# REPRODUCTION, DEVELOPMENT AND BREEDING ACTIVITY IN THE FRESHWATER CRAYFISH, *CHERAX DESTRUCTOR* CLARK

by

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"Ebenso hat mich auch die genäuere Untersuchung unsers Krebses gelehret, dass, so gemein und geringschätzig solcher auch den meisten zu seyn scheinet, sich an selbigem doch so viel Wunderbares findet, dass es auch den grossten Naturforscher schwer fallen sollte solches alles deutlich zu beschreiben."

- Roesel von Rosenhof, 1755.

(Quoted by Huxley 1880).

(Nevertheless a close look at our crayfishes has taught me that, as commonplace and insignificant as they may appear to most, there is so much that is wonderful about them, that the greatest scientist would have difficulty in clearly describing it all.)

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Frontispiece: Cherax destructor (male).

## SUMMARY.

1. As is typical of Parastacidae, the pleopods of Cherax destructor are not modified for sexual function and are absent from the first abdominal segment in both sexes. The male possesses the long, complex, non-calcified genital papilla typical of the genus.
2. The ovaries of C. destructor are typically reptantian, being paired, closely approximated organs connected by a single commissure. The anterior lobes are extended into tubular horns. Histologically, the ovary is of the general decapod pattern. The testes and vasa deferentia are typically reptantian in anatomy and position in the body cavity. The spermatophoric mass is stored in the distal section of the vas deferens, and consists of a tightly coiled spermatophore convoluted randomly throughout a matrix.
3. Five stages of oogenesis, including two previtellogenic stages, are described for C. destructor. The smallest previtellogenic oocytes observed were 50 µm in diameter, with a nuclear diameter of 15 µm. Vitellogenesis begins when the oocyte attains a diameter of about 400 µm. The mature oocyte is 2.0 x 1.2 mm, and is packed with large yolk platelets, up to 80 µm in diameter.
4. Seven macroscopic stages of ovarian maturation are described for C. destructor. These are: I. Immature virgin; II. Maturing virgin 1; III. Maturing virgin 2; IV. Maturing 1; V. Maturing 2; VI. Mature; VII. Spent/Regenerating. Gonosomatic index was shown to be a reliable index of ovarian maturation in C. destructor.
5. Several percent of C. destructor individuals were intersexes, a phenomenon common in parastacids. Intersexual characteristics appear to be hereditary.
6. A gelatinous spermatophore is deposited on the sternal keel of the C. destructor female close to the oviducts. The female does not

undergo a spawning moult.

7. Spawning and egg attachment in C. destructor were similar to those of most macrurans. Spawning began soon after spermatophore deposition and took about three quarters of an hour to complete. Egg attachment was complete within four or five hours of spawning. Glair was present during the spawning process. Fertilization is presumed to be external. Eggs are attached mainly to the endopodites of the pleopods, as is usual in macrurans. Both pinnate and filamentous setae occur on the pleopods, but only filamentous setae are involved in egg attachment.

8. The fertilized egg of C. destructor is large (up to 2.5 x 2.0 mm), light olive-green and densely yolky. Development is direct, larval stages are fully embryonized, and hatching occurs as a juvenile.

9. Three juvenile stages are described for C. destructor. Stages I and II possess the usual undifferentiated telson and uropods and, as is typical for parastacids, bear terminal recurved spines on the fourth and fifth pereiopods which are used for clinging to the maternal pleopodal setae. The stage III juvenile is morphologically similar to the adult. A telson thread attaches the Stage I juvenile to the egg-case for about one day after hatching.

10. Breeding in C. destructor takes place annually during the spring and summer, when a mixture of maturing, mature and spent/regenerating ovaries is found. Oocytes develop during the autumn and winter, and reach maturity in spring when mating and spawning occur. Approximately 25 percent of adult females were found to be berried at any one time during the breeding season. Spermatophoric masses were present in the vasa deferentia of most adult males except during late autumn and early winter.

11. Daylength, and possibly temperature, regulate timing of ovarian

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maturation in C. destructor. Temperature is perhaps the ultimate stimulus to spawning, which begins in spring when the bottom water temperature reaches approximately 15°C. Flooding is not a necessary breeding stimulus.

12. C. destructor eggs hatch after 21 days, at incubation temperatures of 20-25°C. Eggs spawned at the beginning of the breeding season do not hatch until mid-summer, an incubation period of about three months.

13. Repetitive breeding occurs in C. destructor. Regeneration of the ovary begins soon after spawning and, shortly after the juveniles have reached independence, the mother is ready to spawn again.

14. C. destructor females from the study dam reached sexual maturity at a carapace length of approximately 36.5 mm and an age of about two years. The smallest berried females from the study dam and from the study area had carapace lengths of 33.5 mm and 22 mm respectively. Males appear to mature at a smaller size than females. All males with a carapace length greater than 28 mm were mature.

15. The average number of eggs on a berried C. destructor female is about 320; this falls within the range of 30-1300 found amongst species of the Parastacidae. The number of eggs laid was found to increase with the size of female.

## 1. GENERAL INTRODUCTION

Most of the literature dealing with the southern hemisphere family of freshwater crayfishes, the Parastacidae, has been concerned with the systematics of the family. Investigations into life histories have been few. The species of Australian crayfishes whose biology has received attention are Engaeus spp (Clark 1936b), Euastacus kershawi (Clark 1937), Cherax tenuimanus (Shipway 1951, Morrissy 1970, 1974, 1975, 1976a, 1976b), C. davisi (Woodland 1967 - this species, originally identified as albidus, is more recently (Riek 1969) identifiable as davisi), Parastacoides tasmanicus (Lake and Newcombe 1975), Engaeus cisternarius and E. fossor (Suter 1977, Suter and Richardson 1977). The biology of the New Zealand parastacid Paranephrops planifrons has been investigated by Hopkins (1967).

The greatest diversity of species of the Parastacidae is found in Australia, where there are 10 genera and approximately 100 species (Riek 1969, 1972). The most widely distributed genus is Cherax of which the species destructor has the greatest range (Figure 1.1) and, in view of this and its fishery statistics (see below), it is undoubtedly the most abundant. Cherax destructor, Clark 1936, commonly known as the yabby (frontispiece), has adapted to greatly varying environments as indicated by its wide distribution, ranging from the cool, wet regions of Victoria and the Snowy Mountains to the hot, arid parts of central Australia. C. destructor has invaded most freshwater bodies in the Lake Eyre and Murray-Darling basins, and is found in rivers, creeks, billabongs, lakes, irrigation canals, swamps, bore-drains and farm dams.

The importance of C. destructor to the ecology of inland Australia cannot be overemphasised, as it constitutes an important prey species for many freshwater fishes (Hale 1925, Tilzey unpub. data) and birds (Hale 1925, McNally 1957, Carrick 1959, Miller 1976). C. destructor is

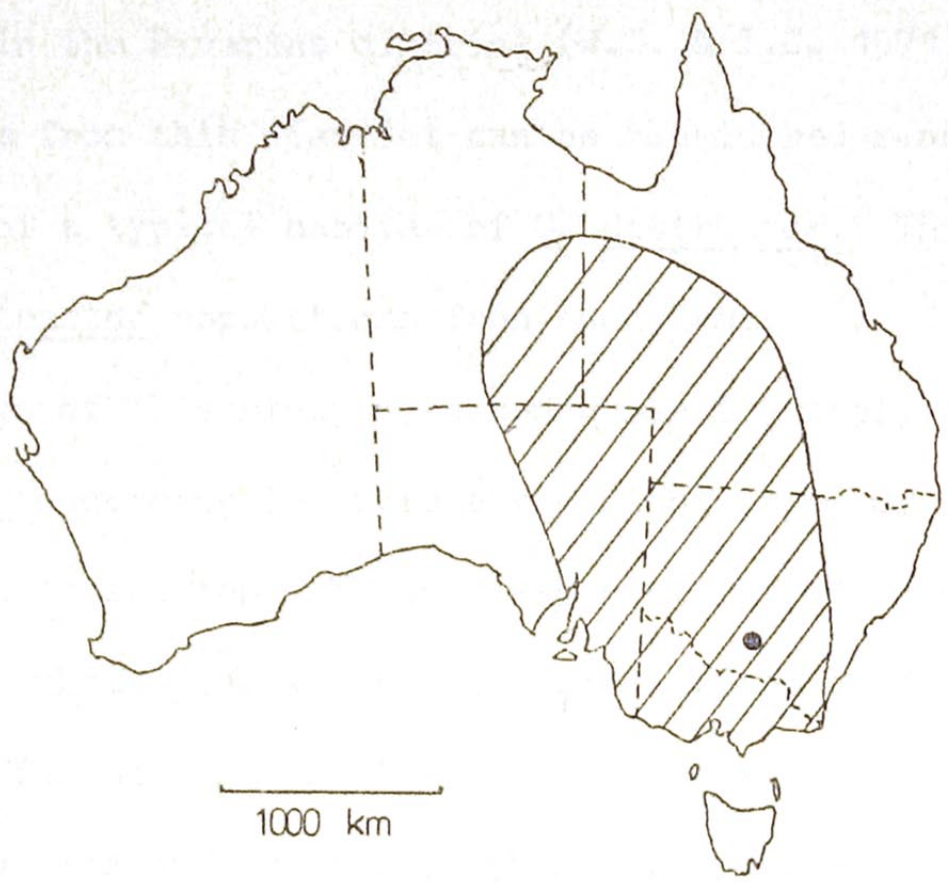


Figure 1.1. The distribution of the yabby, Cherax destructor, according to Riek 1969. The Narrandera study area is indicated by the symbol •

also an economically important crayfish species, supporting a fishery of moderate size (about 100 tonnes per annum; Aust. Bureau of Census & Statistics, 1978).

Resident populations of C. destructor are found in nearly all farm dams within its range, and the highest density of farm dams in New South Wales is found in the Riverina district (W.C. & I.C. 1971). Thus a typical farm dam from this district can be considered reasonably representative of a typical habitat of C. destructor. This study is based on C. destructor populations from such dams.

The findings of this study do not necessarily apply to populations of C. destructor occurring in different habitat types or in other parts of the range of the species. This is especially true when one considers that there is a substantial amount of morphological difference between animals from different parts of the range, this variability possibly being associated with differences in physiology and behaviour.

Little is known about the reproductive biology of C. destructor. This study firstly examines the reproductive system, describing the genitalia and gonads, and the processes of oocyte development and ovarian maturation.

Secondly, reproduction in C. destructor is dealt with, the processes of spawning, and of embryonic and juvenile development being examined.

Finally, various aspects of breeding activity are examined, including the breeding cycle of mature animals and environmental regulation of the cycle, the incubation period, and repetitive breeding. The attainment of sexual maturity and the fecundity of C. destructor are also investigated.

## 2. MATERIALS AND METHODS - GENERAL

### 2.1. INTRODUCTION

This study was carried out between August 1974 and September 1976. Specimens of C. destructor used in this study were collected from farm dams near the town of Narrandera in the Riverina district of New South Wales. This area is well within the geographical boundaries of distribution of C. destructor (Figure 1.1).

One particular farm dam, hereinafter called the study dam, was selected for a study of the pattern of breeding activity within a population. Sampling of the C. destructor population in the study dam was carried out at approximately monthly intervals. During the warmer part of the year, when reproductive activity is enhanced, the frequency of sampling was increased to once a fortnight. Sampling once every six weeks was found to be frequent enough during the colder months.

The data described in results sections 3.3 and 5.3 were obtained by observation of crayfish mainly in the study dam, but also in other farm dams and in aquaria. The data described in results section 4.3 were obtained from crayfish held in aquaria.

### 2.2. COLLECTING SITES

#### 2.2.1. The study dam

The study dam (Plate 2.1) was an excavated tank, the most common type of farm-water storage in New South Wales (W.C. & I.C. 1971). In this type of dam, the storage is entirely below ground level and is filled by runoff from the surrounding country.

The study dam had once been a domestic supply but, at the time of the study, was used solely for watering cattle and sheep. Wheat fields and livestock paddocks were the main catchment areas for the dam. The banks, which sloped at about $15^{o}$ from horizontal, were constructed of

Plate 2.1. View of the south-eastern half of the study dam in April 1975.

red-brown, sandy clay.

The dam was approximately square in shape and, when full, measured 58 metres by 58 metres, with an average depth of about 2 metres, and a maximum depth of 3.2 metres. This represented a surface area of about 0.33 hectare, and a volume of approximately 6¾ megalitres. At the lowest level recorded, which occurred after a twelve-month drought, the water surface was 36 metres by 36 metres, the average depth was around 0.9 metres and the maximum depth was 2 metres. This represented a surface area of 0.13 hectare and a volume of approximately 1 megalitre. Although the mean annual rainfall in the area is fairly low (434 mm), it is unlikely that the study dam would ever dry up, except possibly in extended drought. Changes in water level throughout the study period are tabulated in Appendix 1. The water level is indicative of rainfall throughout the period.

The study dam was a mature dam more than twenty years old and, as such, was a fairly stable ecosystem. No aquatic macrophytes were present. Filamentous green alga was occasionally observed and a bloom of a blue-green alga, Anabaena sp., occurred once, a few weeks after a period of heavy rain.

In addition to C. destructor, fauna in the dam included a population of goldfish, Carassius sp., which preyed upon the yabbies. Other crustaceans were the cladoceran, Daphnia sp., which bloomed from time to time, and several copepods. The predominant insects were the water beetle, Dytiscus sp; the needle-bug, Ranatra sp; backswimmers (Notonectidae); and water-boatmen (Corixidae). Tadpoles were occasionally seen in large numbers. Chironomid larvae were found in bottom mud samples. Ducks, geese, cormorants and ibis were sporadic visitors to the dam. Both cormorants (Miller 1976) and white ibis (Carrick 1959) are reported to prey heavily upon C. destructor.

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As is usual in farm dams in the area, the water had a high silt load and was always very turbid with a Secchi disc reading of never greater than a few centimetres. The crayfish appeared, from examination of stomach contents, to have been feeding mainly upon cow manure which had been either washed into the dam from the catchment area, or defaecated directly into the water by cattle drinking at the dam.

Temperature profile studies showed the water body to have a distinct thermocline within the first 0.75 metres from the surface, in both winter and summer. Bottom water temperature generally did not vary by more than ± one Celsius degree during a 24 hour period.

2.2.2. Other collecting sites

In order to disturb the crayfish population in the study dam as little as possible, only the minimal number of animals, those needed for gonad maturation studies, was collected. Animals needed in laboratory studies on reproduction and seasonal reproductive activity were collected from various sampling sites other than the study dam. These additional sites were farm dams, similar in most respects to the study dam, and with a similar ecosystem.

2.3. SAMPLING METHODS

Routine sampling in the study dam was carried out using a seine net. This method was chosen because it is fast, efficient and non-selective over most of the size range of C. destructor, compared with baited traps and other methods. Berried females and moulting animals are not attracted to bait. Traps tend to select the larger individuals which, being dominant over smaller individuals, enter the traps first. It is considered that the samples obtained using the seine net were representative of the population for animals over 10 mm carapace length. The frequency of smaller animals in the samples was low due to the

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selectivity of the net. (Selectivity is a function of the mesh size of the net). Furthermore, standardization of effort, using the seine net, was relatively easy compared with other methods. The seine net was 55 metres long, 2 metres deep, and made of brown cotton with an 11 mm mesh (stretched). Floats and weights were arranged to give the net a negative buoyancy.

Because of the bottom topography of the dam and because of the presence, in the southern half of the dam, of a fixed pipe and several large boulders, the net could not be worked over the whole of the dam. Sampling was accordingly restricted, on each occasion, to the northern half of the dam. The net was firstly laid out across the pond, using a dinghy, and then worked up to the northern end of the dam where the catch was landed. The catch was examined at the dam-site, specimens were selected and retained for later examination (see Section 2.4.4), and the catch was returned to the water as soon as possible (usually within two or three hours of landing).

Sampling at other sites was carried out by any method found to be convenient at the time. Animals were taken by seine net, baited trap, dredge, trawl net, or by hand.

## 2.4. TREATMENT OF SAMPLES

### 2.4.1. Size of routine samples

The size of a routine sample, taken in a standard haul, was usually about 200 individuals and varied between 43, prior to the annual recruitment of juveniles, to over 600 at the end of the breeding season. Sexes were approximately equal in number.

### 2.4.2. Morphometric measurements

2.4.2.1. Carapace length (CL). Orbital carapace length was used as the standard measurement of length of an individual because of the

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reliability inherent in the method of measurement. Measurement of total length is less reliable due to the lack of rigidity in the abdominal segments; and rostral carapace length is not always measurable, because of the occasional broken rostrum. Carapace length was measured, to the nearest tenth of a millimetre, from the posterior edge of the orbit to the posterior edge of the carapace, parallel to the midline, using Vernier calipers accurate to ± 0.01 mm.

Carapace lengths of animals in the large, routine samples were recorded to the nearest millimetre below the observed value, the centre-point of each size-class of one-millimetre interval being used in expression of the results. As the range of carapace length of animals used in this study extended to about 60 mm, an individual was assigned to one of 60 size-classes.

2.4.2.2. Total weight ($W_t$). This was measured, to the nearest tenth of a gram, after a standard drying which consisted of rolling the animal in an absorbent cotton bath-towel for several seconds. A top-loading torsion balance, accurate to ± 0.05 g, was used.

