OPTIC GLAND IMPLANTS AND THEIR EFFECTS ON THE GONADS OF *OCTOPUS*

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SUMMARY

1. Optic glands transplanted from one *Octopus vulgaris* into another cause enlargement of the gonads and ducts of the recipient.

2. Enlargement occurs whether or not the gland was secreting when implanted and regardless of the sex of the donor or recipient.

3. Glands derived from *Eledone moschata* or *Octopus macropus* implanted into *O. vulgaris* are as effective as glands derived from *O. vulgaris*.

4. Implants derived from *Sepia officinalis* or *Loligo vulgaris* appear to be ineffective.

INTRODUCTION

The optic glands of dibranchiate cephalopods are the source of a hormone that controls the state of the gonad and of its ducts. Animals can be forced into precocious maturity by cutting an inhibitory nerve to the glands from the central supra-oesophageal part of the brain (Wells & Wells, 1959, 1972). Richard (1970) has shown that fragments of the ovary from *Sepia* will carry out oogonia-to-oocyte cell divisions in organ-culture experiments only if optic glands are present, and more recently Wells, O'Dor & Buckley (1975) have demonstrated *in vitro* control of yolk-protein synthesis by extracts from the optic glands.

It should therefore be possible to excise and re-implant optic glands, with the expectation that they will begin to secrete (if not doing so already) and that their secretion will determine the state of the gonad in the recipients. It should further be possible to discover whether the hormone produced by male octopuses is the same as that produced by the females, and to check on the possibility of interspecific and intergeneric grafts. The report that follows deals with such experiments.

METHODS

Animals, and condition of the gonad in controls

Octopus vulgaris (Cuvier) from the Bay of Naples were used. The animals ranged in size from about 50 to about 400 g, with occasional larger animals among those used as donors. Experiments were made during July and August over a 4-year period from 1969 to 1973.

At the size chosen, female octopuses are nearly always immature, with ovaries that together with the oviducts average only about 1/500 of the body weight. Their optic glands are typically minute, less than 0·5 mm in diameter, transparent or pale yellow

in colour. Their condition is quite distinct from that of mature animals, which have much larger glands, dark yellow or orange in colour, and enormous ovaries, which enlarge until they form as much as 1/5 of the total body weight just before egg laying (Wells & Wells, 1959). Females maturing in the sea rarely weigh less than 1 kg (Mangold & Boletzky, 1973).

Occasional individuals of less than 400 g have enlarged glands and ovaries. One such was found during the course of our experiments, in a total of 117 animals examined. This octopus weighed only 90 g, but already had an ovary weighing a little over 1·0 g; it had large, orange-coloured optic glands and was apparently maturing spontaneously in the sea. This individual has been discarded from the control series in the figures and tables that follow. To guard against the possibility of including a spontaneously maturing individual amongst our experimental animals, we examined the optic glands of all the potential graft recipients before making any implants. Apart from the individual already mentioned, none was found with recognizably enlarged optic glands.

In contrast to the females, males of less than 400 g are often mature. Specimens as small as 150 g can have ripe spermatophores in their ducts. There is no obvious correlation between the production of these first few spermatophores and any outwardly visible change in the state of the optic glands. Only in males weighing 1 kg and more, well beyond the size range used in the present series of experiments, do the optic glands become swollen and bright orange as in mature females.

Experimentally, precocious optic-gland enlargement can be produced in males just as in females. The effect on the testis is most marked in small animals; in octopuses from the 50–400 g size range an approximate doubling of testis weight can be expected within 5 weeks of cutting the nerve supply to the glands (Wells & Wells, 1972). This is a rather small change compared with the 100-fold increase in gonad size that can result from the same operation in females. Optic gland implants were therefore made mainly into female animals.

Controls, feeding, and time spent in the laboratory

If the optic nerves are cut, the optic glands and the gonads of octopuses enlarge (Wells & Wells, 1959). If cuttlefish are kept under conditions of reduced daylength, they mature precociously (Richard, 1967). These facts tie in with what is known of the ecology of the animals, which normally breed in the spring (Mangold-Wirz, 1963; Mangold & Boletzky, 1973; Wells & Wells, in preparation).

