thus caught included 1028 males, 49 intersexes and 75 females, thus totalling 1152 (Table 1). In addition to these, on August 8, 1951 several young animals were caught by plankton-netting in shallow water in the same bay. All the animals were fixed *in toto* with 10% formalin solution or Bouin's fluid and, after measurements had been taken of their body and carapace length and the length of interrami of the first and second pleopods, the gonads were removed from some specimens and submitted to microscopical observation. Serial sections were cut 10μ in thickness and were stained with Delafield's haematoxylin and eosin.

Table 1. The number of animals used as the material in the present study.

Date	Immature & ♂	♂–♀	♀	Total
September 17, 1950	355	—	44(35)[1]	399
October 31, 1950	228	—	4(4)	232
November 15 & 20, 1950	59	1	—	60
Apr. 28–May 4, 1951	33	5	2(2)	40
May 31, 1951	93	9	2(2)	104
June 15, 1951	35	10	5(5)	50
July 12, 1951	72	17	10	99
August 5, 1951	40	7	4	51
September 12, 1951	113	—	4	117
Total	1028	49	75(48)	1152

1) In parentheses are given the number of brooding females.

Breeding

The writer could not obtain the material during the winter season owing to the heavy ice covering the sea surface of the Bay. However, so far as the present study goes, egg-bearing females were found to occur in all the months except July and August (Table 1). The same fact is reported by Kubo ('51) for the same species collected from Notsuke Bay and Lake Saroma, Hokkaido. It therefore seems reasonable to assume that the prawn bears eggs almost throughout the year; i.e., from September to June of the succeeding year.

Many species of pandalids are known to require a considerably long period of time for incubating the eggs. Thus, the five species of the Pacific coasts, *Pandalus danae*, *P. borealis*, *P. hypsinotus*, *P. platyceros* and *Pandalopsis dispar*, are reported by A. Berkeley Needler ('37) to "lay their eggs in the autumn, carry them over winter, and hatch them in the spring." Also, nearly one year is required for *Pandalus nipponensis*, according to Tamura ('51). Of the present species, Kubo ('51) surmises that "the incubation interval of an individual may be one or two months." To ascertain the time required for hatching out the eggs of the prawn, the writer examined each month all the eggs held on the pleopods of mother prawns. Fixed sometimes with alcoholic Bouin's fluid, their degree of development was disclosed under binocular microscope. A brief description of the embryonic development is as follows :

The egg of *Pandalus kessleri* is ovoidal in shape and rich in yolk. It is rather large in size (its diameter being approximately 2.3 mm in major axis and 1.6 mm in minor axis). The spawning season seems to begin early in September and go on for about two months. At the end of September (about one month after spawning) the embryo is found most ordinarily in nauplius stage, situated near one pole of the egg facing toward the opposite pole. At the end of October, black pigments become faintly visible on the lateral border of the optic lobes in some embryos, and in approximately two weeks along with the anterior enlargement of the abdomen the base of the last abdominal appendages reaches the anterior end of the optic lobe. Through the winter the embryo grows so large that in four and a half months (or at the end of April of the next year in the case of an egg spawned in early September) it becomes large enough to cover the surface of the egg and almost to conceal the yolk. At that time, the abdomen, with its end extending across the pole to the other hemisphere opposite to the eye, extends over the ommatidia, which are situated along the original axis near the anterior end of the egg. Two and a half months later (or at the middle of June in the above case), the egg becomes slightly larger (approximately 2.4 mm in major axis and 1.8 mm in minor axis) owing to the growth of the embryo, and sometimes the body is seen unfastened, with antennae often extruding out of the egg membrane. The young now seems to be ready to be soon liberated from the mother prawn.

In short, during the course of development, the embryo grows posteriorly in the posterior end of the cephalothorax while it elongates anteriorly in the optic lobe, and the abdomen developed bent ventrally to the cephalothorax extending anteriorly beyond the optic lobe. The abdomen always grows most remarkably, with its ends extending through the one pole of the egg to the other hemisphere. It should be noted, here, that the embryos have never been found in this state before April. Moreover, measurement of embryos attached to mother prawn's pleopods also reveals that the abdominal length of embryos has never been greater than 4 mm during the incubation period excepting toward the middle of March and thereafter (Table 2).

From the results thus obtained from these embryological studies it seems

Table 2. Abdominal length of embryos taken from mother prawns' pleopods monthly.

Date	Abdominal Length of Embryos (Mm)	No. of Mother Prawns Used
November 29, '51	1.3–1.8	3
December 11, '51	2.9–3.0	3
January 11, '51	3.2–3.7	3
February 11, '52	3.2–3.9	3
March 11, '52	3.7–4.0	3
Apr. 28–May 4, '51	3.3–4.0	4
May 31, '51	3.3–3.8	2
June 15, '51	4.1–4.6	5

