J. mar. biol. Ass. U.K. (1959) 38, 481–491
Printed in Great Britain

ON THE SEXUAL BIOLOGY OF *PANDALUS BOREALIS* (CRUSTACEA DECAPODA)

II. THE TERMINATION OF THE MALE PHASE

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Pandalus borealis Krøyer is a protandric hermaphrodite. In some populations, e.g. that found off the Northumberland coast, the hermaphroditism is partial (Allen, 1959). That is to say only certain individuals undergo sex reversal, while others are primary females, never going through a male phase; all the males undergo sex reversal. In the terminology proposed by Carlisle (1959*a*) such populations exhibit partial obligatory protandric hermaphroditism. By contrast, the population of the Gullmarfjord, Sweden, which I have been investigating, appears to exhibit full obligatory protandric hermaphroditism: the population contains no primary females; all the females have passed through a male phase. The data presented in this paper have no bearing, therefore, on the factors regulating the production of primary females, since I have never encountered one.

In most of the southern populations of *P. borealis* (a useful summary is provided by Rasmussen, 1953), the eggs are carried for about 6 months. Since there is only one breeding season per year this means that copulation takes place between animals which are about $1\frac{1}{2}$, $2\frac{1}{2}$, or $3\frac{1}{2}$ years old. The breeding season is somewhat prolonged, however, lasting up to 4 months. The evidence presented by Jägersten (1936), Rasmussen (1953) and Allen (1959) for different southern populations suggests that the male is not mature at 6 months old and cannot therefore breed until about $1\frac{1}{2}$ years old when it is approximately 90 mm long. A few individuals may already by the end of this breeding season be functioning as females. By the next breeding season, when the animals are $2\frac{1}{2}$ years old and measure about 110 mm, the great majority have reversed sex and copulate as females. A year later, at about 140 mm, they copulate again as females. Egg laying follows almost immediately upon copulation, in contrast to the condition found in Reptantia, where the interval may be as long as 6 months. According to Allen (1959) there are in the Northumberland population normally five moults between the fully functional male phase and the assumption of the breeding dress of the female; the last of the five moults is a moult of copulation. In the population of the Gullmarfjord the number of moults appears to be four, giving three intermoults between the male and the functional female states. In some individuals however, especially at certain seasons of the year, this may be cut to two

intermoults or even to one; in experimental conditions a single individual has been observed to complete the change from male to female at one moult and breed as both male and female in one season. An individual taking only two moults (one intermoult) for the completion of sex reversal will also breed in both sexes in the one season under natural conditions; this is rare. The sex reversal takes place in the Gullmarfjord population at between $1\frac{1}{2}$ and $2\frac{1}{2}$ years old. All individuals seem to partake in the breeding season as males in their first season of maturity, all take part as females in their second season; some few may complete sex reversal in time to partake as females in the first season, after having earlier in the same season functioned as males.

In the first paper of this series (Carlisle, 1959*b*) I have described incretory organs which I have reason to believe may exert a controlling influence on the reversal of sex. In this paper I shall be concerned only with influences which bear upon the first part of the sex reversal—the loss of the male characters. For the purposes of these observations all individuals as far as possible were observed through one moult after experimental interference. Results are expressed in terms of loss or otherwise of a functional male condition after this one moult.

EXPERIMENTAL DATA

Vasectomy

Effective vasectomy may be achieved in a prawn by blocking the genital openings with small pellets of plasticine, soft wax, dental cement or cold-cure araldite. It is a commonplace that the operation performed on mammals (by ligating the vas deferens), besides leading to sterility, often leads also to impotence and to loss of libido (e.g. in the tom cat). In my experience the same is also true of prawns, including both *Pandalus borealis* and *Palaemon* (= *Leander*) *serratus*. This is most easily seen in the behaviour. As described by Höglund (1943), Burkenroad (1947) and Forster (1951), a ripe female which has just moulted, i.e. one which has just undergone the moult of copulation, proves attractive to males. Forster states of *P. serratus*: 'If an "attractive" female is placed in a tank with males nothing happens until the antenna of the male touches some part of the female. At once the male's behaviour alters, he swims very rapidly in no particular direction, often making small circles, until he again makes contact....' Essentially the same behaviour characterizes *Pandalus borealis*: the male, once he has touched an attractive female with his antenna, swims rapidly in circles, then with quick sharp motions, once he has found the female again, palpates her all over with his chelae. Copulation normally follows, but if several males are in a tank there may be some fighting.