2.4.2.3. Ovary weight ($W_o$). The paired ovaries (together with the oviducts, which comprised an insignificant fraction of the weight) were removed from the freshly dissected animal and weighed, to the nearest milligram, on a weight-dialling beam balance accurate to ± 0.00005 g.

2.4.2.4. Gonosomatic index (GSI). The gonosomatic index is commonly used in fisheries technology to express objectively the degree of maturation of the gonads, especially the ovaries. Weight of ovaries is usually found to be correlated with degree of maturation, and GSI is usually expressed as a percentage of total weight of the animal (not including the weight of gonads or their products), so:

$$GSI = \frac{W_o}{W_t - W_o - W_e} \times \frac{100}{1}$$

where $W_o$ = gonad (ovary) weight

$W_t$ = total weight of animal

$W_e$ = weight of attached spawn, if any.

2.4.3. Incidence of berried females

Female crayfish which have spawned and are bearing either eggs or juveniles on the pleopods are called berried females (a term common to decapods). The incidence of berried females in each routine sample was recorded.

2.4.4. Collection of specimens for dissection

About four adult and about four juvenile crayfish of each sex (i.e. a total of about sixteen animals) were selected from each routine sample from the study dam, and were returned to the laboratory for dissection and examination.

# 3. ANATOMY OF THE REPRODUCTIVE SYSTEM

## 3.1. INTRODUCTION

In the crayfishes of the northern hemisphere, the pleopods of the first abdominal segment of the male are modified for the sexual function of sperm transmission. In the southern hemisphere crayfishes, the pleopods of the first abdominal segment are absent in both sexes; and this was one of the main criteria used in the erection of the new family, Parastacidae, by Huxley (1878, cited by Clark 1936a).

Sexual dimorphism in, and the genitalia of, Australian parastacids have been described extensively (Huxley 1880, Clark 1936a, Riek 1969, 1972, Suter 1977).

Except for Cherax tenuimanus (Morrissy 1970, 1975), the anatomy and histology of the ovary, and stages of ovarian maturation, have not been previously described for any parastacid species.

The process of oogenesis has not been described previously for Parastacidae.

Intersexes are commonly found in parastacid crayfishes, as reported by Riek (1951, 1956, 1967, 1969, 1972) and Woodland (1967).

Little is known about the reproductive system of C. destructor. This section of the study examines the reproductive system of C. destructor, describing the genitalia and gonads, and the processes of oocyte development and ovarian maturation.

## 3.2. MATERIALS AND METHODS

### 3.2.1. Anatomy of the genitalia; intersexuality

The anatomy of the external genitalia was described from the

examination of specimens of C. destructor collected during routine sampling of the study dam populations (Section 2.1). The various forms of intersexuality and aberrant genitalia were described from dissections of animals collected during routine sampling (see also Section 2.4.1).

3.2.2. Anatomy and histology of the gonads; sequence of ovarian stages; sequence of oogenesis.

The anatomy and histology of the gonads, the sequence of ovarian stages, and the sequence of oogenesis, were described from examination of the specimens collected during routine sampling of the study dam (see Section 2.4.4). The gonads were examined, removed and weighed, then fixed and preserved for histological examination. Large ovaries (those weighing more than one gram) were cut into smaller pieces to facilitate penetration of the fixative; otherwise all gonads were preserved whole.

3.2.2.1. Ovaries. Ovaries were fixed and preserved in buffered formalin. The tissues were prepared for sectioning using a method suitable for eggs and yolk cells (Pantin 1948). The fixed tissues were dehydrated rapidly in a series of alcohols, methyl benzoate, and benzene, finally being embedded in paraffin wax (M.P. 54°C).

Large, yolky specimens (ovaries in stage IV and later, where the GSI is greater than 0.4) were dehydrated, then passed through two baths of a one percent solution of Celloidin in methyl benzoate, the first for 12 to 24 hours, and the second for 48 to 96 hours. The tissue was then passed through benzene and three baths of paraffin wax and, to ensure good wax infiltration, left in the final wax for 12 hours.

Sections were cut at 8 µm and stained with Delafield's haematoxylin by the regressive method (Humason 1972). Eosin was used as a counter-stain. Because of their yolky nature, sections were passed rapidly through the alcohols.

Sections were examined and photographed under a light microscope. Oocyte and nuclear diameters were measured by means of an ocular micrometer.

Gonosomatic indices were calculated as described in Section 2.4.2.4. Range of gonosomatic index values was calculated for each ovarian stage. In order to assess the reliability of the gonosomatic index as an index of ovarian maturation, data from twelve females of various sizes and ovarian maturation stages were selected. The gonosomatic indices were regressed on the mean oocyte lengths using the method of least squares. Mean oocyte length was taken as the mean of the measurements of the major axis of several oocytes of the predominant maturation stage in the ovary.

3.2.2.2. Testes. Testes were fixed for three to six hours in Carnoy fixative (without chloroform), as described by Humason (1972), and were preserved in 70 percent alcohol. The tissues were embedded, sectioned and stained as for ovaries.

## 3.3. RESULTS

### 3.3.1. Anatomy of the genitalia, and sexual dimorphism

The genitalia are similar to those reported for other parastacids (Riek 1969, 1972, Suter 1977).

The male genitalia are a pair of long, complex, non-calcified papillae situated on the ventral surfaces of the coxae of the fifth pereiopods.

In the female, the genital apertures are a pair of oval openings, each surrounded by a ring of short hairs, on the ventral surfaces of the coxae of the third pereiopods. These apertures are usually closed by a non-calcified membrane.

In other respects the sexes are similar, except that the male is usually larger, the great chelae of the male are somewhat bigger, and the abdomen of the female is somewhat broader.

### 3.3.2. Anatomy and histology of the gonads

#### The ovaries. (a) Anatomy.

The ovaries are paired organs which lie above the digestive gland and over the pyloric chamber of the proventriculus. They are connected by a commissure slightly anterior to the level of the third pereicpod, and are closely applied to one another for most of their length except for a short section, immediately posterior to the commissure, at the level of the third pereiopod. (Figure 3.1).

The anterior lobe of each ovary is produced into a tubular horn which passes forward, lateral to the proventriculus, and terminates in a bulb lying on the antero-lateral surface of the cardiac proventriculus. The posterior lobe of each ovary extends backwards to the level of the fourth or fifth pereiopod.

In immature and early maturation stages, oocytes are found in the main body of the ovary and usually in the anterior bulbs, but the

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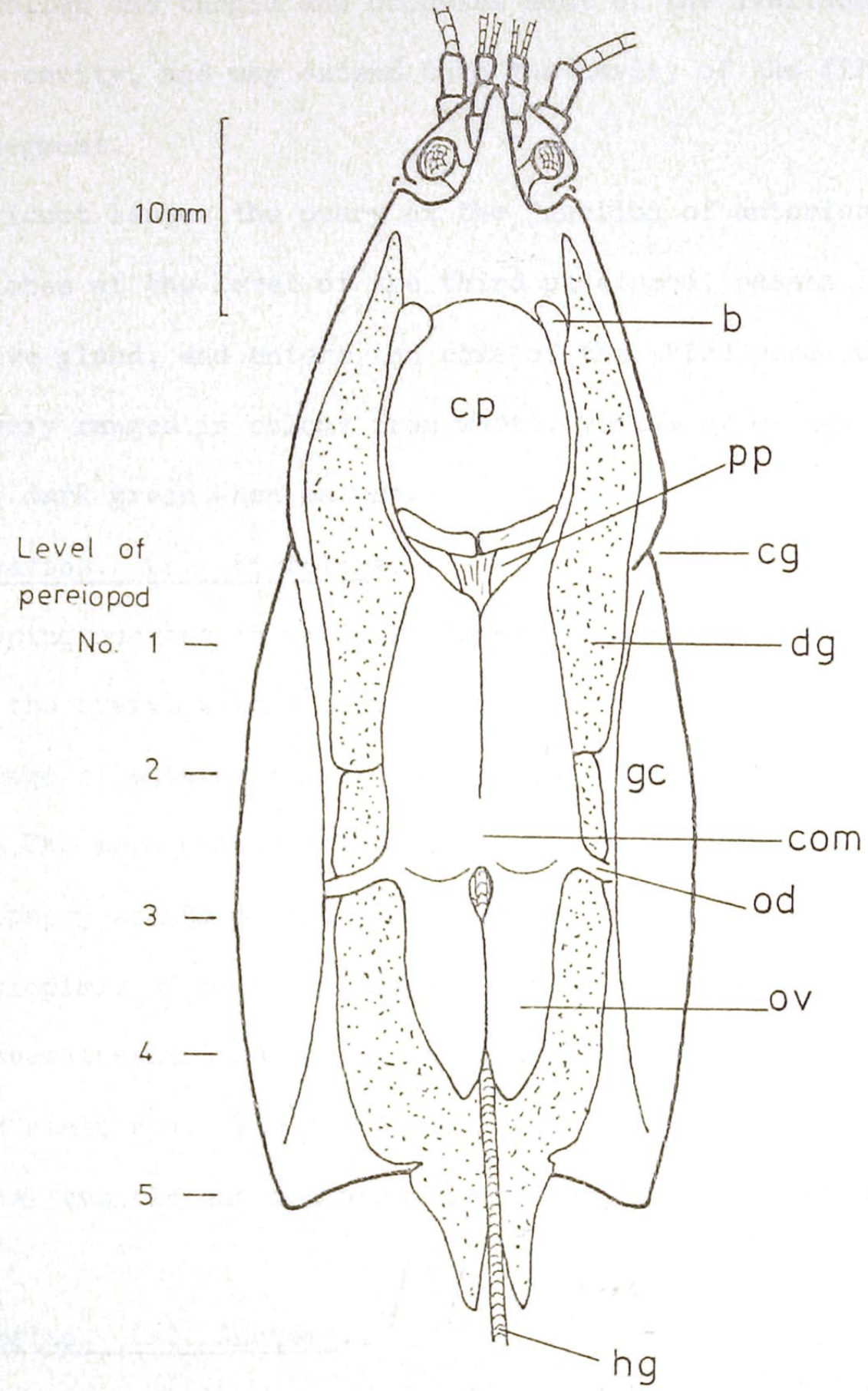



Figure 3.1. Semi-diagrammatic view of the ovaries and their associations in C. destructor. Only the cephalothorax is shown.

b, anterior bulb of ovary; c.g, cervical groove; com, commissure of ovary; c.p, cardiac proventriculus; d.g, digestive gland; g.c, gill chamber; hg, hindgut; od, oviduct; ov, ovary; p.p, pyloric proventriculus.

tubular part of the anterior horn is empty. As the oocytes mature, they fill the tubular part which expands to accommodate them. A mature ovary is swollen and turgid and occupies most of the available space in the body cavity, and may extend into the cavity of the first abdominal segment.

The oviduct leaves the ovary at the junction of anterior and posterior lobes at the level of the third pereiopod, passes lateral to the digestive gland, and enters the coxa of the third pereiopod.

The ovary ranges in colour from white, yellow or orange when immature to dark green when mature.

The ovaries. (b) Histology.

Developing oocytes in various stages of maturation are distributed throughout the ovary, with a particular stage predominating. Oocytes at the same stage of maturation are congregated in large groups throughout the ovary. The more mature stages of oocytes are generally found closer to the periphery of the ovary. Follicle cells surround each oocyte.

The cytoplasm of previtellogenic oocytes is basophilic and stains blue with haematoxylin and eosin. Oocytes containing yolk are acidophilic and stain red. The oocyte nucleus is enlarged in the usual way as a germinal vesicle and contains several nucleoli. The oocyte is elongated.

The testes. (a) Anatomy.

The testes, like the ovaries, are paired organs. They are closely applied to one another in the midline for most of their length, and lie above the digestive gland and behind the proventriculus. They are connected by a transverse commissure about one third of the way from the anterior end. The testes are much smaller than the ovaries and extend approximately from the level of the first to the third or fourth pereiopods (Figure 3.2).

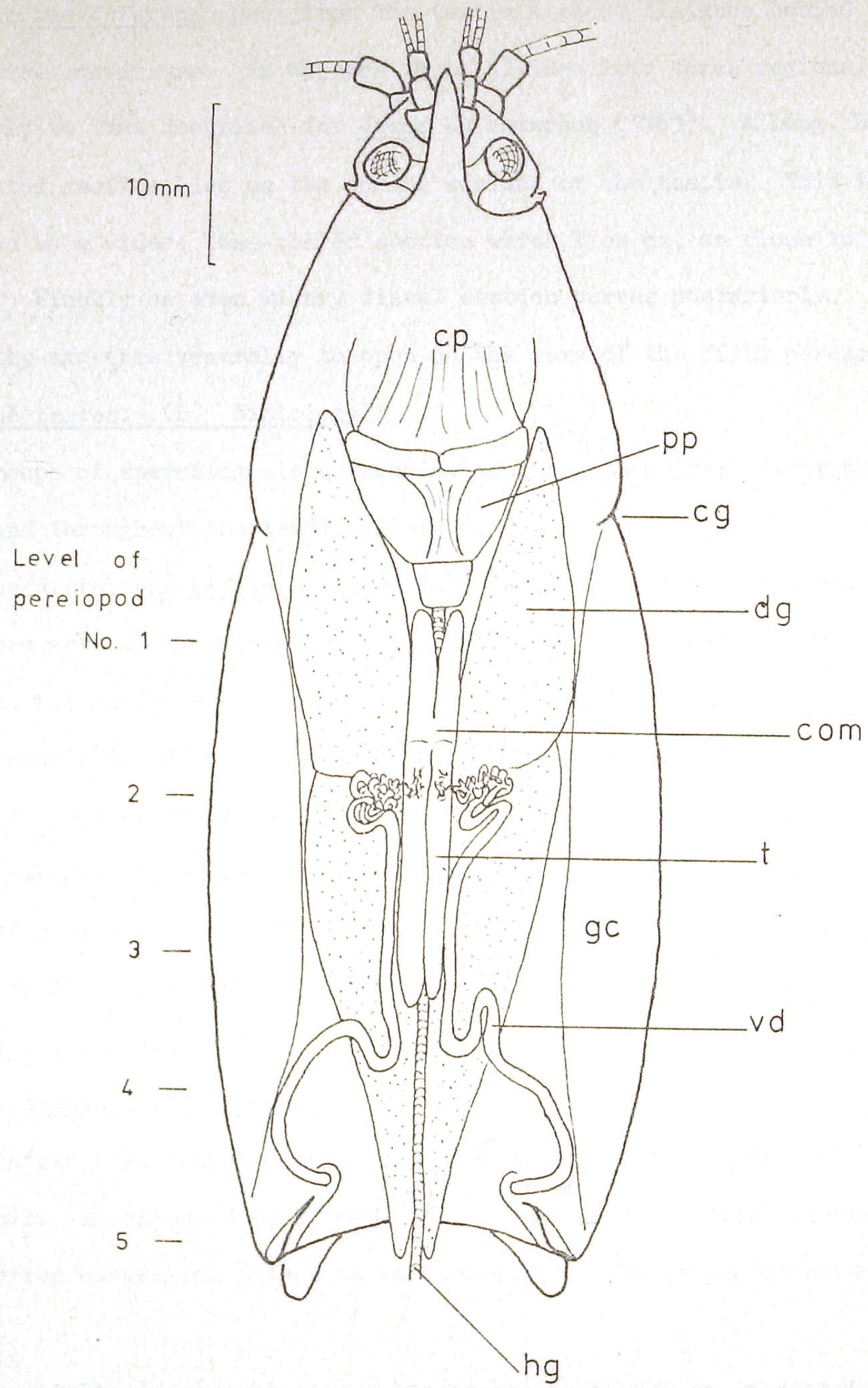



Figure 3.2. Semi-diagrammatic view of the testes and vasa deferentia and their associations in C. destructor.

c.g, cervical groove; com, commissure of testes; c.p, cardiac proventriculus; d.g, digestive gland; g.c, gill chamber; h.g, hindgut; p.p, pyloric proventriculus; t, testis; v.d, vas deferens.

The vas deferens opens from the testis a short distance behind the transverse commissure. It appears to be divided into three regions, similarly to that described for Jasus by Paterson (1969). A long, highly-convoluted section lies on the dorsal surface of the testis. This is followed by a wider, less-coiled section which lies on, or close to the testis. Finally an even wider, distal section curves posteriorly, laterally and then ventrally to open on the coxa of the fifth pereiopod.

The testes. (b) Histology.

Groups of spermatogonia undergoing synchronous meiotic divisions are found throughout the testis (Plate 3.1).

The distal vas deferens stores the spermatophoric material and therefore acts as a seminal vesicle. When empty it appears clear and flaccid, but in the breeding season it appears white and distended and it may double in width (to about 2 mm). The spermatophoric mass contained in the distal section consists of a tightly-coiled spermatophore embedded in an amorphous eosinophilic matrix (Plate 3.2). Upon dissection of the live animal, muscular contractions in the vas deferens often result in the partial expulsion of its contents (c.f. Jasus lalandii, Silberbauer 1971b).

### 3.3.3. Sequence of oogenesis

Histological criteria were used to distinguish five stages of oogenesis. Examples of each stage can be seen in the plates illustrating the ovarian maturation stages in Section 3.3.4. The stages of oogenesis are:

Previtellogenesis, stage 1. The oocyte is 50-130 µm in diameter with a nucleus of 15-40 µm diameter. The cytoplasm is basophilic (see Plate 3.4).