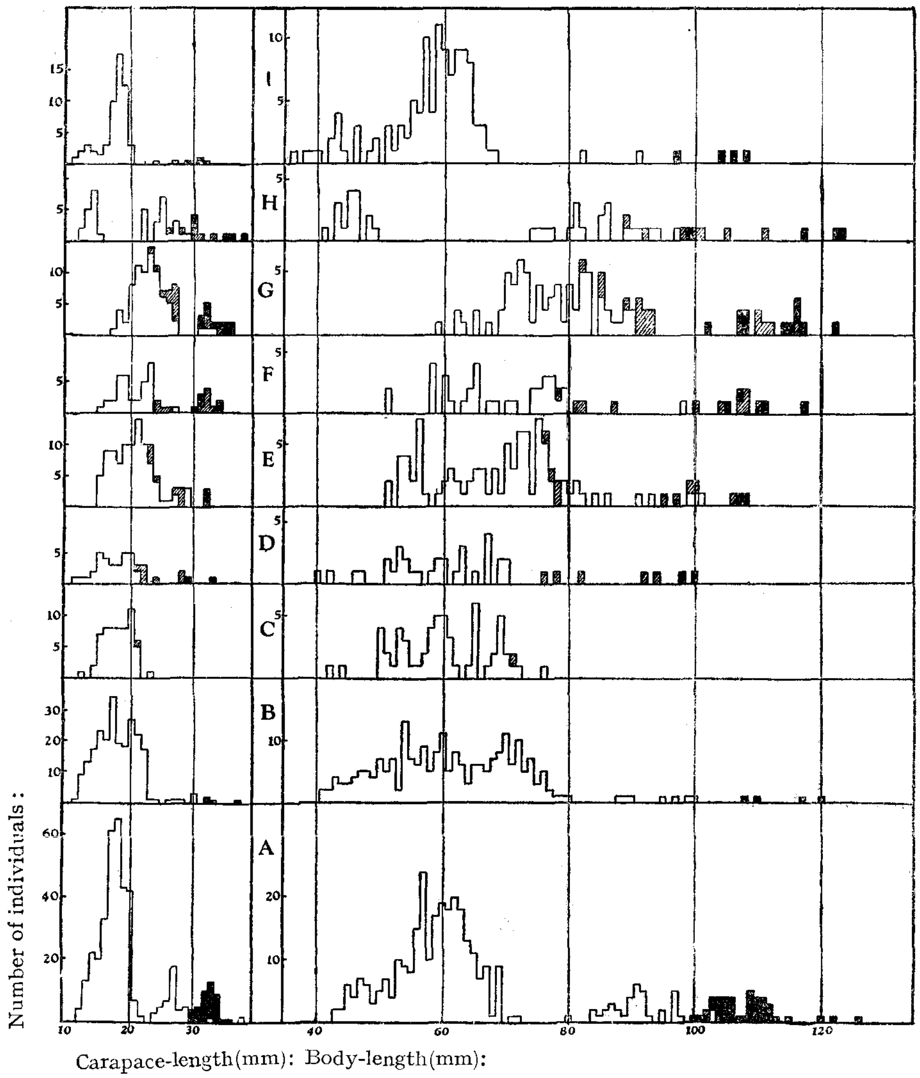
inferable that the incubation period of the prawn begins in early September and ends toward the end of June, covering ten months. But considering that the period for egg-laying and hatching each lasts nearly two months in different individuals, it seems possible to take eight months as really required for the incubation of an egg of the present species.

Growth

The carapace- and body-length, both exclusive of the rostral length, were measured in order to learn the rate of growth and the age of prawns. The results are summarized in text-figure 1. Occurrence of small individuals in the end of April may be likely to lead one to infer that the hatching of embryos takes place in January and February as well as in May and June. But the case seems to be possibly as follows: in some embryos which hatched out late, the larval period has been prolonged and the metamorphosis has been delayed to the next spring by environmental conditions, such as scarcity of food and low temperature of sea-water. Referring to this point, Lebour ('39) stated that in a relative species, *Parapandalus richardii*, "the first and second stages occurred from August to October; — later larvae occurring at any time of year."

Table 3. Sea-water temperature (°C) of Akkeshi Bay in 1951.

Months	Maximum	Minimum	Mean
January	1.1	-1.8	-0.98
February	-1.0	-1.6	-1.20
March	3.0	-1.6	0.34
April	5.5	2.2	3.57
May	10.5	3.1	6.88
June	12.8	9.2	10.68
July	18.0	10.5	14.08
August	22.7	16.2	19.86
September	20.0	14.9	17.49
October	16.0	11.5	13.94
November	10.5	4.0	8.25
December	6.2	0.3	4.07



Text-figure 1. Carapace- and body-length compositions of *Pandalus kessleri* collected in Akkeshi Bay from September 1950 to the next September. Dates of collection are; A, September 17, 1950; B, October 31, 1950; C, November 15 and 20, 1950; D, from April 28 to May 4, 1951; E, May 31, 1951; F, June 15, 1951; G, July 12, 1951; H, August 5, 1951; G, July 12, 1951; and I, September 12, 1951. White, immature and mature males; hatched, animals with their male secondary sex characters degenerated; black, females.

The growth of the prawn either stops entirely or is almost arrested during the winter season, perhaps due to decrease of frequency of molting which is a result of unfavorable conditions of the environment. The temperature of sea-water in Akkeshi Bay drops to point near or below zero during these months (Table 3). Kubo (loc. cit.) mentions that the "frequency percentages of the prawns having empty stomach are 66.66–87.50 in the winter season, and 18.96–38.33 in the other seasons." Recently, Forster ('51) also found in *Leander serratus* that growth of the O-group "almost ceased during the winter and not until June was there a marked change in the size frequencies."

Histological findings

Immature gonads. The youngest animals examined by the writer ranged between 27.0–27.5 mm in body-length (6.5–8.0 mm in carapace-length). They were taken on August 8,1951 from plankton-nets let down in shallow waters where *Zostera* grew in abundance. They were caught probably soon after metamorphosis. In such animals the gonads and ducts are hardly visible under binocular microscope. Serial cross sections of the area, however, revealed that a pair of rudimentary gonads of 0.1 mm diameter are formed half-buried in the underlying liver tissue. The gonads are constituted from rather few young germ cells occupying the central part which is surrounded with densely packed small interstitial cells (Figure 1). The vas deferens is not detectable at this stage.