When an attractive female is offered to 'vasectomized' males the response depends on the interval which has elapsed between the operation and the offer. No 'vasectomized' males were used until 48 h had elapsed, to allow

time for postoperative recovery. Controls were animals in which the pellet was first inserted into each genital opening and then removed.

Up to 5 days after the operation the response of the 'vasectomized' males to the proffered females was normal, even including the fighting between rival suitors. Copulation was attempted but never completed. Six offerings were made to a tank containing nineteen males between 2 and 5 days after the operation. Each female was then removed after the failure at copulation and offered to a similar tank containing twenty controls. In each of these offerings copulation was completed satisfactorily.

Between 5 and 10 days post-operatively the interest exhibited by the operated males dropped progressively. In this interval I was able to offer them seven attractive females. The first of these on the sixth post-operative day elicited all the earlier part of the response without, however, any attempt at copulation. On the tenth day the offer of an attractive female elicited the swimming-in-circles response in about half the animals, but no further response. By the twelfth day an attractive female elicited no response. Every one of these females completed copulation successfully with one of the control animals. One operated animal moulted on the third day after the operation, shedding with the old shell the blocking pellets. The new shell was that of a fully functional male. Five days after the moult an attractive female was offered to him and they completed copulation satisfactorily. Histological examination after this event revealed a normal testis with spermatogenesis continuing, a normal vas deferens gland and a vas deferens still half full of sperm.

Other operated animals moulted from the seventh day onwards, and before my departure from the Kristineberg Laboratory nine had moulted (besides the one mentioned above), together with seven of the controls. All nine of the operated animals after moulting showed male appendages characteristic of the first non-sexual inter-moult, with reduction of the appendix masculina and loss of its setae. All of them at moulting shed the plug from the genital ducts. Histological examination revealed that spermatogenesis had ceased in these animals, the vas deferens was normal, but the vas deferens gland, though not characteristic of a normal first stage non-sexual animal, was greatly reduced and showed many pycnotic nuclei. Of the controls which moulted, only one passed into the non-sexual phase, and histologically it showed the conditions characteristic of this phase, with loss of the massive portion of the vas deferens gland, retaining only the strand. The other controls remained as functional males and retained the histological structures appropriate to this stage.

The conclusion is inevitable that occlusion of the spermduct in *P. borealis* causes sterility, loss of libido, and degeneration of the male genital system, and promotes the assumption of the non-sexual condition at the succeeding moult.

Eyestalk removal

This operation has been shown in other species of Natantia to have a profound effect on the development of the ovary and in particular on vitellogenesis (Panouse, 1943–6; Carlisle, 1953). The operation is followed by the initiation or acceleration of ovarian development and by vitellogenesis. The same is true of crabs (Brown & Jones, 1949), and in male crabs eyestalk removal is followed by a rapid increase in weight of the testes and vasa deferentia (Démeusy, 1953). It might be expected, therefore, that eyestalk removal would have some influence on the change from male to female in a protandric hermaphrodite. On the other hand, in the protandric hermaphrodite *Lysmata seticaudata* I have found neither eyestalk removal nor injection of eyestalk extracts to have any significant effect upon the weight of the testis or of the vas deferens (Carlisle, 1954). Tables 1 and 2 show that the same is true of *P. borealis*. The animals used in these experiments were all mature, sexually active males. Under the conditions of the experiments neither eyestalk removal nor injection of whole eyestalk extracts had any influence upon the weight of the testicular portion of the gonad, nor had starving, while the weight of the vas deferens was affected significantly only by starvation, not by either of the endocrine disturbances. It seems unlikely, therefore, that any tissue within the eyestalk has any effect upon testicular development.

The same result is apparent when the effect on the numbers of individuals losing the external male characters is considered. During April about half the large males which moult (in the samples I have examined) lose their secondary and accessory male characters (93 out of 197 moults observed) and change to the non-sexual phase. Interference with the eyestalks did not in any way alter this proportion. The results are presented in Table 3. Neither eyestalk removal nor injection of eyestalk extracts had any effect on the numbers moulting to the non-sexual state.

The vas deferens gland

The degeneration of this gland shortly before the termination of the male phase made it seem plausible that this event might be the immediate cause of the loss of the male character, since it is known that removal of the gland in *Orchestia* leads to loss of maleness (Charniaux-Cotton, 1954, 1955, 1956). Preliminary attempts at removing the gland by surgical methods in *Palaemon* proved abortive, because of total mortality of all operated animals within 24 h. The use of radio cautery, however, met with some success in *Palaemon* with less than 90% mortality from the operation. The results of these experiments will be reported elsewhere. When the method was applied to *Pandalus borealis*, however, I met with no success at first, most of the animals dying even before completion of the operation, and the remainder within a few

hours. By modifying my techniques I eventually achieved success with no more than 75% mortality, better even than the earlier results with *Palaemon*. The technique of operation which I adopted may therefore perhaps be usefully described in some detail.