Previtellogenesis, stage 2. The oocyte diameter is 130-400 µm and the nuclear diameter 40-80 µm. The cytoplasm remains basophilic which

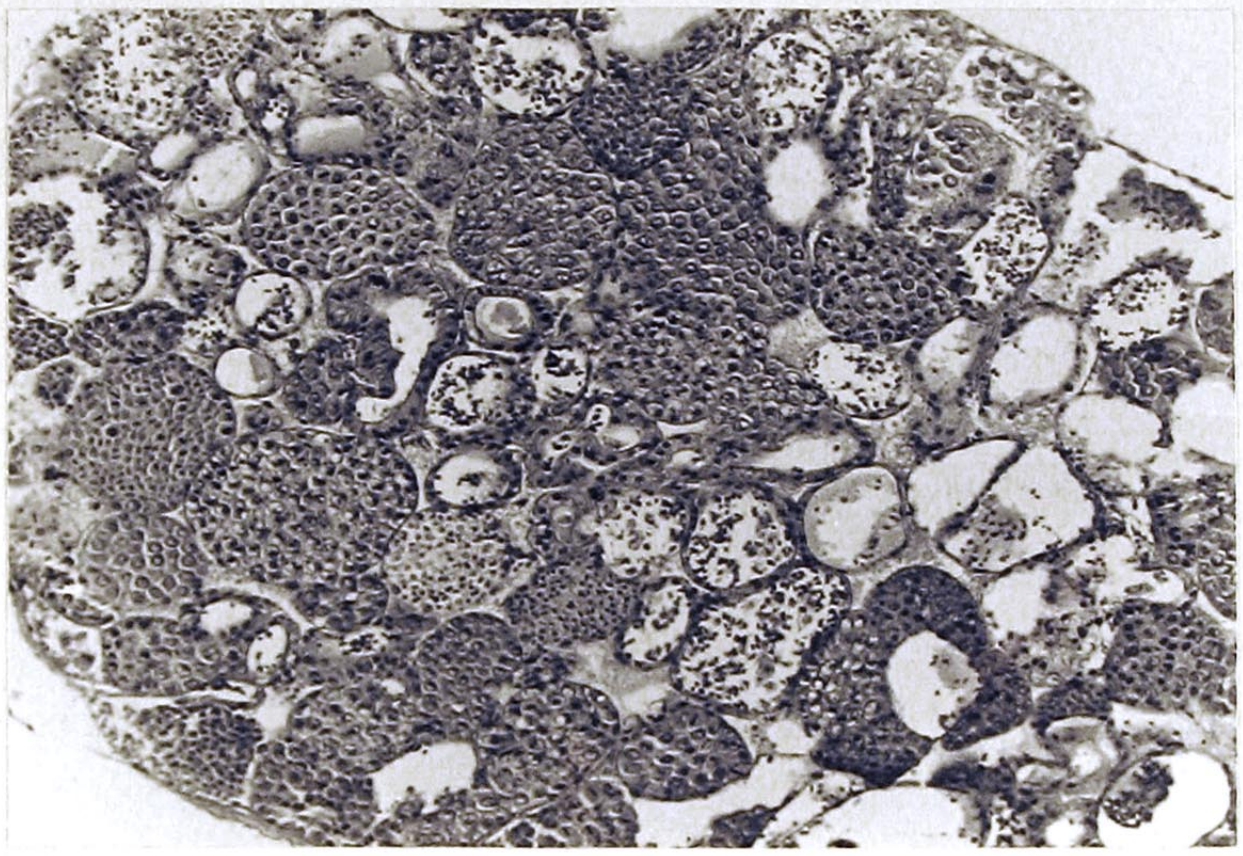



Plate 3.1. Transverse section of the testis of C. destructor, showing groups of spermatogonia undergoing synchronous meiotic division. Delafield's haematoxylin and eosin.

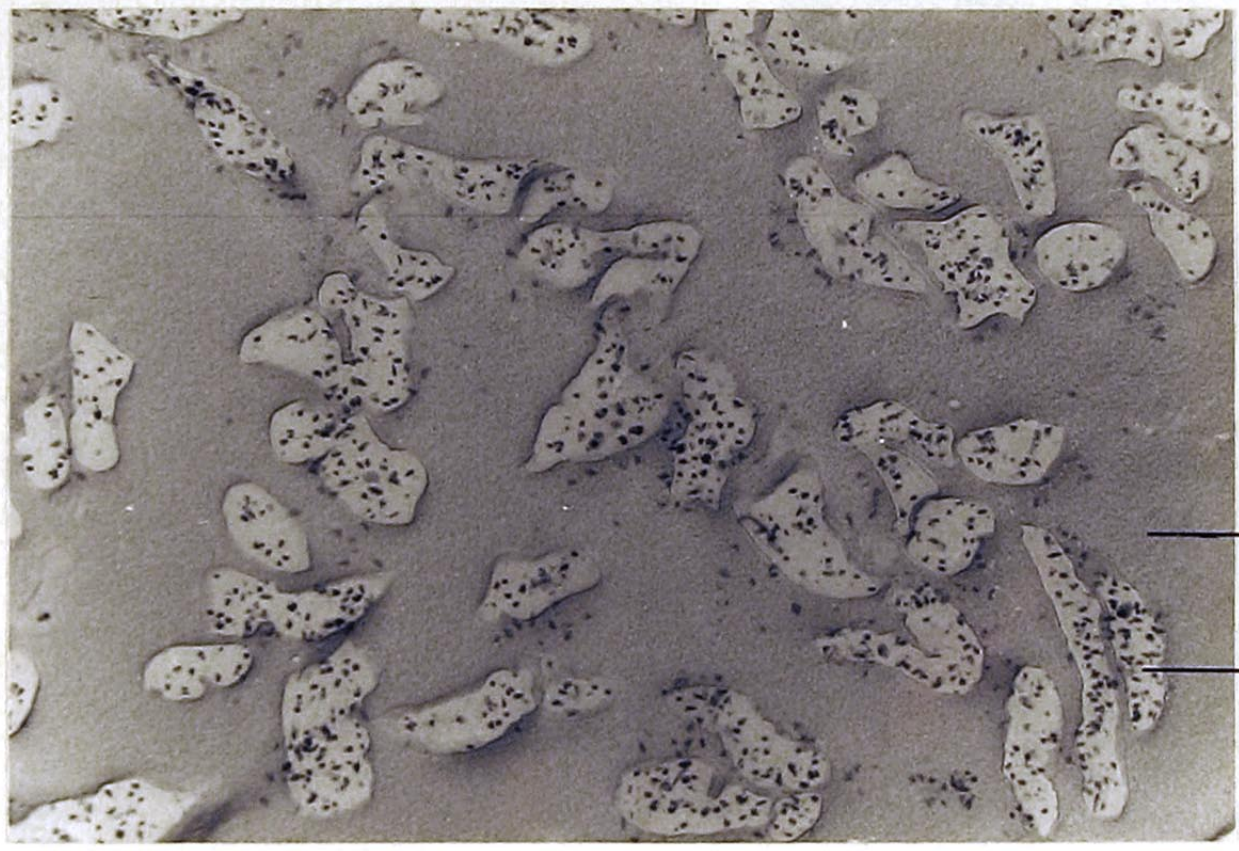
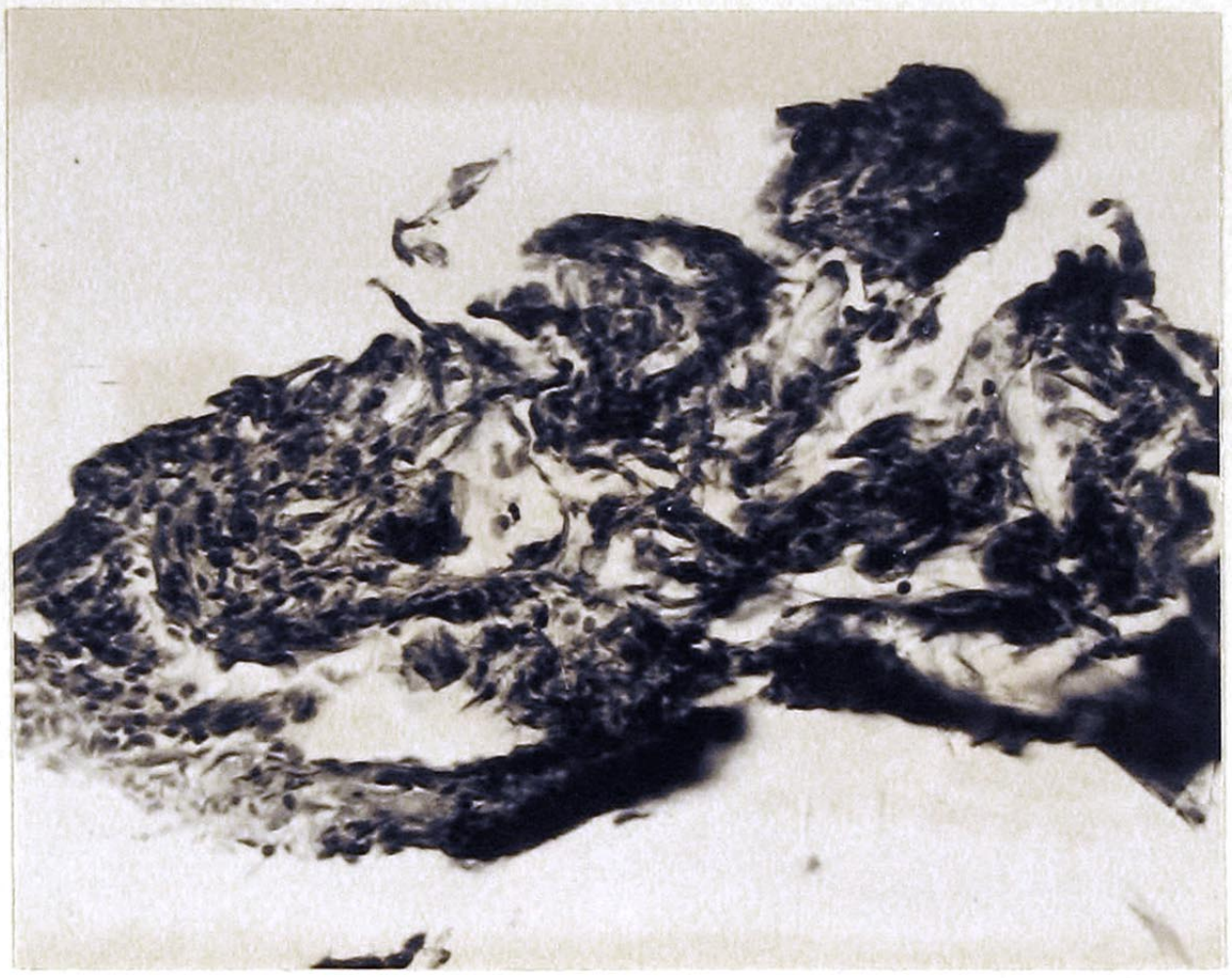



Plate 3.2. Transverse section of the distal part of the vas deferens of C. destructor, illustrating the coiled spermatophore (s) embedded in an amorphous matrix (m). Delafield's haematoxylin and eosin.



100 µm

Plate 3.3. Section through a stage I ovary of C. destructor. No oocytes are present. Delafield's haematoxylin and eosin.

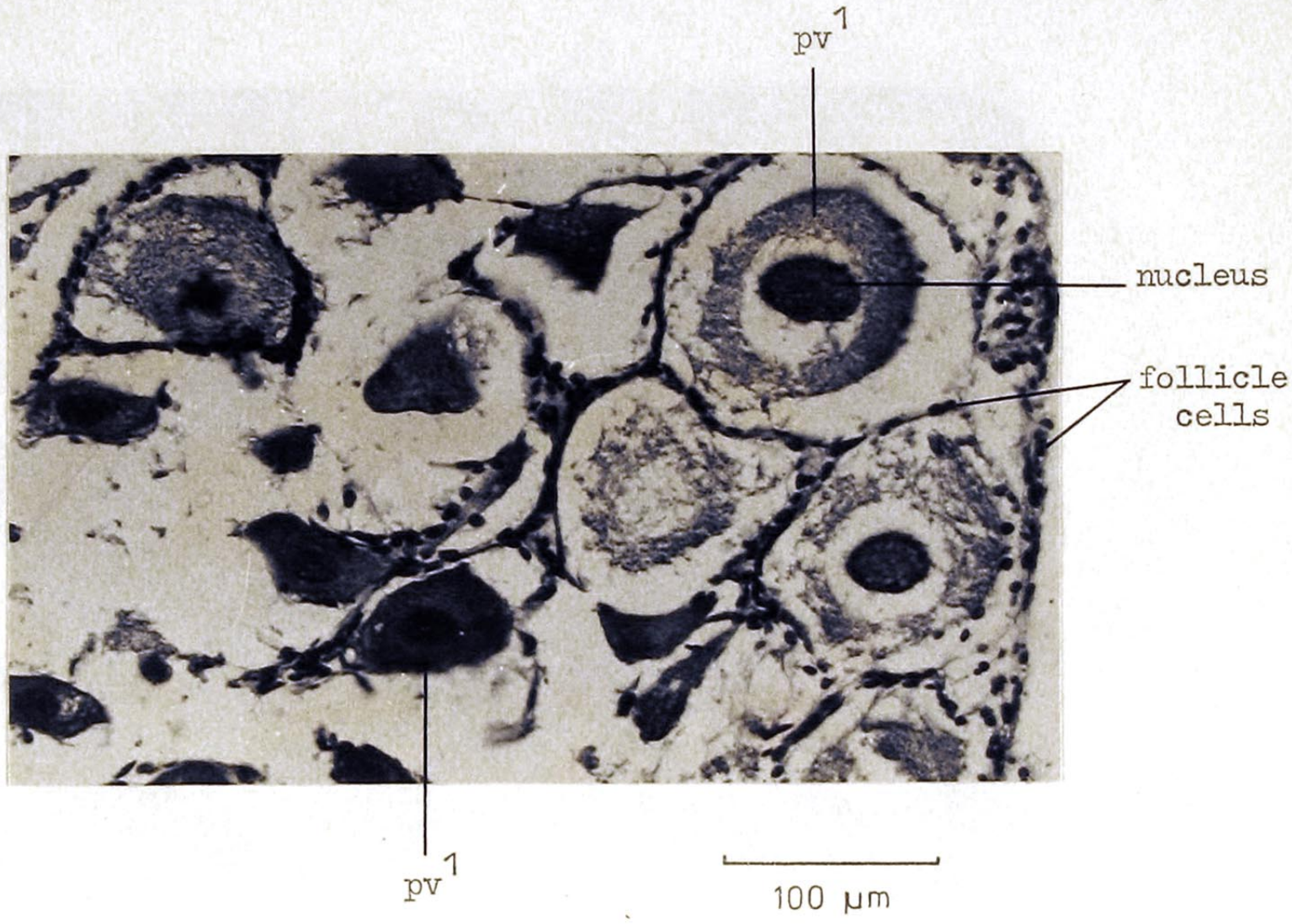



Plate 3.4. Section through a stage II ovary of C. destructor, showing oocytes in the first stage of previtellogenesis ($pv^1$). Delafield's haematoxylin and eosin.

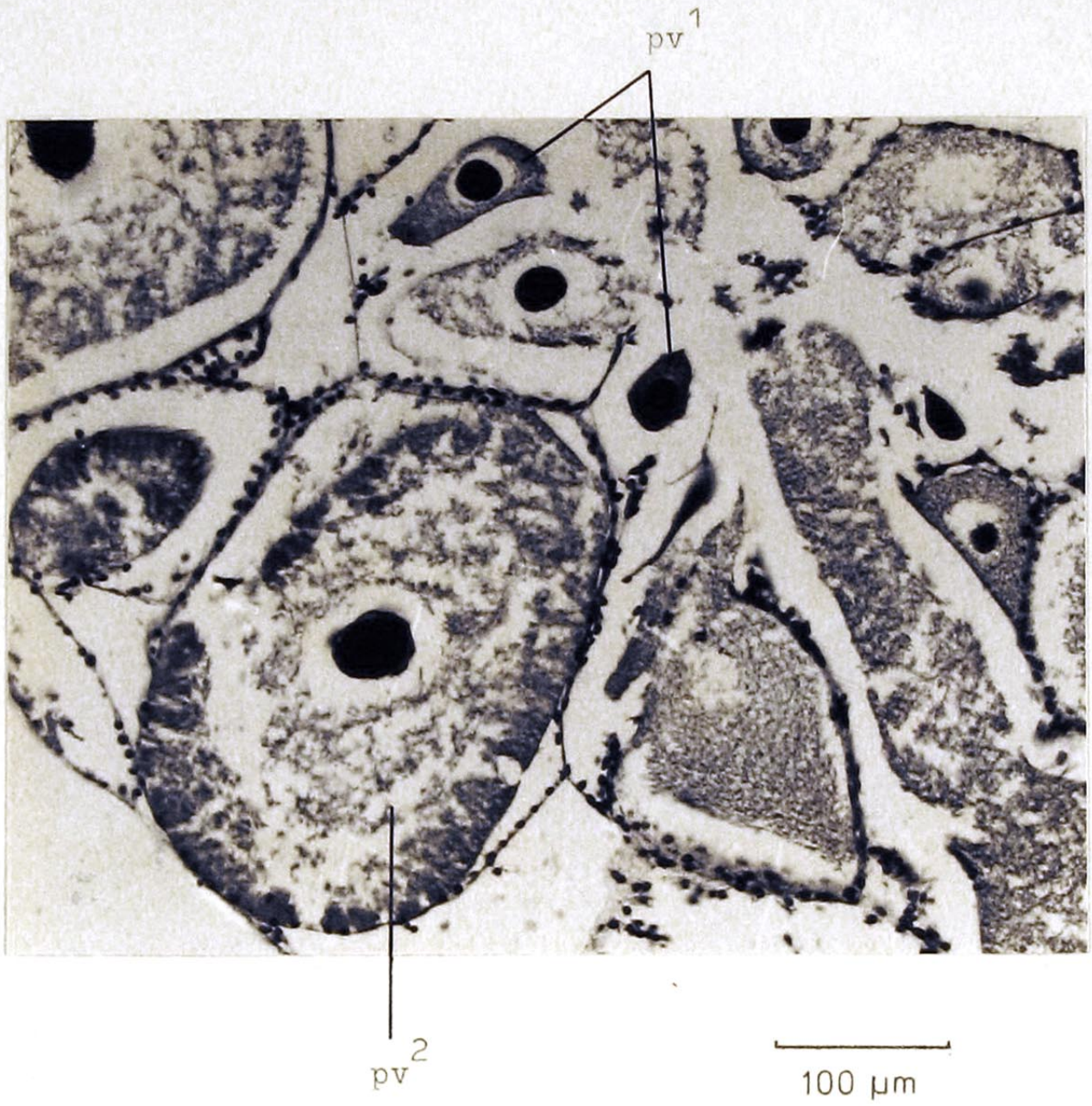



Plate 3.5. Transverse section of a stage III ovary of C. destructor, showing oocytes in the first ($pv^1$) and second ($pv^2$) stages of previtellogenesis. Delafield's haematoxylin and eosin.