At the end of September, the young animals, which hatched out in or before June, grow rapidly up to an average body-length of about 60 mm. In this group of animals various stages of gonadal development from rudimentary to mature testes are seen. The gonia in the central part were found to proliferate and grow to young oocytes. At the same time, intermingled with surrounding interstitial cells there were observed to appear a small number of spermatogonia which are thought to have been originated from those gonia in the central part of the rudmientary gonad (Figure 2). In company with the increase of spermatogonia, the interstitial cells became arranged to form tubules around the central ovarian part (Figures 3 and 4). Spermatocytes in the distal portion of the tubules became advanced in their course of spermatogenesis, while the innermost spermatogonia remained quiescent ; meanwhile, the spermatogenetic wave in centro-peripheral direction could be seen in immature gonads (Figures 5 and 6). Some ooytes in the ovarian part enclosed by these spermatogonial tubules grew to reach about 60μ in diameter but did not increase greatly in number.

The vasa deferentia and the oviducts were found to have differentiated in these animals, though both were still primitive, especially the latter, in smaller animals. The cells of the wall of the vas deferens are small and cuboidal in shape, and they are arranged in a row forming a small lumen within them. Rudimentary oviducts are found in animals reaching more than 41 mm body length (or, 12 mm carapace length). The cells of connective tissue in the oviducts are assembled to wall of the duct.

Testes. Various stages of spermatogenesis are always seen throughout the year. In the male prawns, which are undergoing spermatogenesis, the testicular tissue can be divided into at least two parts on the basis of histological appearance. In the young males before breeding, two parts are at first recognizable ; inner tubules filled with spermatogonia and outer ones with spermatocytes. Meanwhile, the spermatocytes on periphery of the outer tubules begin to undergo spermioteleosis which gradually takes place in all the tubules. Young spermatogonia, adhering in a mass around the central ovarian part of the testes, still remain quiescent during the sperm-formation, but, after the discharge of mature spermatozoa in autumn, they multiply vigorously and tubules containing spermatocytes are newly formed around them (Figures 5–9). They are thought to be homologous in nature with the 'residual spermatogonia' which Makino ('31) found in a hynobid salamander to remain "*in situ* as parent stock for future germ cells." Germ cells in spermioteleosis were observable in the gonads of male prawns collected in August, September and also not rarely in those taken in October. The cell becomes somewhat enlarged and within it is seen a triangulate structure which later becomes the main part of a sperm head (Figure 8). A mature spermatozoan is composed of two parts : a triangular body (head) which sends out three processes of about 20μ radially arranged from its vertices respectively, and a rather long (82.5μ) pin-like structure attached at right angles to the former (Figures 9–12).

There were found two different scalae of body-length in the animals which were collected on September 12, 1951 ; viz., 62–68 mm (or, 18–20 mm in carapace-length) and about 91 mm (27 mm in carapace-length). They seem to belong to two different year-groups, but both had mature spermatozoa in their testes or in their vasa deferentia. At the end of October, some functional males were collected. They were also divided into two groups in respect to body-length, measuring 76 mm (22.4 mm) and 100 mm (30mm). The fact is quite comparable to the former case. The vas deferens of these prawns is a tube well-developed and coiled in the proximal portion. When filled with numberless spermatozoa, it became widened to about 1 mm in interior diameter. The cells forming the wall are somewhat flattened and seem to secret strong acidophilic substance of non-structure. Adjacent to the distal end of the duct is situated a sperm-reservoir which, in view of its well-developed encircling masculatures around the wall of the lumen, seems also to function as a sperm-projectile organ (Figure 12).

In the male of the smaller-sized group, the spermatogenetic process progresses in the following way : the spermatozoa are developed only in the distal (peripheral) portion of the tubules, while the 'residual spermatogonia' are seen in the innermost portion of the testicular part. The spermatocytes, derived from the residual spermatogonia, rapidly increase immediately after the discharge of spermatozoa and eventually fill the entire region of the testicular part. Thus doing, most testes of the O-group seem to resume their testicular activity by the next September. During the breeding season, oocytes in the ovarian part of these gonads are 130–170μ in diamter in mature males and 80–110μ in immature males respectively.

Surviving testes are distinguished from those just referred to by their large size and by the occurrence of interstitial cells thickly packed and occupying the degenerated emptied tubles. In these once-bred animals, the sperm-formation prevails throughout the entire region of the testicular part. During and after the discharge of spermatozoa, oocytes in the central ovarian part become large and attain about 280–350μ in diameter, and the ovarian part containing grown oocytes bulges outward into the emptied tubules. The emptied tubule is of an alveolar structure surrounded by a thick wall densely packed with cells each of which has a small nucleus and poor cytoplasm. There are often found among them rather large round cells which, having several prominent chromatin substances adhered to the wall of the nucleus, show degenerative feature. These may be the same in nature as the "nutritive cells" regarding which Fasten ('26) studying spermatogenesis of the black-clawed crab, *Lophopanopeus bellus*, stated "these have irregular nuclei, and are, undoubtedly, produced from primary spermatogonia which have failed to mature."

On October 30, 1950, while engaging in the present study the writer incidentally obtained one specimen of *P. hypsinotus meridionalis* Balss measuring 29.2 mm in body-length (8.5 mm in carapace-length). The prawn had an ovary filled with grown ova and the vas deferens filled with mature spermatozoa (Figure 13). It seems probale, therefore, that this species also converts sex in the same manner as *P. kessleri* does.