On the stage of the microscope was mounted a heavy, silver-plated brass plate with a central rectangular hole 20 × 7 mm. This was connected to the earth of the radio cautery outfit and served as the neutral electrode. On the brass plate were mounted two containers, whose bottoms were formed by the plate itself, in which could be placed crushed ice. These were mounted at the front and back of the stage to leave room for the hands at the sides. The source of illumination was filtered through copper sulphate solution to remove the infra-red rays. Before operation each animal was refrigerated at -1° C for 30 min. The fingers of my left hand were repeatedly cooled in ice water throughout the operation and were used only for holding and orientating the animal. By transmitted light it is just possible to distinguish the position of the vas deferens and of the gland in the intact animal. With the active electrode of the radio-cautery apparatus (a nickel-chrome (80/20) wire, 40 μ diameter, mounted in a glass holder) and with the apparatus turned to a rather high power I burned a hole near the base of the fifth walking leg immediately over the visible gland. The power was then reduced, the electrode inserted into the gland and the massive portion destroyed on one side. The animal was then immediately returned to the dish of ice-cold sea water and allowed to recover in the constant temperature room maintained at 4° C where the stock of animals was kept. Provided my left hand was kept cold and the operation was completed within about 2–3 min this stage resulted in negligible mortality. The other side of each animal was operated in like manner 24–48 h later. The longer period resulted in less deaths, which in my best series amounted to no more than 50%, while with a period between the two operations of only 24 h, even under the best conditions of cooling, deaths were usually more than 80%. Those animals which survived the first 48 h after the completion of the double operation showed a death rate not significantly different from unoperated controls.

It must be stated that in these experiments only the massive portion of the gland was destroyed, not the strand which runs the entire length of the vas deferens. Destruction has been checked histologically in all specimens. The control animals had the hole burned in the shell over the vas deferens gland, without the destruction of the gland itself; it was apparent that this part of the operation was the prime cause of the heavy mortality, since the deaths in this control group of animals almost equalled those in the operated group.

The operations to be reported here were all performed during the month of September, a few weeks before the start of the mating and egg-laying season or as it was just beginning. In over 300 moults observed amongst large mature males during this period not a single individual was observed

to lose the male characters, while a sample of 2000 animals contained only two specimens in the inter-sexual condition. It is abundantly clear that sex reversal does not normally take place at this time of the year—all individuals seem to enter upon the mating season either as fully functional males or fully functional females. In the females vitellogenesis was well advanced and ovaries dark green. Any augmentation of the sex reversal is therefore most evident at this season.

The results of the operations in terms of loss of external male characters are presented in Table 4, and in terms of testis weight in Table 5. It is obvious that destruction of the massive portion of the vas deferens gland has led to degeneration of the testis and to loss of secondary and accessory male characters.

Examination of sections of the testes of these animals and of the sperm ducts provided additional confirmation of the degeneration of the former. The seminiferous tissue was shrunken and no sign of spermatogenesis could be seen. The sperm ducts by contrast were little altered and remained full of sperm.

A few days after the start of the above experiment a further series of eighty males was operated. Of these thirty-eight survived the operation and were used for injection experiments. Half received an intravenous injection of 0·15 ml. of distilled water 48 h after the completion of the operation and further similar injections 3 and 6 days later; these served as the controls. The other half had a similar series of injections of extract of vas deferens gland. This extract was prepared by homogenizing the fresh glands in distilled water in a Potter all-glass homogenizer. At each injection each animal received the extract of the glands from one individual. The animals were killed nine days after the first injection, 11 days after the end of the operation. The results are presented in Tables 6 and 7. The effects of the destruction of the vas deferens gland are partially but significantly countered by the injection of the extracts. Histological examination of the testes of these animals confirms that although they are somewhat degenerate with shrunken seminiferous tissue and absence of spermatogenesis, yet the shrinkage is not nearly so marked as in the uninjected controls.