One must therefore be wary of the effects of changing the apparent daylength, as is liable to happen when octopuses are kept in lidded tanks with artificial lighting in the laboratory. It is also necessary to be sure that changes in the state of the gonad do not arise from the changes in temperature or diet that are inevitable when the animals are brought in from the sea. Mangold & Boucher-Rodoni (1973), for example, have claimed that starvation tends to bring on early maturity in *Eledone*.

Wary of these possibilities, we have been careful to distinguish between controls fresh in from the sea and controls that have been kept in the laboratory for periods comparable with the experimental animals. The ovary weights of the two were, however, not distinguishable in the present sample (see Fig. 2).

In a further survey, ranging over several years and including periods both before and after that of the present experiments, Buckley (personal communication) has compared
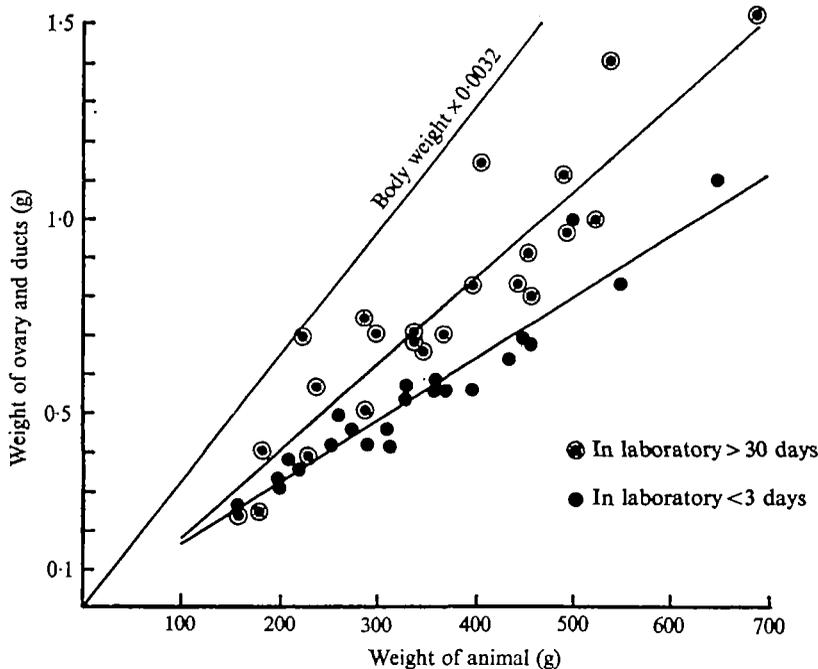
Fig. 1. A comparison of ovary plus oviducts weights from control female *O. vulgaris* kept in the laboratory for less than three and for more than thirty days. The line 'body weight × 0·0032' shows the maximum ovary size found in controls (see Fig. 2).

animals kept in the laboratory for 30+ days at *ca.* 26 °C with animals fresh in from the sea, kept in the laboratory for three days or less. When these extremes are taken some difference in average gonad weight is apparent (Fig. 1). We do not know why. Increased gonad weights seem to result from keeping the animals in the laboratory. But it is also possible that octopuses fresh in from the sea are themselves abnormal due to stress, which certainly affects their feeding and may through this or otherwise affect the degree of hydration and/or the distribution of fluids in their bodies.

The important point for present purposes is that the magnitude of any change in gonad weight that can be attributed simply to keeping the animals in the laboratory is very small compared with the large increases that often follow implantation of optic glands.

Operations and the duration of experiments

Operations (examination of the glands, and implants) were carried out under anaesthetic (3 % urethane in sea water). The implants, whether of optic glands or of other tissues, were placed in the orbital sinus, a large blood space containing the 'white body' behind the eye (Boycott & Young, 1956). The implants were pushed in with forceps and left free-floating in the sinus; successful optic gland implants usually adhered to the membranes surrounding the space. Indian ink injections of the blood system show that they attract a blood supply from the vessels in the walls of the sinus. Other gland implants adhered to the white body where they were later found to be surrounded by a pinkish discoloration, evidently associated with amoebocytes that

congregate around the implant. An account of the histological appearance and fate of implants is in preparation and will be published separately.

Animals with implants, and their controls, were kept for as long as possible before being killed for examination of their gonads and recovery of the implant. The maximum, determined by the available summer working period at Naples, was about 5 weeks. Results with a few animals that survived for less than 14 days have been discarded.