Ovary. Growth of oocytes is not conspicuous in the central part of the male gonads. They usually grow immediately after the maturation of the testicular part. From this fact, it may be inferred that in the male gonads the testicular part inhibits growth of the ovarian part in some way ; for instance, mechanically, pressing the latter so tightly that oocytes are unable to expand, or hormonally, producing some substance from the cortical part. The prospective ovary is found in the animals, in which mature spermatozoa still remain in their vas deferens (Figure 10). The oocytes grow so rapidly soon after the discharge of spermatozoa that the ovarian part is distended enormously in size, its diameter being several times as great as that of grown testes. On the inner lateral side of the ovarian part are seen several oogonia in a mass which are seemingly the same in nature as the "residual spermatogonia" ; successive growth of oocytes is found to begin on the same area interiorly. These 'residual oogonia' are found also among growing oocytes mostly on the periphery of the ovarian part but none in the testicular part. In the course of development of the ovary, degenerating testicular part still remains until the end of April of the next year as several vestigial masses of interstitial and other cells attached to the periphery of the ovary. The oocytes grow enormously during this period, occupying a vast area several times as large as the former testicular part ; surrounding the oocytes are found epithelial cells which lie compactly in a row. A mass of well-developed oocytes now begins to enclose the former ovarian part, in which the residual oogonia and oocytes successively developing are still observable. The formation of yolk in the ovarian eggs was first to be observed in the animal collected in the middle of July. At that time, ova under growth are discernible by their larger size and numberless yolk granules distributed widely in the cytoplasm except for the cortical zone of the egg. In August, the ovary has a tinge of reddish yellow color. Yolk granules become accumulated and finally fill the entire egg, in which the female pronucleus, situated in the center of the cell, is surrounded by alveolar structure in section (Figure 14). The structure is perhaps composed of globules of alcohol-soluble substance. Enclosed by a group of these grown ova, there still remain a number of small oocytes, less than a quarter of the diameter of the former. They occur together with small numbers of oogonia situated on the inner lateral side of the mass of young ocoytes which are separated from grown ova by epithelial cells.

Just before the egg-laying the ovary becomes orange-red in color owing to yolk formation of the eggs. It measures about 17 mm in length and 9 mm in width, and ova are clearly seen by the naked eye. Oviducts are widened distinctly, but vasa deferentia are hardly visible at this stage.

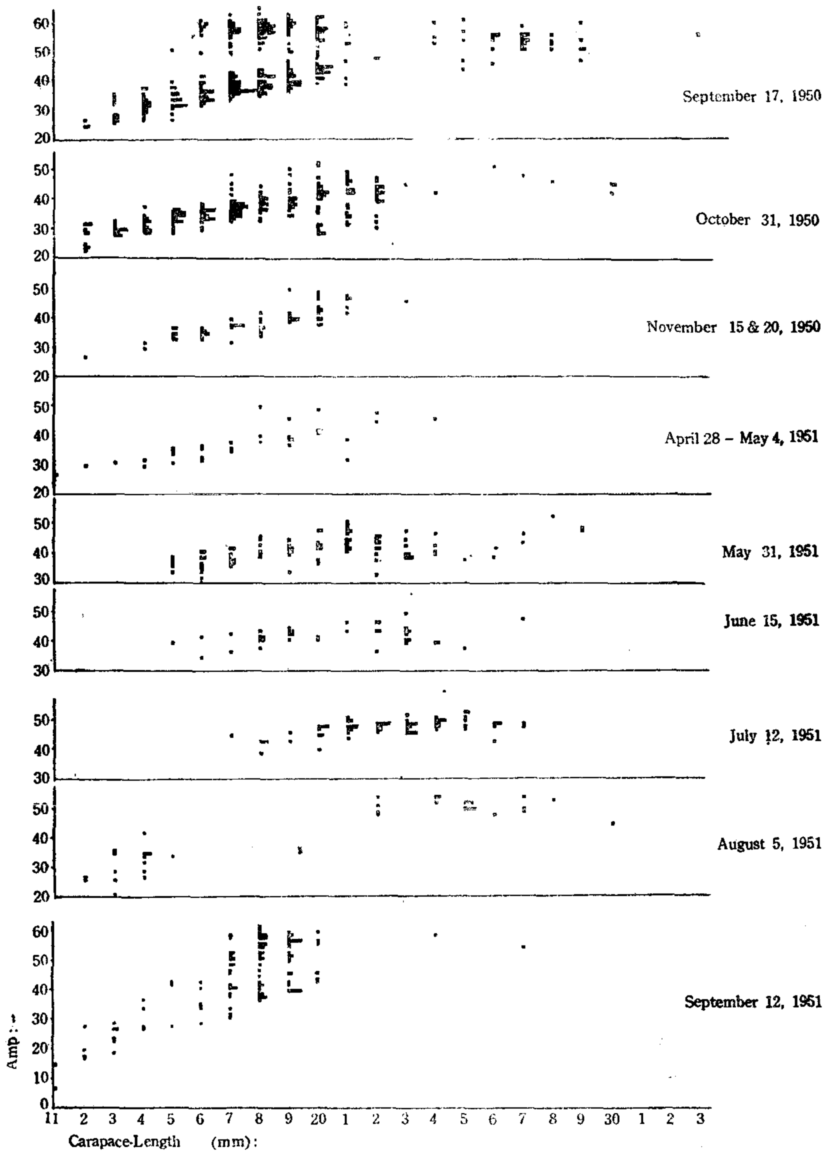
Immediately after the spawning, the ovary becomes almost degenerated to form a small number of degenerating ova intermingling with well-developed interstitial and other cells, and at that time the vas deferens becomes detectable again accompanied with the underdeveloped oviduct. The ovary of the brooding prawns, however, probably recovers its activity during the winter season. The ovarian egg reaches 450–480μ in diameter in April and 580–640μ in June, when the embryos bred on the mother prawn's pleopods were about to hatch out. These eggs are favorably comparable in size with those of other prawns that are changing their sex, have growing ova in their ovaries and that are able to spawn eggs for the first time about three months afterwards (Figures 15 and 16). It is very probable, therefore, that the mother prawns, after the liberation of their hatched larvae, lay eggs at least once more in the following season.