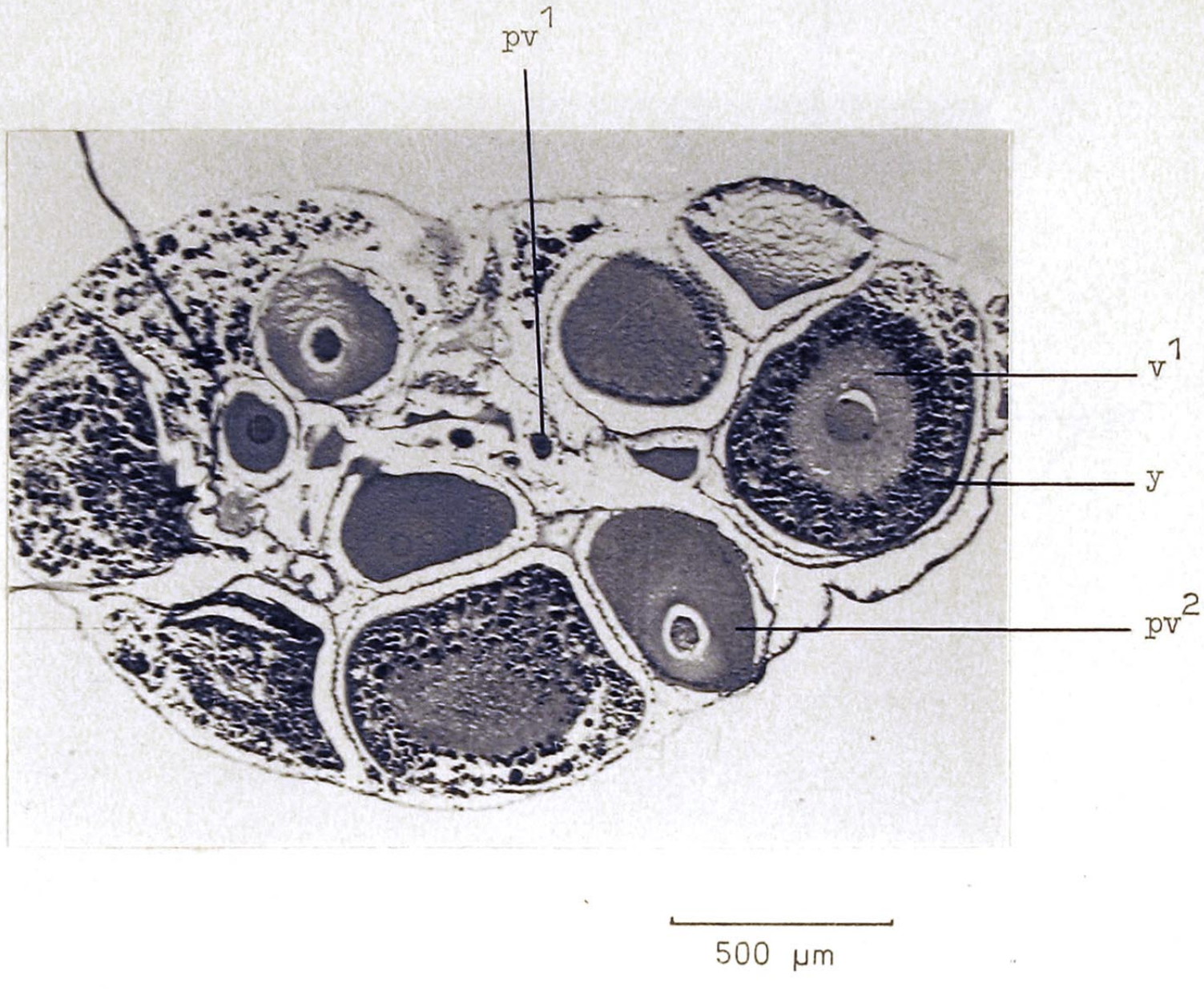



Plate 3.6. Transverse section of a stage IV ovary of C. destructor, showing oocytes in the first stage of vitellogenesis ($v^1$), with yolk (y) forming in the periphery of the cytoplasm. Oocytes in the first ($pv^1$) and second ($pv^2$) stages of previtellogenesis are also present. Delafield's haematoxylin and eosin.

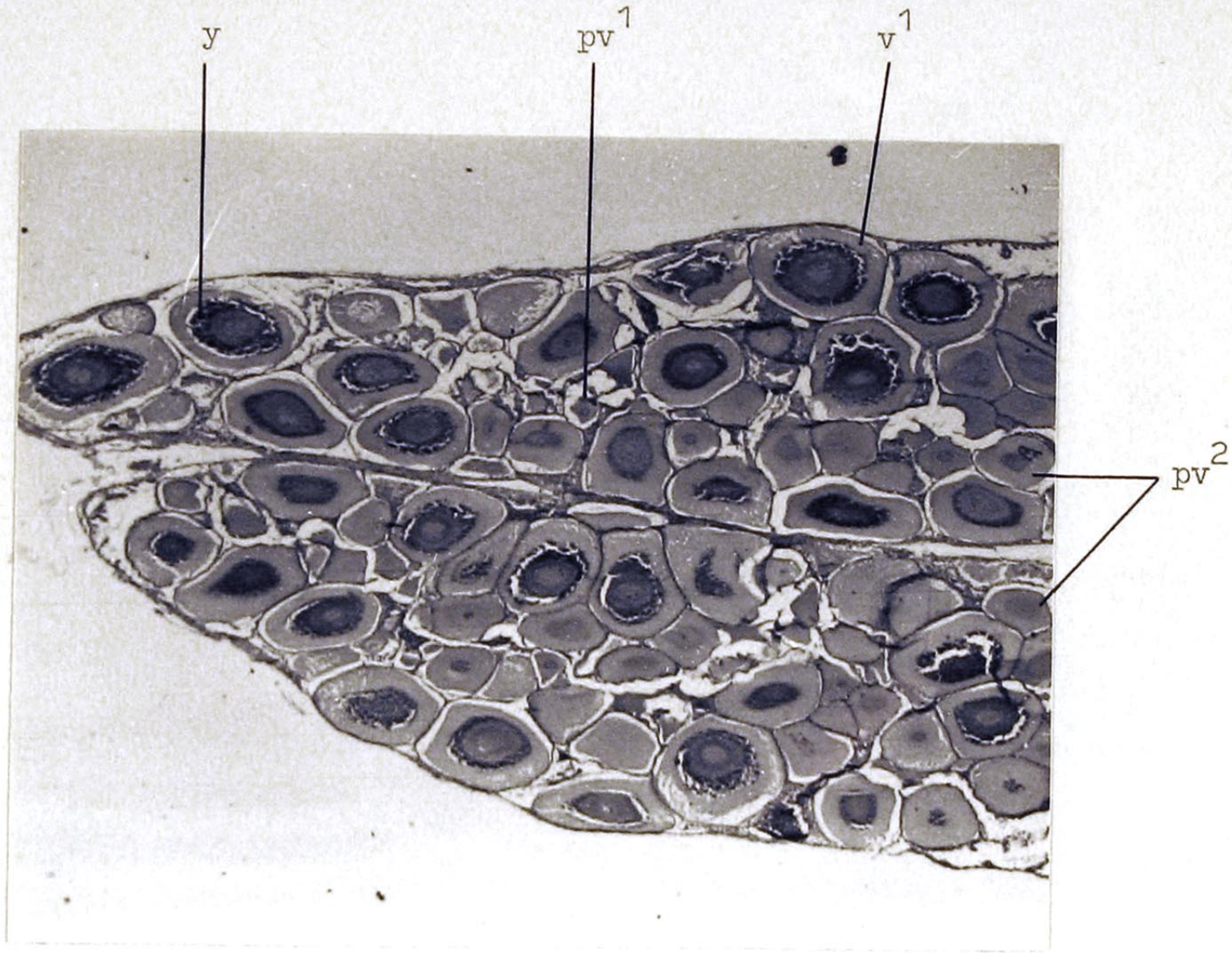



Plate 3.7. Longitudinal section of a stage IV ovary of C. destructor, showing oocytes in the first stage of vitellogenesis ($v^1$), with yolk (y) forming in the perinuclear position. First ($pv^1$) and second ($pv^2$) stage previtellogenic oocytes are present. Delafield's haematoxylin and eosin.

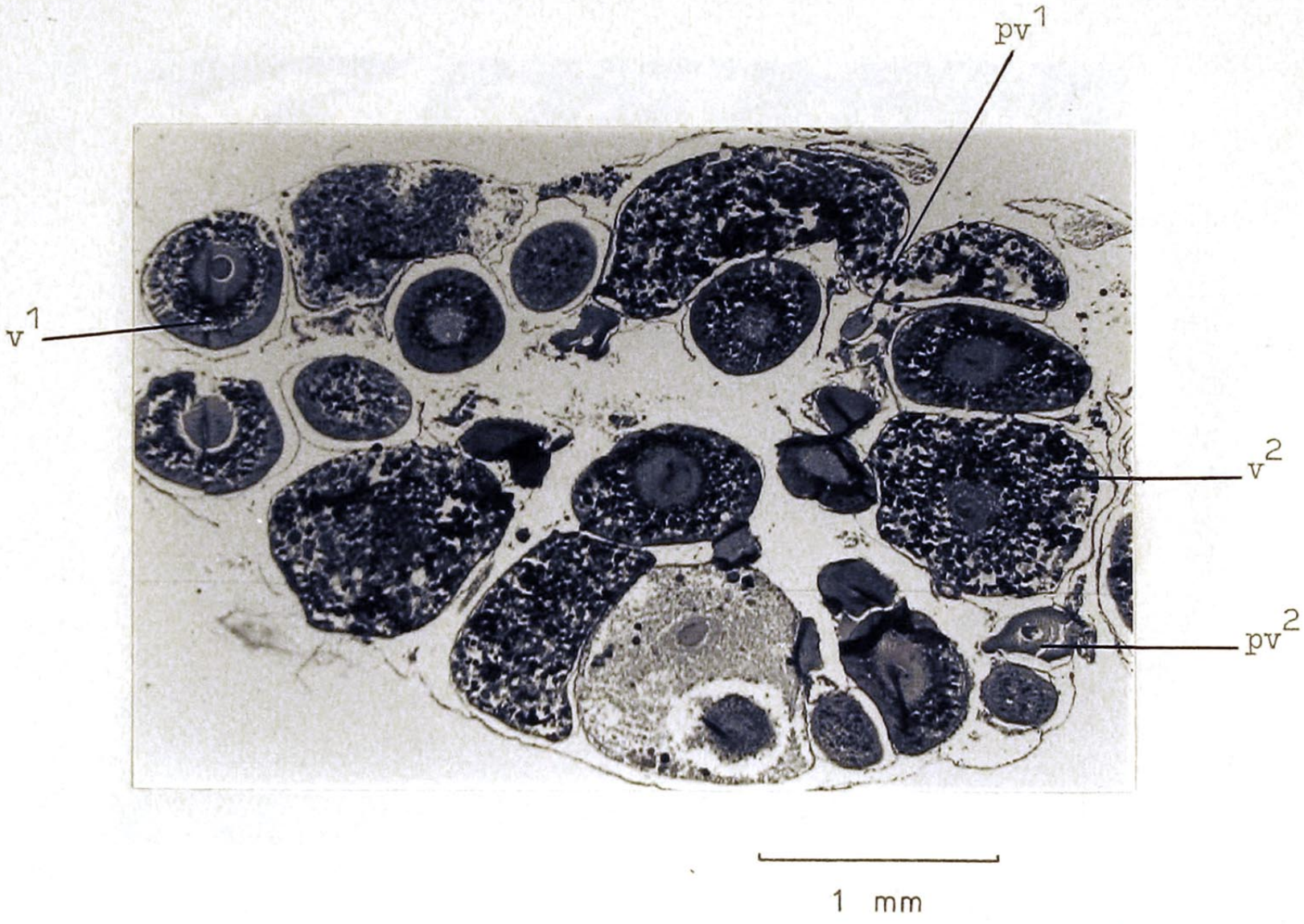



Plate 3.8. Transverse section of a stage V ovary of C. destructor, showing oocytes in the first ($v^1$) and second ($v^2$) stages of vitellogenesis. Previtellogenic oocytes ($pv^1$ and $pv^2$) are also present. Delafield's haemotoxylin and eosin.

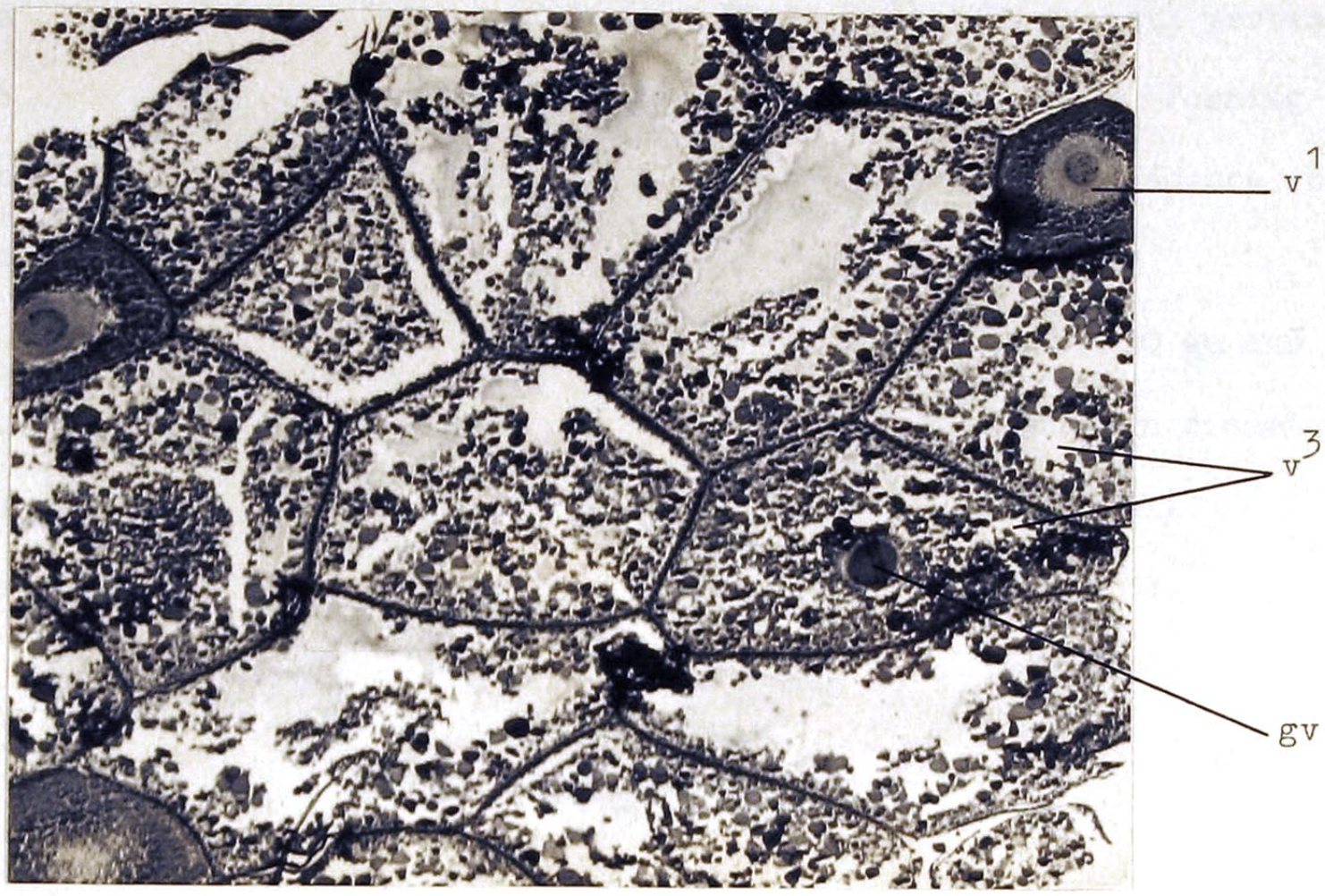



Plate 3.9. Transverse section of an early stage VI ovary of C. destructor, showing oocytes early in the third stage of vitellogenesis ($v^3$), which are packed with yolk platelets, but in which the germinal vesicles (gv) have not yet broken down. A few oocytes in early vitellogenesis ($v^1$) are present. Delafield's haematoxylin and eosin.

becomes acidophilic during this stage (see Plates 3.5 and 3.6).