RESULTS

Animals with their own optic glands enlarged

At the end of the experiments, the animals (controls and animals with implants) were killed and dissected. In each case the animals' own optic glands were examined again. In 8 out of 128 implant recipients (7 females and 1 male) one or both of the glands was enlarged. With the exception of one very small animal, all the females had enlarged ovaries. The male's testis weight was towards the upper limit of the control range. It is possible that the optic glands of all these animals were maturing as a result of damage to their optic stalks incidental to the implantation and/or gland examination operations. Implants were pushed into the region around the optic stalk; examination involved lateral displacement of the optic lobe. Either could damage the optic stalk and the inhibitory nerve supply to the glands. We assume that this is what happened in the eight instances in which the recipients' own glands enlarged. We do not think that the enlargement is an effect of implantation *per se* because (1) it happened in only 5% of all implantations and (2) the series included two animals in which only one of the recipients' optic glands enlarged. This might be expected if damage were the cause, but is rather difficult to explain on any other grounds. The eight animals have been discarded from the experimental series, since the size of their gonads was as likely to be due to the activity of their own optic glands as to any effect of an implant.

This left a total of 120 animals (47 males and 73 females) with normal, inactive optic glands and implants of one sort or another. The results with these animals have been divided into three categories, dependent upon the appearance (or non-appearance) of the implant at terminal dissection. These categories are:

(1) Results from animals with implants clearly recognizable as optic glands; these implants were rounded, yellow and well defined against their background when dissected out under a binocular microscope. We assume that these glands were 'healthy' and secreting.

(2) Results from animals where the condition of the implanted gland was uncertain. These await detailed histological examination. Typically they appeared as areas of discoloured tissue, embedded in the white body or stuck to the membranes bounding the blood sinus around this.

(3) Cases where we were unable to find any trace of the implant on post-mortem dissection of the recipient. *Octopus* tends to pick at stitches, and since this sometimes causes a bigger wound than the original incision, it is generally better to rely on overlap of the skin and orbital membrane, and the animal's own musculature, to close the wound. A not uncommon consequence is that shreds of white body squeeze through the gap, and it is likely that at least some of our failures to recover implants were due to
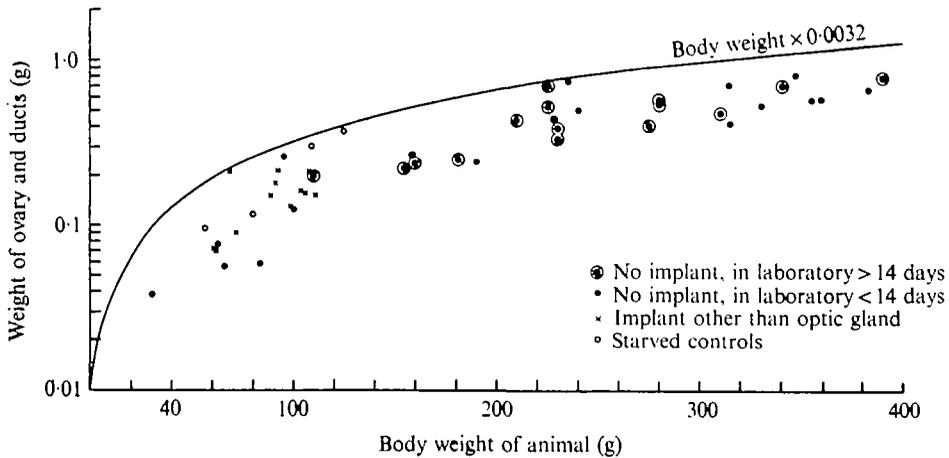
Fig. 2. Ovary plus oviducts weights from control animals and from octopuses with fragments of nervous tissue or testis implanted. The plotted line 'body weight × 0·0032' encloses all these and is used as the maximum value for controls in Figs. 3 and 4.

loss of the gland through the wound shortly after implantation. It other cases it may have been destroyed by host amoebocytes; the likelihood of this will become clearer after examination of the category (2) material.

So far as the present work is concerned, it is clearly the results in category (1), from animals with surviving and apparently 'healthy' implants, that are of greatest interest. This category is distinguished in Figs. 3 and 4 showing the effects of implants. All the firm conclusions that we have reached are based on this unequivocal material.