Chnges of the external sex characters during the course of growth in relation to the gonadal condition

The male of the prawns is most easily discernible from the female by the presence of a prominent process, the *appendix masculina*, on the interramus of the second pleopods both in immature and in male animals. The process appears at first as a bud between interramus and appendix interna in sexually immature young animals, about 30 mm in body-length, but it grows rapidly along with the growth of the animal. To make clear the correlation of condition between appendix masculina and gonad, the length percentage of appendix masgulina to interramus of the second pleopod, AMP (Length of appendix masulina/length of interramus $\times 100$), was measured on all the animals employed in the present study (Text-figure 2). It is evident from the figure that the rate of growth is much higher in appendix masculina than in interramus, the latter part being of no sexual significance. The average value of AMP of all the animals is about 30–50 throughout the year except some animals caught in September, in which AMP is extremely high and reaches 60. These animals seem to be a rather peculiar group, because ordinary group of animals show an ordinary value of AMP at the same time. The specially high value of AMP is probably due to higher activity in the gonads of the former group of animals. In fact, the gonad and vas deferens were found well-developed with mature spermatozoa in these animals (Figure 17).

In the male prawns, the interramus of the first pleopods is bifurcated on its distal end. The inner tip, *appendix interna*, decreases gradually in size after the last discharge of spermatozoa and disappears completely by July when the animal has attained a stage when it has ova in the ovaries. In regard to the process of shrinkage of this appendix interna Kubo reported two phases. However, it should be noticed here that the 'shrinkage' was observed to occur in an animal measuring 81 mm in body-length on June 15, 1951, in which the appendix masculina on interramus of the second pleopods was also degenerating, while in an animal measuring 91 mm on September 12, 1951, the two appendices did not show degeneration at all. Therefore, the 'shrinkage' seems to occur in dependence on gonadal condition rather than on the course of growth of animals. The female can also be distinguished by long hairs growing along the margin of each pleopod.

The size of the lateral end plates differs by sex, larger in the female than in the male. The lateral plate of the second abdominal segment is large, round in shape and demarcated from the main part by two small notches, one anterior and another posterior. The length (L) denotes the distance from the anterior notch to the posterior distal margin of the plate as measured on thirty-two animals larger than 30 mm in carapace-length (105–126 mm in body-length) (Table 4). It becomes apparent that the L was larger, in general, in larger animals. However, it should be noted here that the L's were never larger in the brooding females collected on June 15, 1951, when they were going to liberate their litter, than in those of the same size, which had just laid eggs and were collected on September 17, 1950. Further, the L's of the animals which were collected on June 15, 1951, and were to be in brood about three-months afterwards, were extremely small when compared with the L's of those in brood on the same date. Moreover, three females presumably just before egg-laying on September 17, 1950 showed rather small values of the L's, each being 15.5, 16.0 and 17.5 respectively, or 16.3 on the



Text-figure 2. Compositions of AMPs taken each month on male prawns of each carapace-length.

Table 4. Size of the lateral plates in the prawns measuring more than 30 mm in carapace-length.

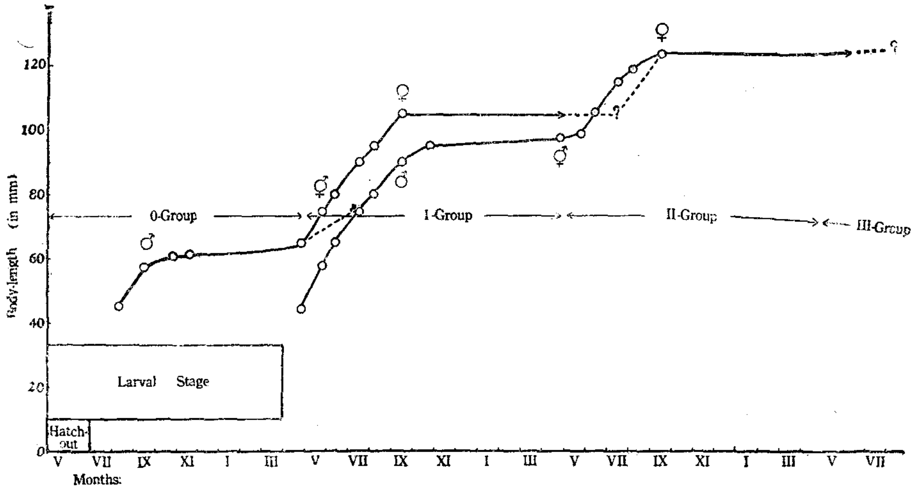
Carapace-Length (Mm)	September 17, 1950				June 15, 1951			
	Br. ♀		Non-Br. ♀		Br. ♀		Non-Br. ♀	
	L.	No. of Animals	L.	No. of Animals	L.	No. of Animals	L.	No. of Animals
30	18.0	1	—	—	17.1	1	—	—
31	18.1	3	—	—	17.5	2	15.0	1
32	17.9	2	16.3	3	—	—	16.1	4
33	18.2	4	—	—	—	—	16.3	1
34	18.9	7	—	—	19.9	2	—	—
35	—	—	—	—	—	—	—	—
36	19.5	1	—	—	—	—	—	—
37	—	—	—	—	—	—	—	—
38	21.4	2	—	—	—	—	—	—

average. From these facts, it can be concluded that the increase of size of the lateral plates is possibly affected by some internal physiological conditions, though they may be slightly enlarged with growth, and that in the present prawn there probably occurs the same parturial molting as that reported by Kajishima ('49) in the shrimp, *Leander pacificus*.

Relation between the sexual phase and age of the prawn.