Vitellogenesis, stage 1. The diameter of the oocyte is 400-750 µm and the diameter of the nucleus 80-110 µm. There is a layer of yolk platelets, 10-30 µm in diameter, in the now acidophilic cytoplasm, around the periphery of the oocyte (see Plate 3.6). In a small percentage of ovaries, the yolk platelet layer is seen instead to be forming closer to the nucleus (see Plate 3.7). Both layers are in evidence in the oocytes of some ovaries.

Vitellogenesis, stage 2. The oocyte diameter is 750-1000 µm and the nuclear diameter, 110-150 µm. Yolk platelets, 30-60 µm in diameter, are scattered throughout the acidophilic cytoplasm. Many small platelets of 5-10 µm diameter are also present (see Plate 3.8).

Vitellogenesis, stage 3. The oocyte is mature, 1100-1200 µm in diameter, and over 2000 µm in length. The cytoplasm is acidophilic and densely packed with yolk platelets 30-80 µm in diameter. The nucleus has broken down (oocytes in early stage 3 vitellogenesis, prior to nuclear breakdown, are seen in Plate 3.9).

### 3.3.4. Sequence of ovarian stages

Seven macroscopic stages are discernible in the maturing ovary. These stages are similar to those described for *Jasus* by Fielder (1964) and Heydorn (1969). Gonosomatic index (GSI) was shown to be a reliable index of ovarian maturation as it correlates significantly ($P < 0.05$) with oocyte length (major axis) and is independent of size of animal. The regression equation is: GSI = 2.04 x oocyte length (mm) - 0.42; $n = 12$; $r = 0.97$; range of GSI = 0.03 - 4.5. The range of GSI is shown for each stage. The stages are:

I. Immature virgin. The ovaries are very small and are difficult to demonstrate; they are almost translucent and no oocytes are discernible. Gonosomatic index is less than 0.03 (Plate 3.3).

II. Maturing virgin 1. The ovaries are larger, about the size of an adult testis; the colour is either white, off-white, yellow or orange; individual oocytes are discernible. Only oocytes in the first stage of previtellogenesis are present. Gonosomatic index is approximately 0.03 to 0.1 (Plate 3.4).

III. Maturing virgin 2. Swelling has progressed; the colouring is similar to that of stage II; oocytes may be 0.5 mm or more in length. The majority of oocytes are in the second stage of prèvitellogenesis, with a smaller number in the first previtellogenic stage. Gonosomatic index is approximately 0.1 to 0.4 (Plate 3.5).

IV. Maturing 1. The ovary is larger and has changed in colour to pale olive to bright green; oocytes may be up to 1 mm in length. Oocytes in the first stage of vitellogenesis are predominant, and some oocytes are in the second stage of previtellogenesis. An occasional oocyte in stage one previtellogenesis is present. Gonosomatic index is approximately 0.4 to 0.9 (Plates 3.6 and 3.7).

V. Maturing 2. The ovary has entered a stage of fast growth. It is much larger, is becoming turgid and has changed to a dark green colour, often with orange or yellow flecks. Oocytes are beginning to occupy the tubules of the anterior horns. The predominant oocytes are large (over 1 mm in length) and are in stage two of vitellogenesis, with a smaller number in stage one. A few oocytes are in the second stage of previtellogenesis, and an occasional oocyte in stage one is seen. Gonosomatic index is approximately 0.9 to 3.5 (Plate 3.8).

VI. Mature. Spawning follows almost immediately this stage is reached. The ovaries are grossly swollen and occupy all available space in the thoracic cavity and may protrude into the first abdominal segment. The colour is a deep dark-green. Individual oocytes may be more than 1.5 mm in length. The ovarian wall is fragile and easily

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ruptured. The anterior horns are full and ova are pushing into the proximal ends of the oviducts. The majority of oocytes are in stage three of vitellogenesis, with an occasional previtellogenic or early vitellogenic oocyte being present. Gonosomatic index is greater than 3.5 (with a maximum of around 5) (Plate 3.9).

VII. Spent/Regenerating. This stage is found only in females carrying eggs. The ovary is collapsed and flaccid and much reduced in size. A few residual oocytes (stage three vitellogenesis) are found in the oviducts, along the lateral edge of the ovary, and at the extremities of the lobes. The colour is translucent to dirty cream or grey, and previtellogenic oocytes are present. GSI is around 0.7. Following spawning, regeneration begins immediately and most oocytes are in vitellogenesis (ovarian stage IV) within one or two days (see Section 5.3.4). This stage (VII), therefore, is very short.

Regeneration now proceeds, and the ovary passes quickly into stage IV. However, when regeneration is fast, as in summer, gonosomatic indices remain a little higher, due to the persistence of the thickened ovarian walls, than in females not bearing eggs. At stage IV, for example, the gonosomatic index may be as high as 1.5. The difference disappears during stage V. Mature oocytes, and ovaries of the corresponding stage VI, are not found in egg-bearing females.

On the other hand, if stage VI is reached at the season's end, regeneration is slow and the gonosomatic indices in stages IV and V, which are those stages normally present in adults during winter, return to the more usual values, as the ovarian walls return to normal thickness.

3.3.5. Intersexuality

Intersexuality was found to occur in several percent of the crayfish of the study population. Most of the animals examined were found, upon dissection, to be intersexes either bearing gonads of only

one sex, or not possessing functional mechanisms for dealing with the gonad products.

The most common form of intersex encountered was that in which three gonopores were present. Usually these were either functional males, in which case the gonopore on the third pereiopod was blind; or functional females with a blind, aberrant gonopore on a fifth pereiopod. However, one instance was recorded in which a functional male had a small island of ovarian tissue (two or three yellow oocytes) amongst the testicular tissue. In another instance, a functional female was examined, having testicular tissue at the posterior end of the ovaries, and an apparently normal vas deferens leading from the testicular tissue to the gonopore on the fifth pereiopod. Both oviducts were present.

An otherwise normal female was found to possess four gonopores, the male ones being blind. In another instance, a true hermaphrodite was found, in which a normal testis lay on the right and a normal ovary lay on the left; male and female gonopores were present on the right side only, and a vas deferens and an oviduct connected them to testis and ovary respectively.

## 3.4. DISCUSSION

### 3.4.1. Anatomy of the genitalia and sexual dimorphism

The position, in C. destructor, of the male genital apertures on the last thoracic somite, and of the female genital apertures on the third last thoracic somite, is typical of decapods, and of the majority of eumalacostracans (Barnes 1974).

The long, complex, non-calcified genital papilla of the male C. destructor is distinctive, and is used as an identifying characteristic for the genus Cherax. The male genital papilla in most other parastacids is shorter and structurally less complex (Riek 1972).

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Although marked sexual dimorphism is common in crustacea, the similarity in general body form found between the sexes in C. destructor is typical of macrurans. Most parastacids exhibit the characteristics seen in C. destructor, i.e. the larger size, narrower abdomen, and large great chelae of the male (Riek 1951). Other macrurans, such as Jasus lalandii (von Bonde 1936) also have these characteristics. The male of Homarus americanus tends to have larger great chelae (Herrick 1895). Broader abdomens are found in the females of Astacus astacus and Pacifastacus leniusculus (Abrahamsson 1971). The broad abdomen of the female astacid was considered by Goeller (1943, cited by Abrahamsson 1971) to be an egg-carrying adaptation.

3.4.2. Anatomy and histology of the gonads.

The ovaries.

The basic structure of the ovaries of C. destructor, that of paired, closely approximated organs connected by a single commissure, and the position in the body cavity of these organs, is similar to that described for other reptantian decapod crustaceans, including the parastacid crayfish, C. tenuimanus (Morrissy 1970), the spiny lobsters, Jasus lalandii (von Bonde 1936, Silberbauer 1971b), J. novaehollandiae (Fielder 1964 - at the time, this species was known as J. lalandei), and Panulirus homarus (Berry 1971), and the crab, Portunus sanguinolentus (Ryan 1967). The ovary of the northern hemisphere freshwater crayfish, Astacus fluviatilis, has a trilobed appearance (Huxley 1877, 1880).

Histologically the structure of the ovary of C. destructor appears to follow the general decapod pattern. The predominance, in a given ovary, of a particular oocyte stage has also been recorded for the North American freshwater crayfishes, Cambarus sp., Orconectes sp., and Procambarus sp. (Beams and Kessel 1963). The distribution of ova of different stages in all regions of the ovary is homogeneous, as is seen

in other decapods, including Parapenaeopsis stylifera (Shaikmahmud and Tembe 1961), Penaeus duorarum (Cummings 1961), J. novaehollandiae (Fielder 1964) and Portunus sanguinolentus (Ryan 1967). The peripheral location of the larger oocytes in the ovary has also been described for J. novaehollandiae (Fielder 1964) and for the Atlantic lobster, Homarus (Kessel 1968).

The presence of follicle cells around the oocyte has been recorded for other decapods (Huxley 1880, Shaikmahmud and Tembe 1961, Beams and Kessel 1963, Fielder 1964, Ryan 1967, Heydorn 1969).

The location in the ovary of the germinal epithelium was not established for C. destructor but in other respects the histological structure of the ovary is similar to that described for J. novaehollandiae (Fielder 1964), and P. sanguinolentus (Ryan 1967).

The testes.

To the extent to which they were examined, the testes and the vasa deferentia of C. destructor appear to be similar both anatomically and histologically to those of the palinurid lobster, J. lalandii, as described by von Bonde (1936), Paterson (1968), Berry and Heydorn (1970).

The spermatophore is like that of J. lalandii in that it is convoluted randomly throughout the matrix and not localised ventrally in the vas deferens as in Palinurus spp. (Berry and Heydorn 1970).

### 3.4.3. Sequence of oogenesis

Five stages of oogenesis, including two previtellogenic stages, are distinguished for C. destructor. Germinative stages are not described. Oocytes are seen to develop in the usual way by increase in cell diameter and enlargement of the nucleus to form a germinal vesicle.

The diameter of 50-130 µm of the stage 1 previtellogenic oocyte is large for a young oocyte which has recently emerged from the germinal zone. The young oocyte of Homarus has a diameter of less than 15 µm

(Kessel 1968). The smallest oocyte described by Fielder (1964), for Jasus novaehollandiae, has a diameter of about 100 µm. The previtellogenic oocytes of C. destructor have the usual basophilic, homogeneous-appearing cytoplasm, which becomes acidophilic prior to vitellogenesis.

By the time yolk begins forming in the cytoplasm, the diameter of the oocyte of C. destructor is approximately 400 µm, whereas the oocyte of J. novaehollandiae is around 150 µm (Fielder 1964), and that of Homarus only 10-15 µm (Kessel 1968). The initial occurrence of yolk platelets in a layer in the periphery of the oocyte, seen in C. destructor, has also been recorded for Cambarus sp., Orconectes sp., and Procambarus sp. (Beams and Kessel 1963) and Homarus (Kessel 1968). The deposition of yolk in a perinuclear layer, observed in some ovaries of C. destructor, occurs in a number of other crustaceans (Woods 1969).

The mature oocyte of C. destructor is about 1.2 mm in diameter and more than 2 mm in length. The mature oocyte of Astacus fluviatilis (Huxley 1880) is of a similar size, but that of the spiny lobster J. novaehollandiae is only 500 µm in diameter (Fielder 1964). According to Morrissy (1975) the oocyte of the Western Australian crayfish, Cherax tenuimanus, can reach a length of 4.5 mm.

The yolk platelets, up to 80 µm in diameter, present in the mature oocyte of C. destructor are much larger than those of Cambarus sp., Orconectes sp. and Procambarus sp. described as "several microns in diameter" (Beams and Kessel 1963).

3.4.4. Sequence of ovarian stages

Seven macroscopic stages are identified in C. destructor, these stages being similar to those described for Jasus novaehollandiae by Fielder (1964) and J. lalandii by Heydorn (1969).

Morrissy (1975) described three ovarian stages for Cherax tenuimanus.

The first stage, which he called Type I, is immature, with no discernible oocytes, and is equivalent to stage I of C. destructor. The Type II ovary of C. tenuimanus contains oocytes similar in description to the second stage previtellogenic oocytes of C. destructor, and is thus equivalent to the stage III ovary of C. destructor. The Type III ovary of C. tenuimanus contains mainly vitellogenic oocytes and is equivalent to stages IV, V and VI of C. destructor. The Type III ovary of C. tenuimanus also contains previtellogenic oocytes, as do stages IV to VI of C. destructor. Mature oocytes of C. tenuimanus may be up to 4.5 mm in length, i.e. two to three times the length of those of C. destructor. The yolk in C. tenuimanus is grey-black (Morrissy 1975) rather than green as in C. destructor.

At each maturation stage of C. destructor most of the oocytes are of approximately the same size and stage of oogenesis, but there are always earlier oocytes in evidence, even in the mature ovary. This was also observed in Cambarus sp., Orconectes sp. and Procambarus sp. by Beams and Kessel (1963).

Regeneration of the ovary of C. destructor begins immediately after spawning and yolk deposition in oocytes is seen within two or three days. The same phenomenon is seen in other decapods such as the spiny lobster J. lalandii (von Bonde 1936, Heydorn 1969) and the freshwater prawn Macrobrachium rosenbergii (Raman 1967).

Gonosomatic index, commonly used in fisheries research, was shown to be a reliable index of ovarian maturation in C. destructor, as it correlates significantly with oocyte length, and is independent of size of animal.

### 3.4.5. Intersexuality

Intersexuality occurs in many crustaceans. It is not uncommon amongst parastacids and has been recorded for the genera Cherax,

Euastacus and Parastacoides (Riek, 1951, 1956, 1967, 1969, 1972, Woodland 1967). Woodland (1967) studied a farm dam population of Cherax davisi (previously identified as C. albidus; see Section 1) where he found that up to 25 percent of the animals were intersexes, but many of these had no more than a depression in the exoskeleton in the gonopore position. This depression was not observed in any specimens of C. destructor examined by the author.

Riek (1969) considered the possibility of C. davisi being a subspecies of C. destructor. If this is so, comparisons become more meaningful.

The varieties of intersexes for parastacid species described by Riek (1951, 1956, 1967) are similar to those found in C. destructor as described in this study. However, the relatively common occurrence, in the author's experience, of C. destructor males with three gonopores, is at variance with the rarity (one animal) ascribed to this abnormality amongst parastacids by Riek (1951, 1972).

Interestingly, Riek (1951) recorded a solitary case of a true gynandromorph, a specimen of Cherax punctatus, occurring in a collection of several thousand of this species. Gynandromorphism is very rare and only a few cases have been described amongst the crustaceans (Charniaux-Cotton 1960).

It appears that, in Cherax, specific intersexual traits may occur in isolated populations (the examples described by Riek, Woodland and the author are from populations geographically isolated from one another). Such intersexual characteristics are likely to be hereditary. The genetic nature of some types of intersexuality in certain isopod and amphipod species has been demonstrated by crossbreeding (Charniaux-Cotton 1960).

# 4. REPRODUCTION AND DEVELOPMENT

## 4.1. INTRODUCTION

Little is known about reproduction and development in Cherax destructor. Copulation and spermatophore transfer have not been described for any parastacid crayfish, but evidence of spermatophoric mass deposition has been recorded for C. tenuimanus (Shipway 1951).

Spawning and fertilization have not been examined previously in parastacids, but the mode of egg attachment has been described for a number of parastacid species (Clark 1937, Hopkins 1967, Woodland 1967, Suter 1977).

Shipway (1951), Woodland (1967) and Suter (1977) have described the eggs in several parastacid species, but development of a parastacid embryo has not been described previously.

Juvenile stages of parastacids have been less neglected. The first description of a parastacid juvenile was by Wood-Mason in 1876 (cited by Huxley 1880) of the New Zealand crayfish Paranephrops. The juvenile stages of C. destructor were incompletely described by Hale in 1925. Juveniles of other parastacids have been described, in more or less detail, by Clark (1937), Shipway (1951), Hopkins (1967), Woodland (1967), Lake and Newcombe (1975), and Suter (1977).

This section of the study examines reproduction in C. destructor, particularly the process of spawning, and embryonic and juvenile development.

## 4.2. MATERIALS AND METHODS

Specimens of C. destructor used in reproduction studies were collected from waters other than the study dam, as described in Section 2.2.2.

4.2.1. Spermatophore transfer

Spermatophores were observed attached to female crayfish from the study dam as well as from other water bodies. They were also observed attached to captive animals in aquaria.

4.2.2. Spawning, fertilization and egg attachment

These processes were observed in only one individual, a captive female maintained in an aquarium at a constant water temperature of 20-21°C. Spawning of infertile eggs was recorded for several other captive females. Terms used to describe setae are according to Roberts (1968).