Implants of tissues other than optic glands (segments of the nervous system, or testis) were more difficult to locate on dissection because they are not coloured. Some were recovered, but no attempt has been made to separate these results into categories; implants other than optic glands never caused enlargement of the hosts' gonads.

Female controls and animals with implants other than optic glands

Fig. 2 shows the weight of ovaries plus oviducts from controls and animals with implants other than optic glands. The controls include animals fresh from the sea, killed within 14 days of collection ($n = 15$) and unoperated animals, kept in the laboratory for longer than 14 days ($n = 18$).

Implantation of fragments of optic lobes or testis ($n = 12$) has no obvious effect, nor, on this sample, does keeping the animals in the laboratory (but see Fig. 1). The largest ovary (plus ducts)/body weight ratios from the 45 animals all fall below a line drawn at 0·0032 × body weight, and this has been taken as representing the upper limit for controls in Figs. 3 and 4. It is the same upper limit as we found in a previous sample of 72 controls in the 200–900 g range (Wells & Wells, 1959).

In addition to these octopuses four small animals were starved for periods of from 34 to 41 days. Details of these are given in Table 1 and Fig. 2. They, too, fall well within the control range. Mangold & Boucher-Rodoni (1973) have claimed that starvation will induce precocious maturity in female *Eledone*. Their four animals had gonad/body weight ratios of up to 0·28, and although they were kept for considerably longer than ours (3½ months, mid-September to January; against just over 1 month in July–

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Table 1. *Details of starvation controls*

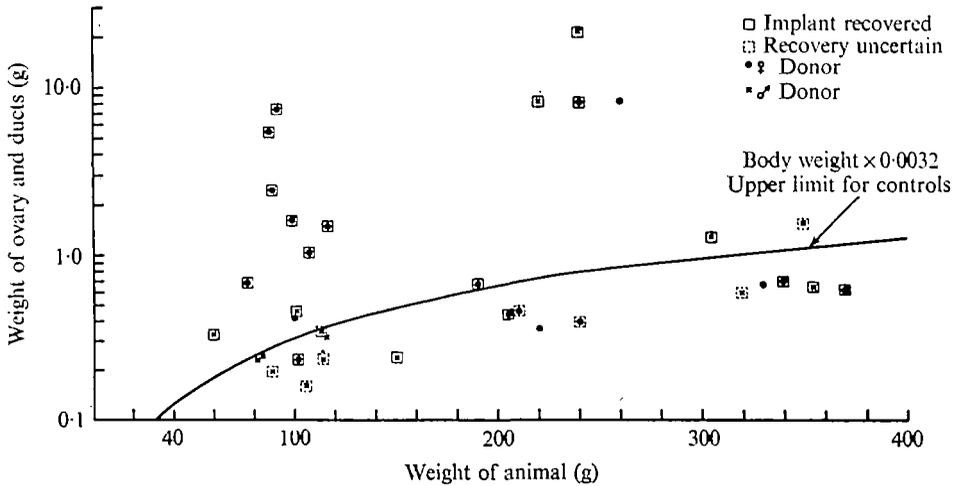| Initial weight (g)	Death weight (g)	No. of days in laboratory	No. of days starved	Wt. of ovary and ducts (g)	Ratio of ovary and ducts to body weight
175	110	41	38	0·285	0·003
200	125	41	38	0·392	0·003
88	80	54	34	0·120	0·002
58	58	61	41	0·105	0·002

Fig. 3. Ovary plus oviducts weights for female octopuses with *O. vulgaris* optic glands implanted into the orbital sinus. The line indicates the upper limit for control animals (see Fig. 2).

August) the two sets of results are, on the face of it, incompatible; one would have expected at least some signs of enlargement during the first month in *O. vulgaris*, particularly in view of the relatively high temperature at which our animals were kept. *Eledone* could differ from *Octopus* in this respect, but it is also possible that their animals were maturing for reasons other than starvation, since the evidence that they were unfed is based on their being in each case the smallest from a group of five or six individuals kept together. In such a group, the larger animals do indeed tend to grab any crabs placed in the tank. But it is also true that octopuses stop trying to feed as their gonads mature (Wells & Wells, 1959; van Heukelem, 1973).

Ovary and duct weights in animals with implanted optic glands

Fig. 3 shows the results of implanting optic glands into 35 female animals. Sixteen of these had glands taken from male and 19 from female octopuses. Six of those with male implants, and 11 of those with female implants, had ovaries larger than the largest found in controls. The animals with enlarged ovaries include nearly all of the instances where the implant was successfully recovered (category 1, see p. 582).