Chiefly owing to the small number of specimens observed, especially during the winter months, it was rather difficult to decide the age of the prawns from size frequencies. However, from the facts above described, successive changes of sexual phase of the prawn may be summarized as follows (Text-figure 3):

Collection of larvae was not successful. Youngest animals belonging to the O-group were taken in abundance from shallow waters in early August. Microscopical observation of this group disclosed that the testicular part of the hermaphroditic gonad was then highly active. Some of these animals produced mature spermatozoa later in the middle of September. From the fact that the body-length of mature males can be arranged in two different scalae, it can be concluded that these prawns are probably divided into two groups: the O-group and the I-group, or the I-group and the II-group, the latter case being less probable. Among the prawns showing male characters the largest two were collected on September 17, 1950, and both measured 103 mm long. They were provided with a long appendix masculina and their AMPs were 54 and 46 respectively. There was no evidence, however, to lead one to assume that these two had functioned as males thrice or more. Thus, most animals seem to function as male twice in succession in the first two year. Among the animals, in which the male secondary sex characters were in process of degeneration, the smallest one was caught on November 20, 1950. The animal, measuring 70.5 mm long (21 mm in carapace-length) and having broken appendix masculina (its AMP was 12), seems to have begun reversal of sex probably in the first year of its life. All the year round except in September, animals in a sexually transitional state were very commonly found ; they showed two scalae of body-legnth distinctly as in the case of males. However, no animals in such a state could be found with onset of the breeding season in September. The prawns seem thus to transform their sex from male to female either in the first or the second year of their lifetime just after the discharge of spermatozoa.



Text-figure 3. Diagram showing the relation between sexual phase and age of *Pandalus kessleri*.

In *P. kessleri* since the egg-laying, incubating and spawning seasons last over a long period of time, great divergence of growth is quite naturally to be expected among individuals of the same year group. This makes it difficult to make a conclusive statement about the relation between the age and its sexual aspects, but the animals are considered mostly to find their places between the two curved lines of the figure. Whether the small-sized functional males belong to the 0-group, as the present writer believes, or to the I-group remains to further study for affirmation.

Of the European species, *P. borealis*, it is believed that the animal is functional as male in the second year (the I-group) and functional as female in the third year (the II-group) (Berkeley, '30 ; Jägersten, '36 ; Hjort and Rund, '38). Also Tamura ('50) gave a diagram to show that *P. nipponensis* becomes functional as male about two years after hatching, attains a sexually transitional state about six months later and transforms into an ovigerous female just three years after hatching. The present species, *P. kessleri*, differs from the two species above referred in that it is functional as male in the first year of its hatching. As the age of the prawns is very difficult to ascertain as mentioned above, the writer wishes to avoid any precise statement on this point.

Discussion

As to the breeding habits of *Pandalus kessleri* there has been very little known. Recently, Kubo ('51), in his excellent investigation on the bionomics of the present species, stated that the breeding season "seems to be covering a long period," and that there are "two, spring- and autumn-peaks of spawning," and surmised that "the incubation interval of an individual may be about one or two months." Examination of embryonic development by the present writer reveals that the breeding season begins in early September and lasts about two months. Occurrence of young animals 30–40 mm long in late winter and early spring seemingly supports the assumption that the spawning and hatching of embryos take place twice a year with two peaks. In fact, however, the embryos were found to continue development during the winter and spring months and become liberated from mother prawns up to the end of June of the succeeding year. Therefore, it can be concluded that the metamorphosis must probably have been delayed in the larvae of winter and spring, and that the incubation period would last about eight months in the present prawn. The delayed metamorphosis and the stoppage of growth of the animals seem to be caused by unfavorable conditions such as cold water-temperature and lack of food.

Leopoldseder ('34) described the occurrence of four heterochromosomes in the second spermatocytes of *Pandalus borealis*. In *P. kessleri*, germ cells appear first in the medullary portion of the early gonad, surrounded by dense interstitial cells. And later, intermingling with these interstitial cells in the cortical portion occur numerous spermatogonia which undoubtedly have been derived form those young germ cells in the medulla. The medullary portion of immature gonads, being occupied by oocytes and gonial cells, later becomes separated by epithelial cells from those spermatogonia in the cortical portion. Hence, the writer cannot help but assume that the definitive spermatogonia have been originated from the primordial germ cells, which at first appear in the medulla, and that, therefore, chromosomal complexes are not possibly different in the sexes of the present species.

The residual spermatogonia were found to exist in the testicular part of the male gonads. They, adhering as a mass around the central ovarian part, proliferate and provide daughter cells for future functional gametes. On the other hand, the oocytes in the central part of male gonads, undoubtedly originated from residual oogonia, increase in number and become slightly enlarged during the course of spermatogenesis in the testicular part. Growth of oocytes in these animals is so great just after the last discharge of spermatozoa that the ovarian part becomes enlarged and a few remaining spermatogonia are pressed against wall of the emptied tubules to become unable to develop. Hence, there possibly exists an antagonistic relation between the testicular and the ovarian parts of hermaphroditic gonads. If that is the case, it appears strikingly similar to the antagonism well known to exist between the medulla and cortex in early gonads of amphibians.

As mentioned above, the present prawn functions as male once or twice, and, thence, two scalae of body-length are seen in functional males in September. There can be found to exist the following cases ; some were functional as male twice for two years successively, some failed to mature in the first year becoming functional in the next season, while some were functional as male in the first year and as female in the next year, etc. On the other hand, the sex reversal from female to male does not occur. From the fact that the ovarian eggs grow during the period in brood, it is highly probable that a female prawn is able to spawn twice or more in her lifetime, and that the natural life span of the present prawn is about four years.