DISCUSSION

The experiments reported in this paper make it plain that the hormones of the eyestalks have little or nothing to do with the loss of the male characters at the termination of the male phase in *Pandalus borealis*. On the other hand, it seems probable that this event is in large measure directly controlled by the secretions of the vas deferens gland. The gland degenerates in animals which are approaching the end of the male phase, its extirpation has resulted experimentally in the premature termination of the male phase at a time of

year when this does not normally happen, and injection of extracts of the gland have partially prevented this result of operation. It seems probable also that the similar results which followed occlusion of the male aperture may in fact be ascribed to interference with this gland. Certainly it is most likely that some hormonal influence is involved since such occlusion results also in behavioural changes.

Such a conclusion about the importance of the role of the vas deferens gland does not of course preclude the possibility, or indeed the probability, that other endocrine organs are playing a part. Allen (1959) has implicated the follicle cells of the testis, for example, while it seems *a priori* probable that the endocrine complex of the protocerebrum might be exerting an over-riding endocrinokinetic control (Carlisle & Jenkin, 1959).

Despite such considerations, however, it seems to me that the immediate cause of the termination of the male phase is the degeneration of the vas deferens gland which was described in the first paper of this series (Carlisle, 1959). Other endocrine factors may exert a modifying influence or may operate through the vas deferens gland.

I should like to acknowledge the friendly assistance afforded me in this work by the former director, Dr G. Gustafson, and the staff of K. Svenska Vetenskapakadamien Kristinebergs Zoologiska Station.

SUMMARY

In the Gullmarfjord population of *Pandalus borealis* all individuals seem to be protandric hermaphrodites; no primary females have been seen by me in the years 1956, 1957 and 1958. Bilateral destruction of the vas deferens gland in mature males is followed by degeneration of the testes and by loss of the external male characters at the next moult, so that the animal moults into the non-sexual condition characteristic of the first stage of the normal sex reversal. These two effects of destruction of the gland have been partially prevented by injection of extracts from fresh glands. The same results follow occlusion of the male openings by means of pellets of cement. It is surmised that this is a result of damage to the vas deferens gland. Such occlusion of the vasa deferentia is followed also by loss of libido. Eyestalk removal and injection of eyestalk extracts have no significant effect on the testis, vasa deferentia, or rate of loss of male characters. Prolonged starvation results in loss of weight of the sperm-filled vasa deferentia, but has no noticeable effect on testis weight or histology. It is concluded that the immediate cause of the termination of the male phase in *P. borealis* is the degeneration (and consequent cessation of endocrine activity) of the vas deferens gland which always precedes this event.

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TABLE 1

Group	Treatment	No. in group	A	B
1	Intact. Fed *ad lib.*	27	3·157 ± 0·432	7·710 ± 0·554
2	Intact. Starved	26	3·263 ± 0·391	7·419 ± 0·516
3	Both eyestalks removed. Fed *ad lib.*	23	3·315 ± 0·302	7·895 ± 0·600
4	Both eyestalks removed. Starved	17	3·240 ± 0·611	7·314 ± 0·708

All animals were killed 15 days after the start of the experiment; animals dying before the close are not included. The animals which are recorded as 'starved' obtained a little food by scavenging in the tank.

A, mean weights and standard deviations (mg) of pairs of testes; B, mean weights and standard deviations (mg) of pairs of vasa deferentia.

ANALYSES OF VARIANCE

Nature of variation	Degrees of freedom	Sum of squares	Mean square	Probability
Testis weights				
Operation	1	0·122	0·122	N.S.
Feeding	1	0·013	0·013	N.S.
Interaction	1	0·228	0·228	N.S.
Error	89	16·292	0·183	—
Total	92	16·654	—	—
	$s = 0·428$.			
Vas deferens weights				
Operation	1	0·144	0·144	N.S.
Feeding	1	4·064	4·064	< 0·001
Interaction	1	0·172	0·172	N.S.
Error	89	26·197	0·294	—
Total	92	30·576	—	—
	$s = 0·543$.			

N.S. = not significant.

TABLE 2

Group	Treatment	No. in group	A	B
5	Intact. Injected distilled water. Fed *ad lib.*	23	3·258 ± 0·408	7·777 ± 0·532
6	Intact. Injected distilled water. Starved.	25	3·250 ± 0·439	7·431 ± 0·517
7	Intact. Injected eyestalk extract. Fed *ad lib.*	20	3·201 ± 0·397	7·938 ± 0·505
8	Intact. Injected eyestalk extract. Starved.	22	3·111 ± 0·515	7·505 ± 0·539
9	Both eyestalks removed. Injected distilled water. Fed *ad lib.*	18	3·330 ± 0·430	7·818 ± 0·590
10	Both eyestalks removed. Injected distilled water. Starved.	16	3·090 ± 0·319	7·475 ± 0·611
11	Both eyestalks removed. Injected eyestalk extract. Fed *ad lib.*	20	3·210 ± 0·592	7·793 ± 0·58
12	Both eyestalks removed. Injected eyestalk extract.	18	3·227 ± 0·577	7·390 ± 0·680
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All animals were killed 13 days after the start of the experiment; animals dying before the close are not included. The animals which are recorded as starved obtained a little food by scavenging in the tank. All animals were injected at the start and again on the sixth day with 0·05 ml. either of distilled water or of a whole eyestalk extract made by grinding fresh male eyestalks with sand and distilled water and centrifuging; the extract was equivalent to 40 eyestalks/ml.