A further analysis of these results is given in Table 2, which shows the sex of the donor and condition of the glands when implanted in relation to the results produced. Although nearly twice as many enlargements resulted from female–female as male–female implants, the numbers are too small to be sure that this is a real effect, and all

Table 2. *The effect of implants into* Octopus vulgaris, *considered in relation to the sex and state of the glands implanted. All donors were* O. vulgaris

Sex of recipient	Sex of donor	Condition of host's gonad at death	Condition of the gland when implanted	
			Enlarged	Not enlarged
♀	♂	Enlarged 6	2	4
		Normal 10	4	6
	♀	Enlarged 11	3	8
		Normal 8	4	4
♂	♂	Enlarged 8	1	7
		Normal 16	2	14
	♀	Enlarged 2	2	—
		Normal 9	6	3

Table 3. *Cases where the two optic glands from a single* O. vulgaris *donor were implanted into two different female recipients of about the same weight (the two glands were in the same state when implanted)*

(Enl. = ovary (host) or glands (donor) enlarged; — = not enlarged; ? = recovery of implant uncertain (category 2, p. 582).)

Donor			Recipient 1			Recipient 2		
Wt. (g)	Sex	Condition of glands	Wt.	Effect of implant on ovary	Recovery of implant	Wt.	Effect of implant on ovary	Recovery of implant
400	♂	—	100	—	?	90	—	?
480	♀	—	80	Enl.	Yes	85	Enl.	Yes
190	♀	—	150	Enl.	—	140	—	Yes
1850	♂	Enl.	110	—	Yes	170	Enl.	Yes
480	♂	—	170	—	?	210	Enl.	Yes
390	♀	Enl.	265	—	—	150	—	?
310	♀	Enl.	261	—	—	180	—	Yes
370	♂	—	180	—	—	180	Enl.	Yes

that can be stated with certainty is that both female and male glands can cause gross enlargement to the ovaries of recipients (Fig. 3). It seems also that it makes no difference if the glands are inactive at the time of implantation. Twenty-two out of the 35 female octopuses received implants of inactive glands: 12 of these subsequently developed enlarged ovaries, 4 as a result of male and 8 of female implants. The proportion of 'successes' is just as high as in the 13 female recipients of enlarged glands, 5 of which matured (Table 2).

If the sex and/or state of maturity of the donor are unimportant, variations in the response to implantation must depend upon the recipient. The view is borne out by examination of the few results obtained from pairs of recipients each receiving one of the two glands available from each donor. In 4 out of the 8 instances, one recipient enlarged and the other did not (Table 3). In all probability, successful implantation is largely a matter of chance. If the implant lies against a suitable membrane, it attracts a blood supply and continues to grow; if, by chance, it lies loose in the white body, a tissue dedicated to the production of amoebocytes (Bolognani, 1951), it is liable to become encapsulated and prevented from further growth. It seems likely that a higher proportion of 'successful' implantations could be achieved by more careful placing of the glands.
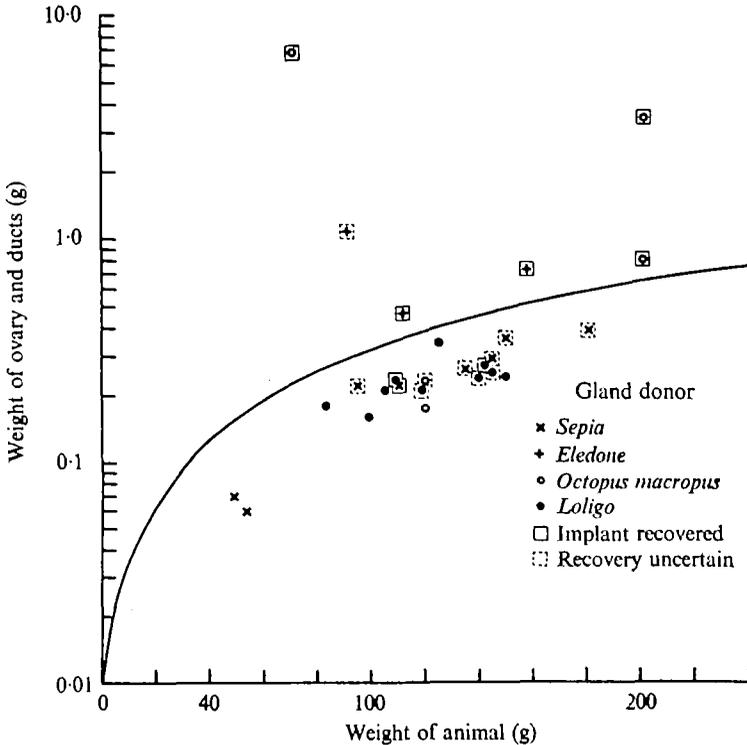
Fig. 4. Ovary plus oviducts weights from female *O. vulgaris* with implanted optic glands derived from other species of octopod and decapod. The line shows the upper limit for control animals as in Figs. 2 and 3.