There was found a distinct correlation between the following sexual character and the following non-sexual part of the body ; the length percentage of appendix masculina to interramus of the secnd pleopods (AMP) is generally 30-50 in animals with body-length larger than 40 mm in all months excepting September. In that month another group of animals was also found. They are larger than the ordinary ones and reach about 60 in AMP. Regarding this simultaneous occurrence of two groups of animals with high and low AMPs in the corresponding size, either of the two following interpretations may be reasonable : (1) the animals with lower AMP have already bred and are provided with shorter appendix masculina immediately after the molting which has taken place following the discharge of spermatozoa, or (2) different values of AMP are due to difference of sexual race. Referring to the latter interpretation, Witschi ('30) described three sexual races in the European grass frog, *Rana temporaria* : differentiated, semidifferentiated and undifferentiated races in connection with isotherm of the area. Geographically, Akkeshi Bay is situated on the eastern coast of Hokkaido, open to the Pacific Ocean and connected directly with the brackish Lake Akkeshi, so that the habitat of the prawns is possibly exposed to an incressant fluctuation of the salinity.

In males, gradual degeneration of appendix interna of bifurcated interramus of the first pleopods can be seen after the last discharge of the spermatozoa. On the other hand, the interramus of the definitely female animal is leaf-shaped, provided with numberless long hairs and lacking appendix interna. The degeneration of this interramus was found to be correlated with the gonadal condition rather than with the age of the animals. Sudden growth of lateral plates on the second abdominal segment of females can be caused by parturial molting just before the spawning. The lateral plates of the female prawn are very similar to the oostegites of isopod crustaceans in bearing their eggs. Whether these characters are affected by gonads or not remains for future study. It is noticeable that Takewaki and Nakamura ('44) studying the sex characters of *Armadillidium vulgare* (Isopoda) found that the characters were "independent of the gonad," because they were not modified after gonadectomy.

Distinct cases of protandrous hermaphroditism are also reported by Kinoshita ('36, '39) on three species of the genus *Sparus* (Teleostei) : *S. longispinis*, *S. aries* and *S. latus*, in which the testicular part of young hermaphroditic gonad is functional and envelopes the inner ovarian part. In these animals, sexual character shifts toward either male or female later when one of the sexual parts degenerates. The present prawn is very similar to *Sparus* having hermaphroditic gonads in all young individuals. They at first produce mature spermatozoa. These two animals, however, cannot be placed in the same categotry, for the present prawn has the definite female alone and no definite male. In this respect the present case is rather like to that of the parasitic isopod, *Danalia curvata*, which is at first functional as male in free-living pelagic stage and finally transform into female parasitic on the rhizocephale, *Sacculina neglecta*.

Summary

1. Sexual phases of the gonads were studied histologically in the prawn, *Pandalus kessleri* Czerniavski, with special reference to its reversal of sex.

2. Berried females occur during the months from early September to the end of June of the next year, and about eight months are estimated to be required for the incubation of the eggs.

3. In early August there appear the smallest specimens of this animal which, measuring about 17 mm long (about 7.5 mm in carapace-length), have rudimentary gonads composed of small numbers of young germ cells in center and numerous interstitial cells around them.

4. In due time, new young germ cells come into appearance among dense interstitial cells on periphery and proliferate vigorously to form a mass of spermatocytes, while in center the young oocytes are found to grow to some extent.

5. Thus, the prawn shows protandric consecutive hermaphroditism during the one or two years after hatching. In these animals, hermaphroditic gonads

are constituted from the central ovarian part and the peripheral testicular part, the latter enveloping the former and being functional in producing mature spermatozoa.

6. After the last (the first or second) discharge of spermatozoa, the testicular part diminishes gradually and the ovarian part becomes enlarged and grown ova finally occupy the entire gonad.

7. In both sexes, the new gonads are again formed by 'residual' gonial cells.

Addendum : Recently the writer read a note on the sex of the deep-water prawn, *Pandalus bonnieri* Caullery, in the Annual Report of the Scottish Marine Biological Station (1950–1951). It reads, "Dr. Pike has established that in this species the sexes differentiate into male and female at the end of their first year, instead of being protandric hermaphrodites as in *P. borealis* K., as well as in some of the North American species." So far as the writer is aware, this is the first *Pandalus* species reported as to be in non-hermaphroditic tendency.

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Explanation of Plate I

Date of fixation, body- and carapace-lengths and AMP of animals employed as material in this PLATE are shown in table 5.