A, mean weights and standard deviations (mg) of pairs of testes; B, mean weights and standard deviations (mg) of pairs of vasa deferentia.

ANALYSES OF VARIANCE

Nature of variation	Degrees of freedom	Sum of squares	Mean square	Probability
Testis weights				
Operation	1	0·001	0·001	N.S.
Feeding	1	0·269	0·269	N.S.
Injection	1	0·155	0·155	N.S.
Interaction-OF	1	0·007	0·007	N.S.
Interaction-FI	1	0·063	0·063	N.S.
Interaction-OI	1	0·170	0·170	N.S.
Triple interaction	1	0·255	0·255	N.S.
Error	154	32·920	0·214	—
Total	161	33·840	—	—
		$s = 0·462$.		
Vas Deferens Weights				
Operation	1	0·022	0·022	N.S.
Feeding	1	5·856	5·856	< 0·001
Injection	1	0·059	0·059	N.S.
Interaction-OF	1	0·095	0·095	N.S.
Interaction-FI	1	0·036	0·036	N.S.
Interaction-OI	1	0·304	0·304	N.S.
Triple interaction	1	0·034	0·034	N.S.
Error	154	43·119	0·280	—
Total	161	49·491	—	—
		$s = 0·529$.		

N.S. = not significant.

TABLE 3. THE LOSS OF THE EXTERNAL MALE CHARACTERS AT THE MOULT FOLLOWING EYESTALK REMOVAL OR INJECTION OF EYESTALK EXTRACT

Group	Eyestalks removed	Injected	No. of moults	No. losing male characters	Proportion losing male characters
14	−	−	197	93	0·472
15	+	−	94	43	0·457
16	−	+	87	40	0·460
17	+	+	103	49	0·476
Total			481	225	0·468

$P \simeq 0{\cdot}7$.

TABLE 4. THE LOSS OF EXTERNAL MALE CHARACTERS AT THE MOULT FOLLOWING DESTRUCTION OF THE VAS DEFERENS GLAND

	Operated	Controls
Retaining male characters	0	9 (11)
Losing male characters	7 (11)	0

$P < 0{\cdot}001$.

Moults 4 or more days after the completion of the operation; two earlier moults in the control group are omitted. The figures in parentheses include animals which were sufficiently advanced in proecdysis at the end of the experiment for the new appendages to be dissected free of the old integument.

TABLE 5. TESTIS WEIGHT 12 DAYS AFTER VAS DEFERENS GLAND DESTRUCTION

	No. surviving	Testis weight (mg) and standard deviation
Gland destroyed	16	$2{\cdot}110 \pm 0{\cdot}225$
Controls	20	$3{\cdot}725 \pm 0{\cdot}371$

$P < 0{\cdot}001$.

TABLE 6. THE LOSS OF EXTERNAL MALE CHARACTERS AT THE MOULT FOLLOWING DESTRUCTION OF THE VAS DEFERENS AND INJECTIONS OF EXTRACTS OF THE GLAND

	Injected	Controls
Retaining male characters	0	4 (5)
Losing male characters	6 (7)	3

$P = 0{\cdot}049$, $P' = 0{\cdot}022$.

Moults 4 or more days after the completion of the operation. The figures in parentheses include animals which were sufficiently advanced in proecdysis at the end of the experiment for the new appendages to be dissected free of the old integument. P' is the probability including these animals, P that excluding them, utilizing only the actual moult figures.

TABLE 7. TESTIS WEIGHT 11 DAYS AFTER VAS DEFERENS GLAND DESTRUCTION FOLLOWED BY INJECTION OF EXTRACTS OF THE GLAND

	No. surviving	Testis weight (mg) and standard deviation
Injected	14	$2{\cdot}795 \pm 0{\cdot}230$
Controls	15	$2{\cdot}011 \pm 0{\cdot}222$

$P < 0{\cdot}001$.