Female recipients with optic gland implants derived from other species

As well as *O. vulgaris*, *O. macropus* (Risso), *Eledone moschata* (Lamarck), *Sepia officinalis* (L.) and *Loligo vulgaris* (Lamarck) were used as donors. The results are summarized in Fig. 4. Three out of five *O. macropus* implants caused enlargement, as did all three of the glands taken from *Eledone*. None of the 18 decapod implants was effective. With so little data available it is impossible to be sure that the rate of development caused by *O. macropus* and *Eledone* implants is the same as that brought about by *O. vulgaris* glands, though this appears to be the case.

Results with male recipients of optic gland implants

Forty-seven implantation experiments were run with male recipients. Thirty-five of these had implants from male or female *O. vulgaris*. A summary of the results is included in Table 2 with an analysis in terms of the sex of the donor and the condition of the glands at implantation. Of the 35, 24 had implants derived from males, and 11 from females: 10 of the recipients (2 with female and 8 with male gland implants) had testis and duct weights greater than the largest found in 42 controls ($0.008 \times$ body weight). A further five had gonad/body weight ratios exceeding those of all but two of the controls.

Neither the sex of the donor nor the state of maturity of the donor's glands appeared to be relevant to the result obtained (Table 2). The rate of recovery of implants from

'males was about the same as that from females (21 out of 35 *O. vulgaris* implants recovered and classed as category 1 (p. 582) from males, 20 out of 35 from females).

Twelve male octopuses had implants derived from other species of cephalopod. One, with a gland derived from a female *O. macropus*, had the largest male gonad weight found in the size range 50–200 g. None of the others had testis and duct weights above the maximum found in controls, though a further three out of the 12 approached this, being above the range defined by all but two of the 42 controls. Interestingly, one of these three had an *Eledone* implant while the other two had implants derived from *Loligo* and *Sepia*.

DISCUSSION

The series of experiments summarized above has shown that optic glands excised from one octopus and implanted into another will survive and begin to secrete, causing precocious maturation of the recipient. Granted survival of the implant, maturation was to be expected from the results of previous work on the physiology of hormonal control of sexual maturity in octopuses. In addition, and not predictable from previous experiments, we now know: (1) that the optic gland hormone from males and females is probably the same; and indeed (2) that the hormone is probably very similar in all octopods, since interspecific and intergeneric implants appear to be as effective as intraspecific. It should be noticed, in passing, that the ripe glands of *O. macropus* are white, not orange as in *vulgaris*: the yellow colour of extracts from *O. vulgaris* glands is evidently not a necessary characteristic of the hormone.

The situation with regard to decapods is uncertain. Implanted glands from *Sepia* or *Loligo* do not seem to cause enlargement of the gonads of *O. vulgaris*, which would suggest that the hormone they are producing is different from that secreted by the octopods. Alternatively, it is possible that decapod glands are more readily recognized as 'foreign' and destroyed by the host. Out of 23 decapod implants, only 4 (with 13 additional 'doubtful' cases, category 2, see p. 582) were recovered. This is in marked contrast to the interspecific octopod implants, where the score was 9 out of 15 recoveries of glands in good condition. Clearly identifiable *O. vulgaris* glands implanted into the same species were recovered on 41 out of 70 occasions, with a further 19 'possibles' in category 2. The decapod implants were in general of smaller glands, and so more difficult to find on dissection, but even allowing for this the difference between 4 out of 23 and 50 out of 85 seems a little large to attribute to chance.

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