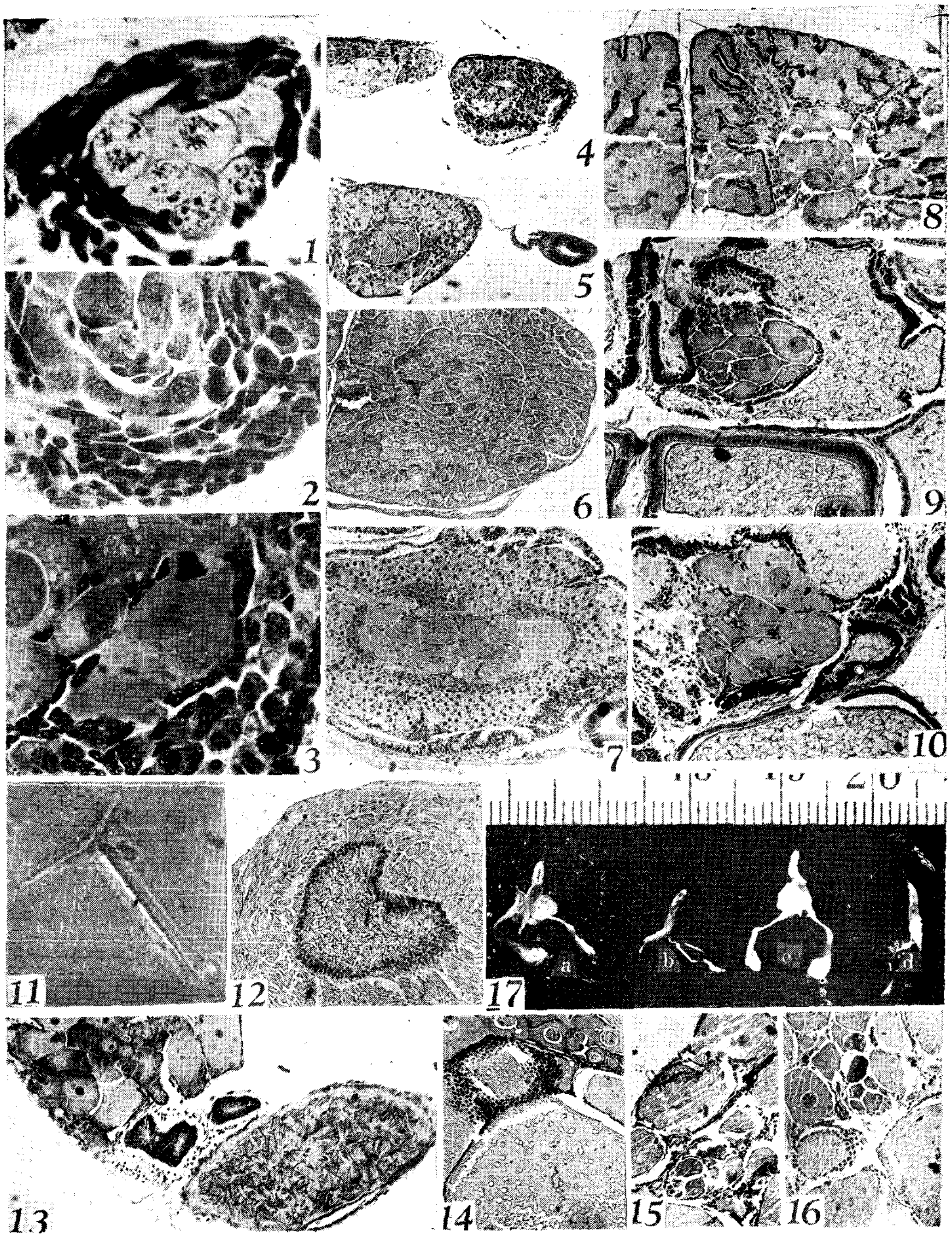
Table 5.

No. of Figure	No. of Animal	Date of Fixation	Body-Length (Mm)	Carapace-Length (Mm)	AMP
1	300B	51- 8- 8	27	7	19
2	413	51- 9- 2	42	12	17
3	298	51- 8- 5	41	12	26
4	290	51- 8- 5	46	13	34
5	395	51- 9-12	52	16	43
6	363	51- 9-12	55	17	49
7	281	51- 8- 5	74	22	48
8	362	51- 9-12	59	19	50
9	307	51- 9-12	68	20	60
10	305	51- 9-12	91	27	55
11	—	51- 9	—	—	—
12	881	50-11-20	72	21	47
13	630	50-10-30	29.2	8.5	—
14	114	51- 6-15	81	25	—
15	105	51- 6-15	105	31	—
16	253	51- 8- 5	122	36	—
17a	313	51- 9-12	66	20	57
17b	321	51- 9-12	66	20	43
17c	340	51- 9-12	60	18	60
17d	336	51- 9-12	61	18	38

1. Around young oocytes in the central part of the rudimentary gonad are seen several gonial cells appearing to intermingle with dense interstitial cells. ×410.
2. On periphery, young germ cells increase in number and two histologically different parts are distinguished. ×250.
3. Formation of tubules is in progress in peripheral part. ×410.
4. The ovarian and testicular parts are separated from each other by a thin epithelial tissue. ×41.
5. On periphery of the testicular part are seen advanced stages of spermatogenesis. ×41.
6. At least three different stages of spermatogenesis are recognizable in the testicular part ; innermost spermatogonia around the central ovarian part, first spermatocytes in median layer, and second spermatocytes in periphery. ×41.
7. Thus, spermatogenetic wave in centro-peripheral direction can be seen in the testicular part of immature gonads. ×410.
8. Sperm-formation is in progress in tubules of the testicular part. A triangulate structure in spermatids is thought to become later a proximal portion of the sperm head. ×41.
9. Numberless spermatozoa fill up the vas deferens and the whole tersticular part excepting only its innermost portion, where a thin layer of residual spermatogonia still remains. ×41.
10. Gonad of a once-bred male. Note the absence of residual spermatogonia and the enlargement of oocytes in the ovarian part. ×41.

11. Photomicrograph of a mature spermatozoan taken *in vitro* by a phase microscope. ×510.
12. Cross section of "sperm-reservoir" which, provided with well-developed musculatures around the wall, is filled with numberless spermatozoa. ×41.
13. One specimen of *Pandalus hypsinotus meridionalis* which has mature spermatozoa in the vas deferens and growing ova in the gonad. ×41.
14. True ovary of an animal with its male secondary sex characters in way of degeneration. The testicular tissue diminishes until completely gone and growing oocytes come to periphery of the gonad. ×41.
15. Ovary of brooding female. In size the growing oocytes compare favorably with those of non-brooding female on the same date (Fig. 13). ×41.
16. Ovary just before spawning. Accumulation of yolk granules is distinct in ovum reaching about 1 mm in diameter. Residual oogonia and successive growth of oocytes are still to be seen in an area of these ovaries. ×41.

17a-d. Remarkable differences of gonadal condition were found in these testes of the prawns which show correlative high and low values of AMP respectively. ×1.3.



T. Aoto: Sex reversal of Pandalus kessleri