4.2.3. Egg and embryonic development

Twenty-two berried females were captured during the 1975/76 breeding season and held in the laboratory until incubation was complete and the juveniles had escaped from the parent. The berried females were held in individual baskets to avoid loss of eggs due to aggressive encounters. The baskets were placed in a fish-hatchery trough where the animals were provided with a continuous flow of clean, oxygenated water at ambient summer water temperatures (20-25°C). Temperature was monitored continuously by means of a recording chart thermometer which was regularly calibrated with a standard thermometer.

Eggs and juveniles were examined daily and their external appearance recorded. During the period of incubation, a sample of three or four eggs was removed, approximately daily, from each of four particular berried females; and less frequently from another nine berried females.

Eggs with a high yolk content (i.e. those which had been incubating less than 14 days and which contained early embryos) were pricked, to ensure thorough penetration of the fixative, and placed in Smith fixative for 24 hours. The eggs were then washed overnight and transferred to buffered formalin. Eggs containing late embryos were

fixed in Alcoholic Bouin solution for a few days before transferring to 70 percent alcohol.

The embryo was located on the surface of the egg using a binocular dissecting microscope. The egg-membranes were teased away and the unstained embryo examined in situ. Use of high-power, incident-light sources, placed at suitable angles, allowed embryonic features to be more easily distinguished by the method of shadow-relief. A camera lucida was used for most of the drawings. Measurements were made using an ocular micrometer.

4.2.4. Hatching and juvenile stages

As described in Section 4.2.3., daily examination of the juveniles continued after the eggs had hatched. Except for gross anatomy and measurement of carapace length, the characteristics of each stage were described from live specimens. This was found to be preferable to examination of preserved specimens, because certain characteristics of the live juvenile, such as pigmentation of the cuticle, are lost during the process of fixation.

A small sample of juveniles was removed from five adult females, approximately daily, and less frequently from another nine females. The juveniles were fixed and preserved in buffered formalin and were examined, unstained, under a binocular dissecting microscope. The drawings were done with the aid of a camera lucida. Measurements were made using an ocular micrometer. Carapace length was measured as described in Section 2.4.2.1 and recorded to the nearest tenth of a millimetre. Carapace lengths given in the results are mean values of measurements of about 20 individuals.

## 4.3. RESULTS

### 4.3.1. Spermatophore transfer

Mating was not observed, despite numerous attempts to do so. It was seen, however, that the female mates when in intermoult or late post-moult, and does not moult prior to mating as is the case with many decapods.

The spermatophore is a white, semi-translucent, gelatinous mass which is extruded through the male genital papillae and deposited onto the sternal keel of the female, approximately in the area bounded by the coxae of the third and fourth pereiopods (Plate 4.1). On extrusion, the spermatophore is sticky, enabling adhesion to the exoskeleton of the female. It remains in place for a day or two, when it either falls off or is removed.

### 4.3.2. Spawning, fertilization and egg attachment

The complete process of spawning was observed in only one individual. Spawning began soon after spermatophore deposition. The female slowly manoeuvred her body until she was lying on one side, supported by the legs and the carapace (Plate 4.1). She then flexed the abdomen until the uropods reached the gonopores on the third pereiopods and, at this stage, began extruding ova from the gonopores. The olive-green ova, guided by the uropods, passed over the spermatophore (when, presumably, fertilization occurred) and into the cup formed by the abdomen. (The abdominal pleura overlapped one another to complete the formation of a secure repository for the newly-fertilized eggs). The female alternated between lying on the right and the left sides during the spawning process. A number of times, throughout the process, spasms of the whole body occurred, and the antennules and various pereiopods were occasionally seen to twitch. On two occasions the female rose and walked about for a few minutes before resuming the spawning position.

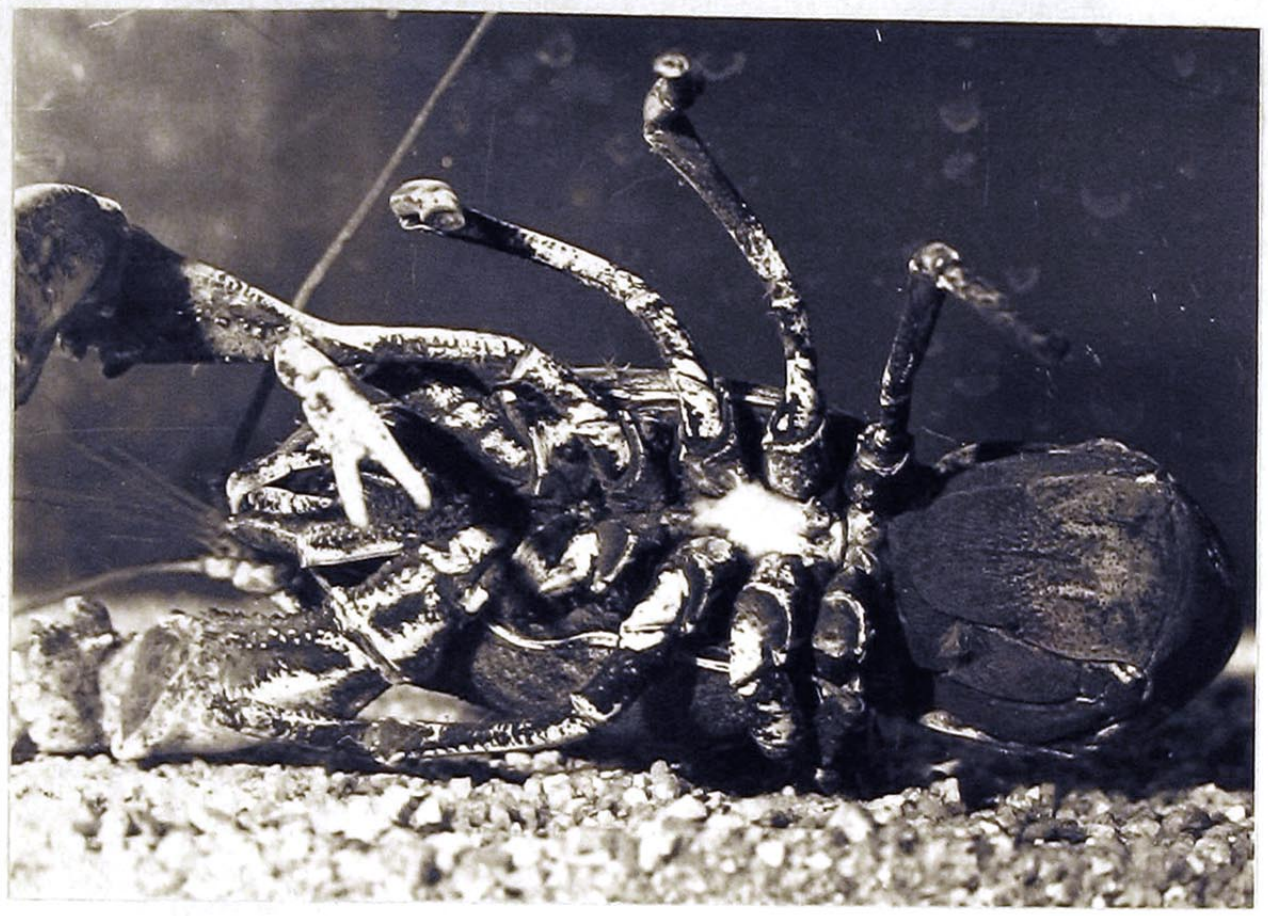

Plate 4.1. A female C. destructor in the spawning position, lying on the side, supported by the pereiopods. Note the tightly-flexed abdomen, and the white spermatophore adhering to the thoracic sterna between the third and fourth pereicpods. (Shown actual size).

Plate 4.2. Ventral view of the abdomen of a berried C. destructor female, showing the eggs attached to the pleopods. The exopodites of the pleopods are not encumbered with eggs, and can be seen lying on the egg-clumps. (Shown twice actual size).

The time taken for spawning was approximately three-quarters of an hour and, on completion, the eggs were seen to be floating in what appeared to be a sac of clear, viscous liquid in the tightly-cupped abdomen. The abdomen was forcibly extended two hours after completion of spawning, when the visible eggs were seen to be still unattached and floating free in the liquid. The abdomen was again forcibly extended about two and a half hours later, when all the eggs were seen to be attached to the pleopods by individual stalks, or funiculi (Plate 4.2). Close examination revealed each egg to be completely enclosed in a thin, clear membrane twisted at one end to form a stalk 1 to 2 mm long which was attached to the pleopod (Figure 4.1).

Examination of a number of berried females showed that most eggs are attached to the setae of the endopodite, while the exopodite remains fairly free of eggs (Figure 4.2). Eggs are attached to the proximal, lateral edge of the exopodite and also to the mesial surface of the basipodite.

In the female C. destructor, pinnate setae are found on both exopodite and endopodite. The endopodite, in addition, bears groups of 10-15 fine, filamentous setae between the pinnate setae. Filamentous setae also occur between the pinnate setae on the proximal, lateral edge of the exopodite, and in a fringe on the mesial surface of the basipodite. During spawning, the filamentous setae become twisted into matted clumps, 10-15 setae to each clump, to which individual egg-stalks are attached. Pinnate setae are not involved in egg attachment.

The arrangement of pleopodal setae is similar in the male C. destructor, except that only 2-8 filamentous setae are found between the pinnate setae on the endopodite.

Examination of several berried females showed that newly-spawned eggs are very soft, and it was frequently seen that they would die if

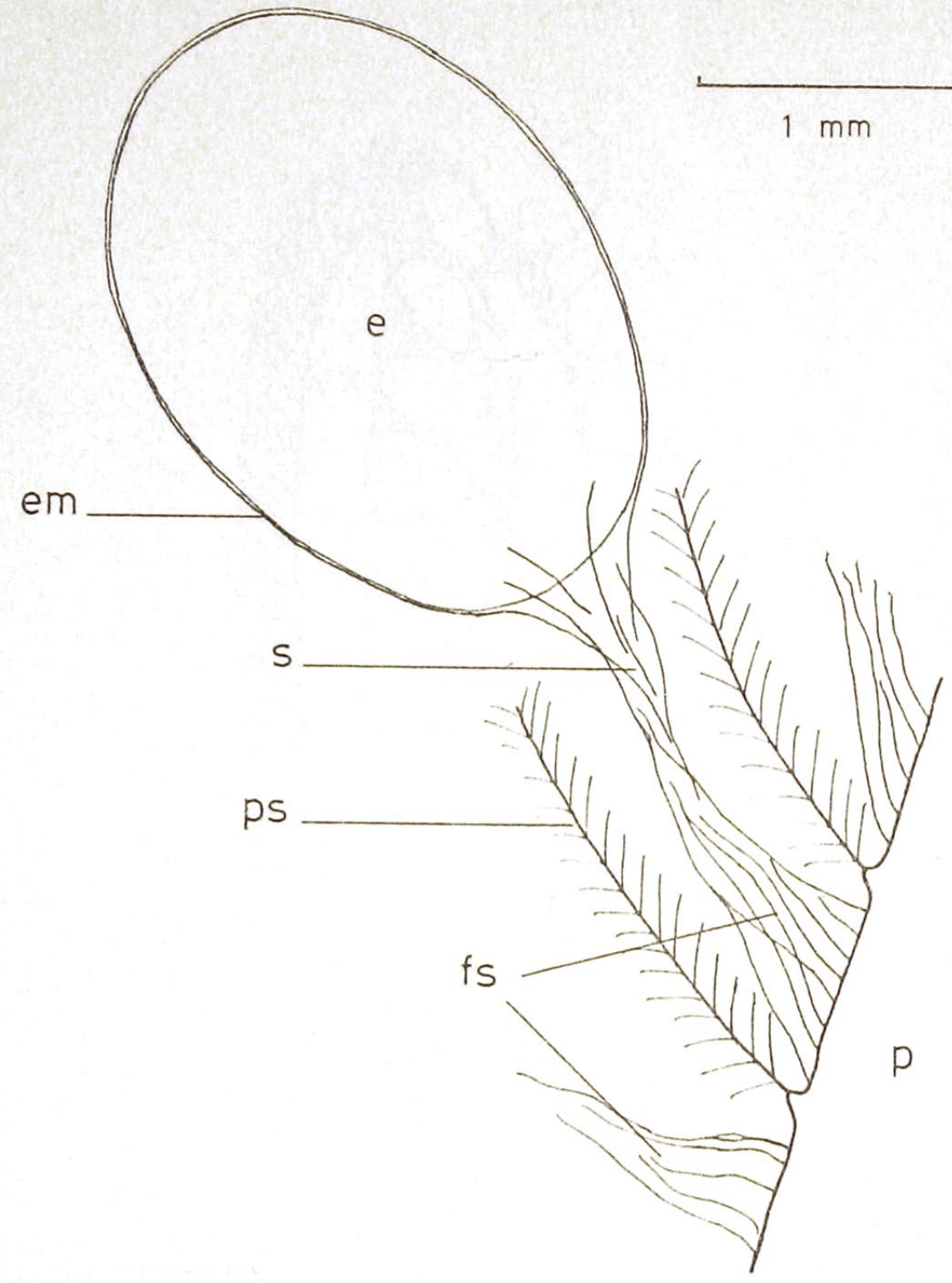


Figure 4.1. Mode of egg attachment in C. destructor. Note the clumped filamentous setae forming the stalk.

e, egg; e.m, egg membrane; f.s, filamentous setae; p, pleopod; p.s, pinnate setae; s, stalk.

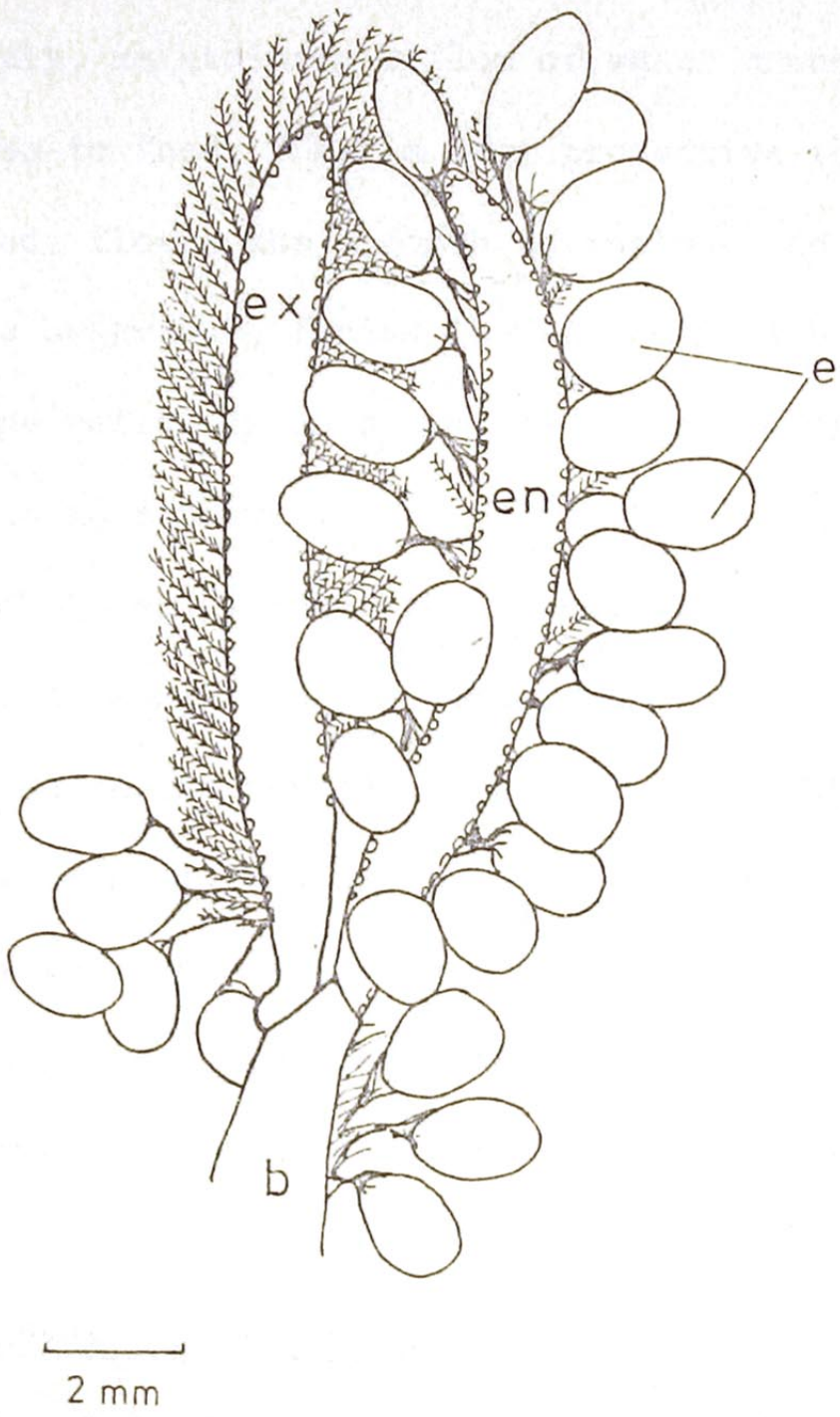


Figure 4.2. Pleopod of a berried female of C. destructor, showing points of attachment of the eggs. Note that the majority of the eggs are attached to the endopodite.

b, basipodite of pleopod; e, egg; en, endopodite; ex, exopodite.

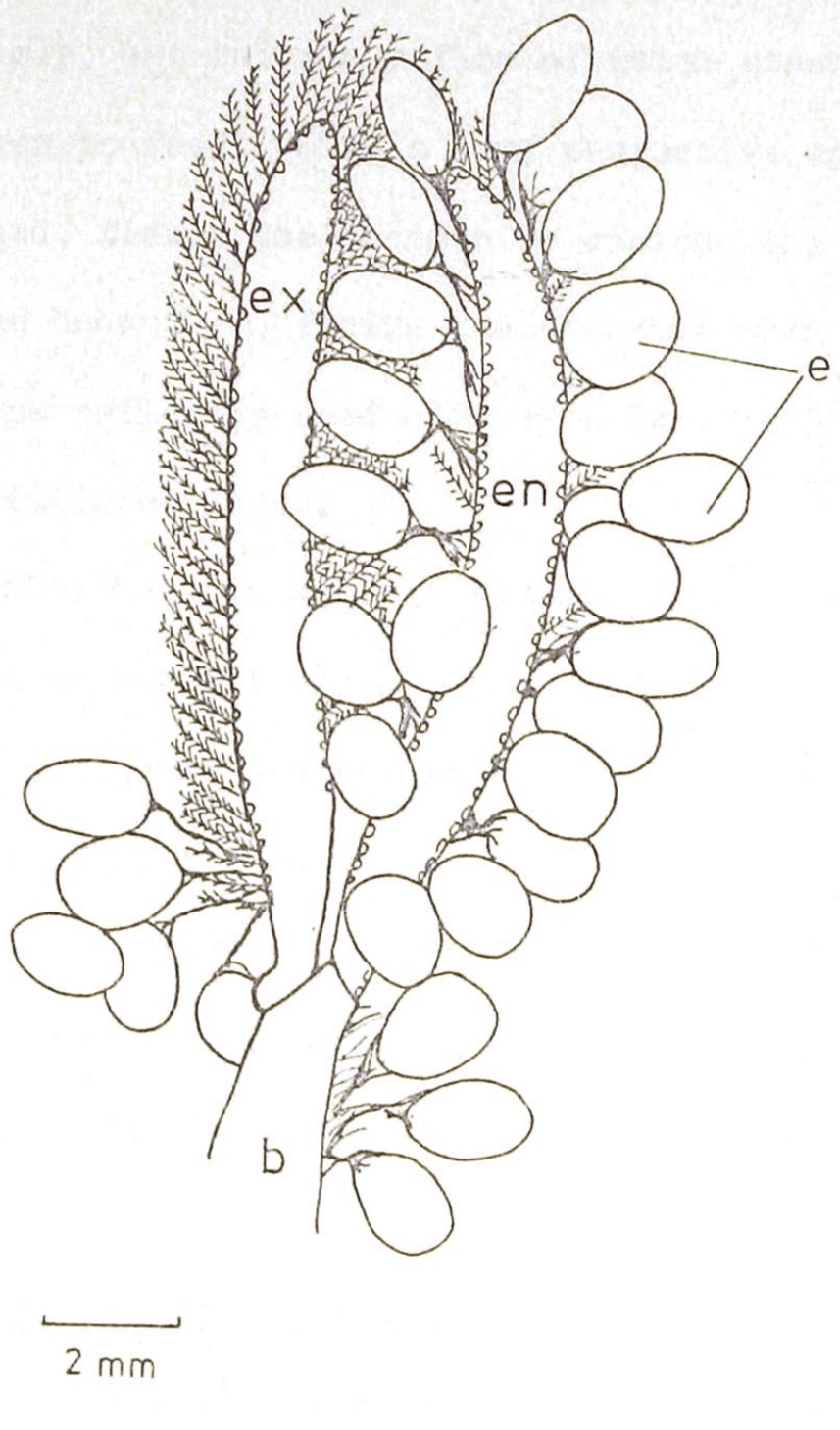


Figure 4.2. Pleopod of a berried female of C. destructor, showing points of attachment of the eggs. Note that the majority of the eggs are attached to the endopodite.

b, basipodite of pleopod; e, egg; en, endopodite; ex, exopodite.

the yabby was handled roughly. After two or three days, the eggs become hardened and less vulnerable.

The female now usually keeps the abdomen extended and moves the pleopods continuously, maintaining a flow of water around the developing eggs. She continues to feed. She is very protective towards her eggs and, when threatened, flexes the abdomen to enclose the eggs. She exhibits aggressive behaviour, facing the attacker with raised chelae. The abdominal escape reflex is used only as a last resort, and eggs are often lost if it is employed.

Sometimes females held in aquaria spawned very early in the breeding season, at an ambient water temperature of about 15°C, apparently without mating, as no spermatophores were ever seen. The spawned eggs were always unequal in size, and were blue-green with an alabaster-like translucence. They were apparently infertile as, without exception, they turned yellow-brown and deteriorated within a day or so. They were all either lost or removed by the adult within a few days (see also Section 5.3.2).

4.3.3. Egg and embryonic development

The egg is elongated, with a major axis of 2.0-2.5 mm and a minor axis of 1.5-2.0 mm. It is densely yolky and opaque and a light olive-green when first spawned.

Five-day embryo.

During the next few days, the colour darkens to an olive-green. No other changes are visible externally. By about the fifth day, the egg has become dark olive and the embryo is just discernible, by the naked eye, on the surface of the egg about one third of the distance from one end. Microscopic examination shows the embryo, ventrum outermost, to be oriented along the major axis of the egg with the posterior towards the nearer pole of the egg. The embryo is an early

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embryonized nauplius, about 200 µm long (Figure 4.3). Rudiments of the labrum, of the thorax and abdomen and of the first three paired appendages (first antennae, second antennae and mandibles) are just discernible.

Eight-day embryo.

No colour change occurs over the next few days but, on approximately the eighth day after spawning, a post-naupliar embryo stage, about 550 µm in length, is seen to have developed. Its anterior end is in the same position as in the five-day embryo, but the abdomen has grown in the direction of the nearer pole of the egg (Figure 4.4). Both first and second antennary rudiments have lengthened and bifurcated, and the mandibular rudiment has lengthened. An additional six discrete pairs of rudiments are now present, these being the first and second maxillae, the first, second and third maxillipeds, and the first pereiopods. The rudiments of the remaining pereiopods have not yet differentiated. The forwardly-folded abdomen is undifferentiated except for distal bifurcation into a caudal fork. A large, pentagonal rudiment, about 150 µm in diameter, occupies a medial anterior position - this is a complex rudiment consisting of the labrum posteriorly, protocerebrum anteriorly and deutocerebrum and tritocerebrum laterally. There is no discernible separation of these several rudiments at this stage. Paired rudiments of the ganglia of the three segments of the mandible and first and second maxillae are visible in the midline between the abdomen and the labrum. There is a suggestion of paired optic lobes at the anterior of the embryo.

Eleven-day embryo.

At some stage in the next three days the egg turns black and, by about the eleventh day, the embryo has reached a length of 1 mm. Its anterior end is located in the usual position but the posterior edge has

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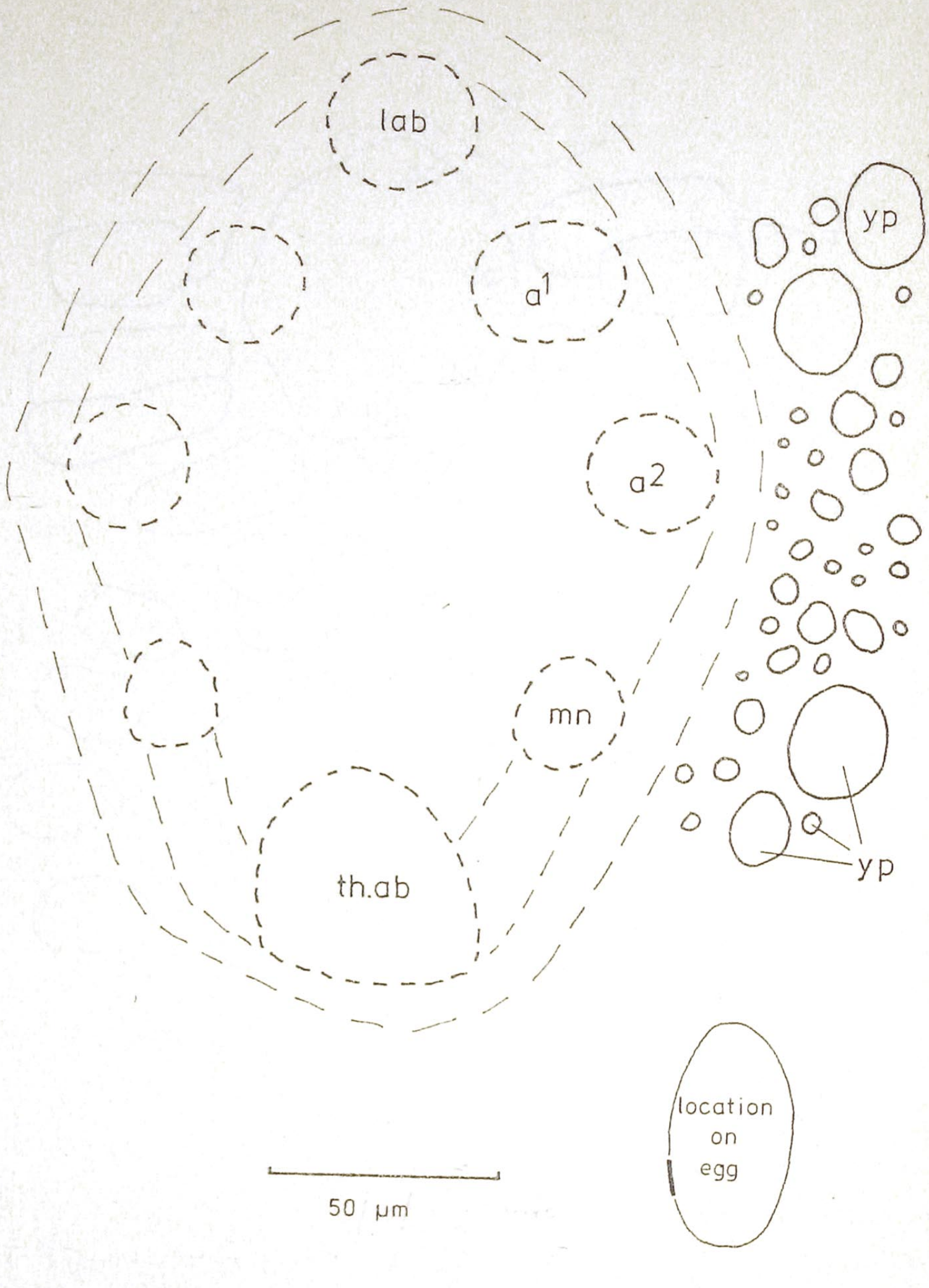


Figure 4.3. Ventral view of the five-day C. destructor embryo - an early embryonized nauplius - showing rudiments of the labrum (lab), first antenna ($a^1$), second antenna ($a^2$), mandible (mn), and thorax and abdomen (th.ab). y.p, yolk platelets.

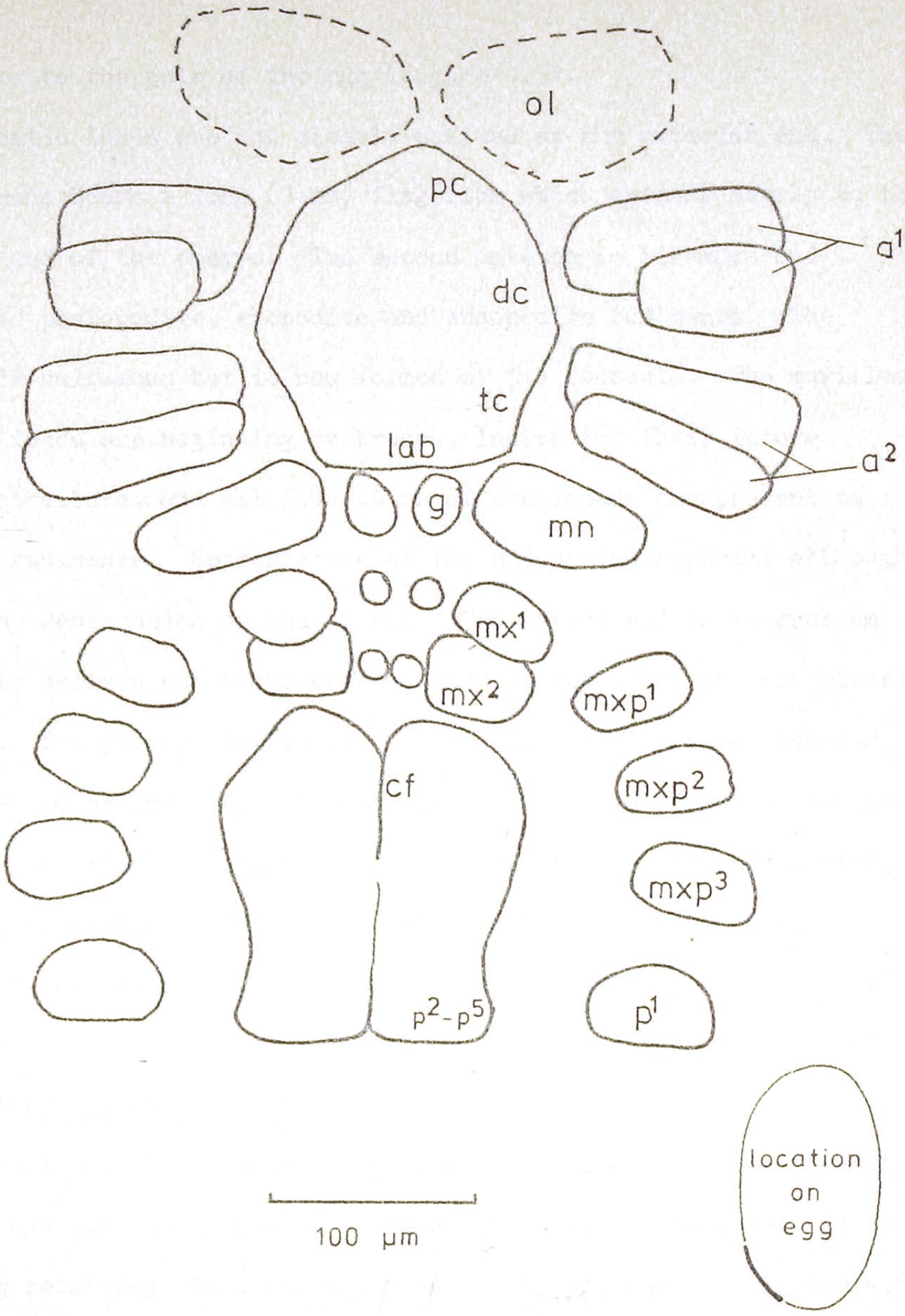


Figure 4.4. Ventral view of the eight-day C. destructor embryo - a late embryonized protozoea.

$a^1$, first antenna; $a^2$, second antenna; c.f, caudal fork; dc, deutocerebrum; g, ganglion; lab, labrum; mn, mandible; $mx^1$, first maxilla; $mx^2$, second maxilla; $mxp^1$ - $mxp^3$, maxillipeds; o.l, optic lobe; $p^1$ - $p^5$, pereiopods; pc, protocerebrum; tc, tritocerebrum.

pushed down to the pole of the egg (Figure 4.5).

The optic lobes are now clearly defined at the anterior end. The first antenna bears a long (1 mm) flagellum which extends nearly to the posterior end of the embryo. The second antenna is biramous and consists of protopodite, exopodite and endopodite rudiments. The mandible is uniramous but is now formed of two segments. The maxillae and maxillipeds are beginning to branch, indicating their future biramous structure, and all five pairs of pereiopods are present as uniramous rudiments. Segmentation of the abdomen is apparent although there is no demarcation of the telson. The labrum and tritocerebrum are clearly defined but protocerebrum and deutocerebrum are not clearly separated. The paired ganglia of mandible and maxillae are seen and, if the abdomen be prised away, the ganglia of subsequent segments can be discerned. A carapace is now evident, enclosing the yolk mass, with the branchiostegites bordering the embryo laterally. The heart, situated on the posterior dorsal surface of the embryo, is beating at this stage.

Fourteen-day embryo.

On about the 14th day after spawning, the embryo is approximately 2 mm long and extends around one pole of the egg, the anterior end of the embryo retaining its usual position on the egg surface (Figure 4.6). In profile, it appears as a whitish crescent occupying 10-15 percent of the egg. The bulk of the egg is yolk which may still be black, but may have turned dark brown or dark red.

The optic lobes are well-developed, but there is no visual pigment. The endopodite of the second antenna has begun to develop the long flagellum. The first antenna and mandible have altered little, and the main changes shown by the maxillae and maxillipeds are increases in length. The pereiopods have developed markedly, the first three being

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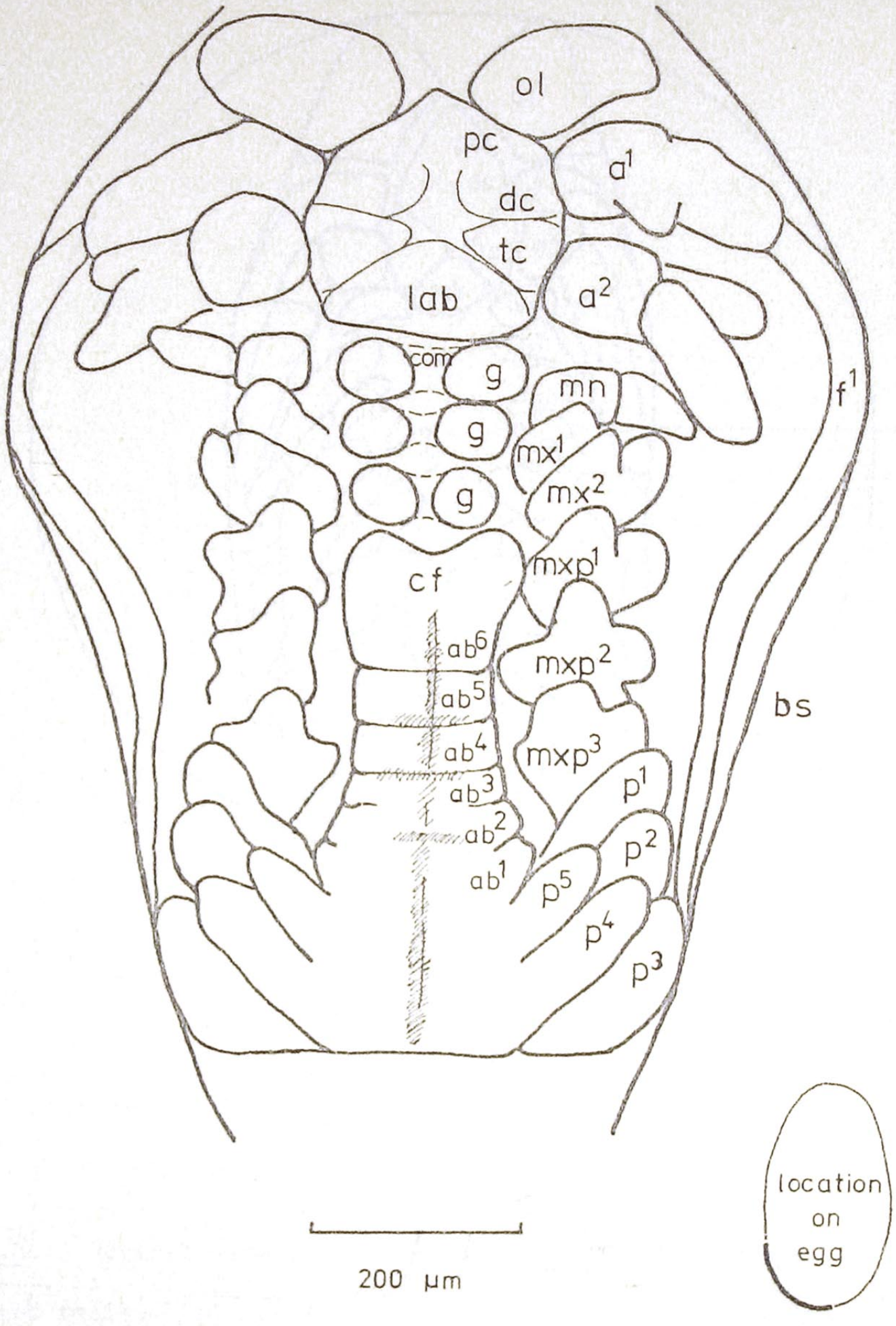


Figure 4.5. Ventral view of the eleven-day embryo of C. destructor. $a^1$, first antenna; $a^2$, second antenna; $ab^1$ - $ab^6$, abdominal segments; bs, branchiostegite; c.f, caudal fork; com, transverse commissure of ganglia; dc, deutocerebrum; $f^1$, flagellum of first antenna; g, ganglion; lab, labrum; mn, mandible; $mx^1$, first maxilla; $mx^2$, second maxilla; $mxp^1$ - $mxp^3$, maxillipeds; o.l, optic lobe; $p^1$ - $p^5$, pereiopods; pc, protocerebrum; tc, tritocerebrum.

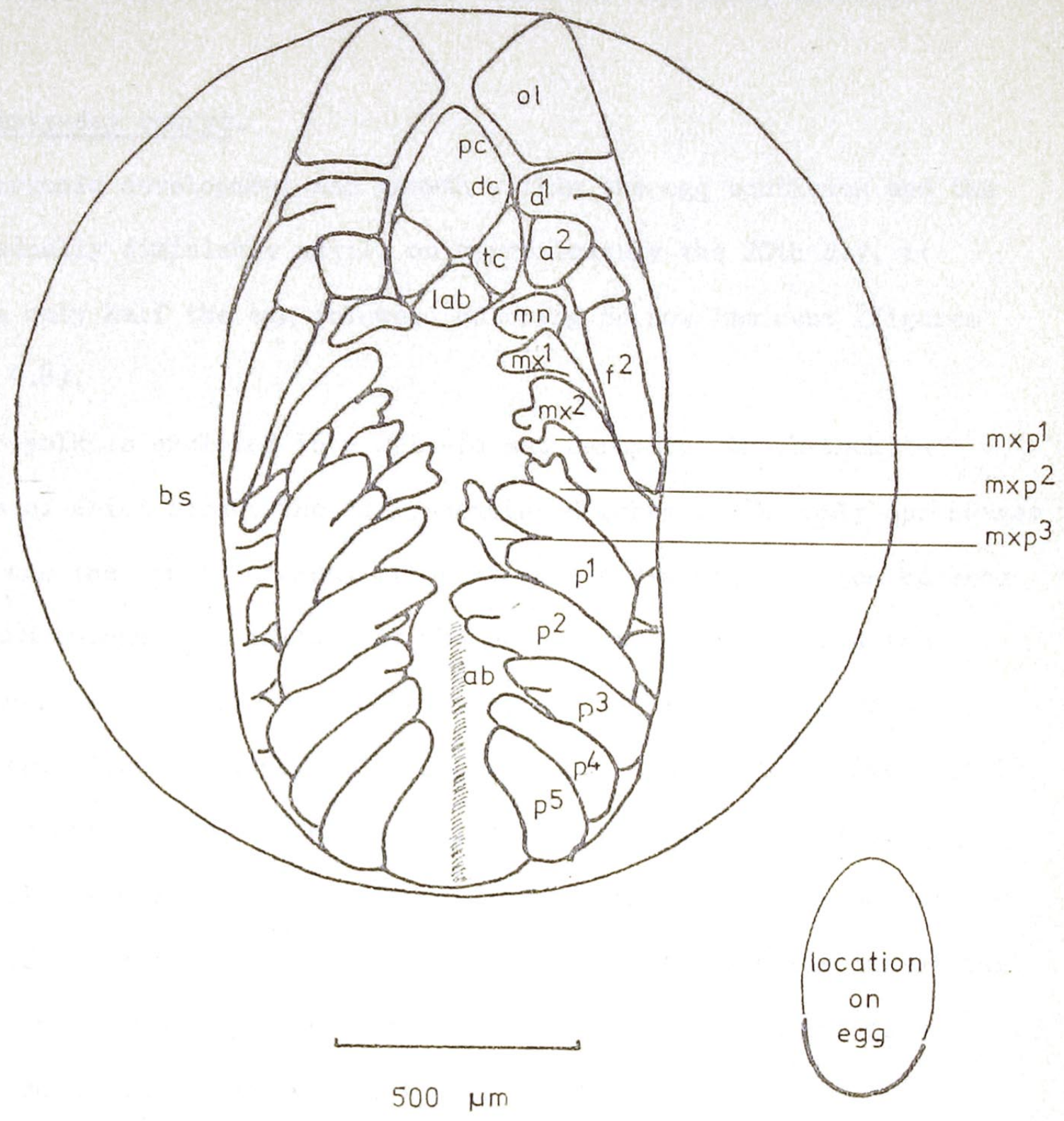


Figure 4.6. Ventral view of the 14 day C. destructor embryo - an embryonized mysis.

$a^1$, first antenna; $a^2$, second antenna; ab, abdomen; bs, branchiostegite; dc, deutocerebrum; $f^2$, flagellum of second antenna; lab, labrum; mn, mandible; $mx^1$, first maxilla; $mx^2$, second maxilla; $mxp^1$ - $mxp^3$, maxillipeds; o.l, optic lobe; $p^1$ - $p^5$, pereiopods; pc, protocerebrum; tc, tritocerebrum.

branched distally, foreshadowing the chelae of the fully-developed limbs.

Twenty-day embryo.

Embryonic development and growth within the egg continues and the yolk gradually diminishes until, on approximately the 20th day, it occupies only half the egg volume. Hatching is now imminent (Figures 4.7 and 4.8).

The yolk is enclosed in a well-formed carapace, the branchiostegites of which border the fully-developed embryo. The only appendages visible are the first antenna, of which only a few segments can be seen; the second antenna, of which only the more distal segments and the flagellum of the endopodite can be seen; and the five well-developed pereiopods. Only the four distal segments of each pereiopod are visible, the more proximal segments being obscured by the flagellum of the second antenna and the branchiostegite of the carapace. The dorsal surface of the abdomen is partially visible at the posterior end of the embryo. The eyes are prominent and visual pigment is present as a thin crescent inside the lateral border of the eye.

4.3.4. Hatching and juvenile stages

Hatching takes place on about the 21st day, with the emergence of a juvenile possessing the basic features of the adult (Figure 4.9).

Stage I juvenile.

Characteristically, this stage I juvenile has a rounded, egg-like carapace packed with brown or red-brown yolk. The rostrum is depressed between sessile eyes. The abdomen is undeveloped by comparison with the cephalothorax, the pleopods being little more than biramous rudiments, and there is no external evidence of separate telson and uropods. There is a prominent anal papilla. The body bears no setae, and the antennal scale is small and narrow. The distal terminations of

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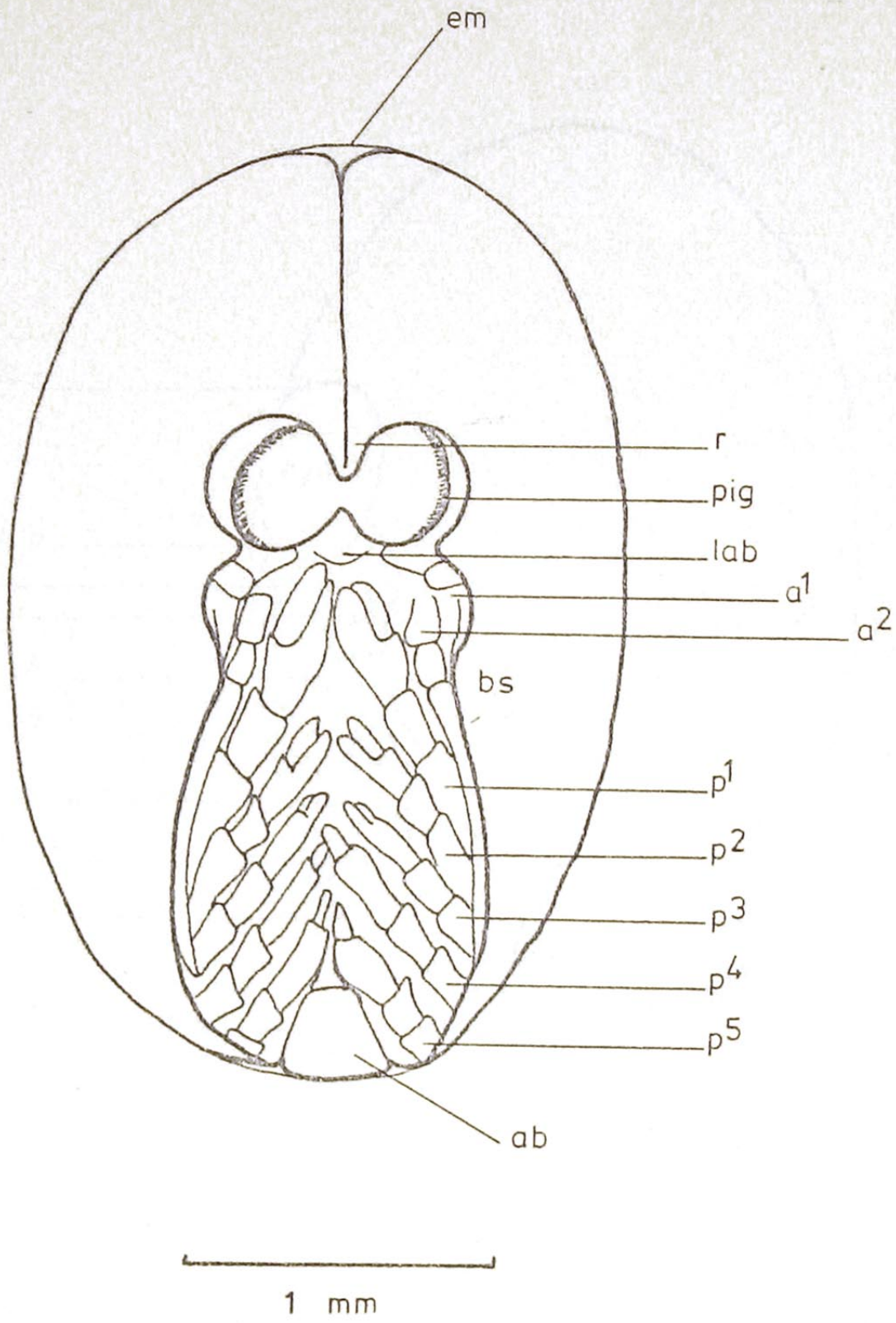



Figure 4.7. Ventral view of the 20 day embryo of C. destructor - about to hatch.

$a^1$, first antenna; $a^2$, second antenna; ab, abdomen; bs, branchiostegite; e.m, eggmembrane; lab, labrum; $p^1$ - $p^5$, pereiopods; pig, visual pigment; r, rostrum.

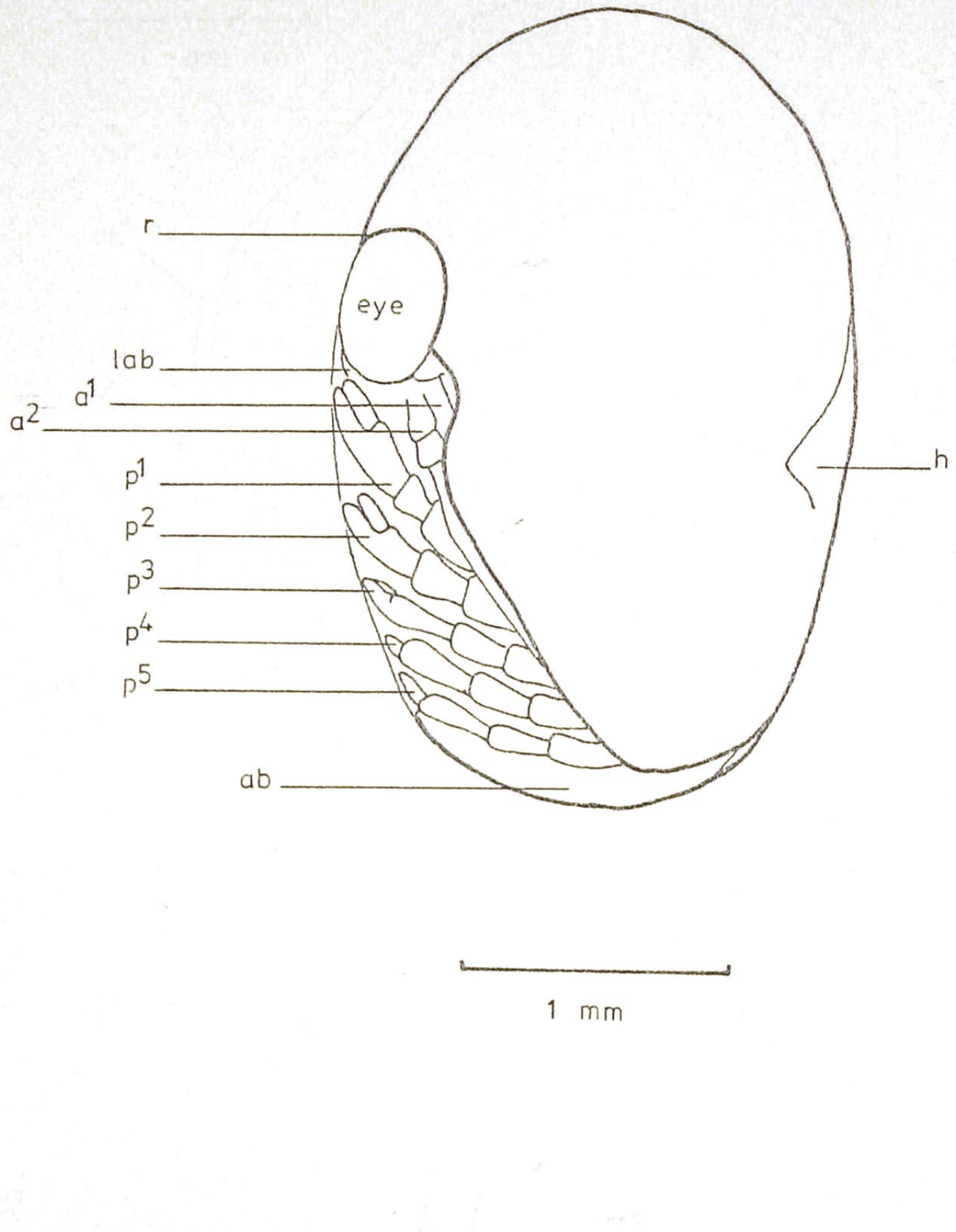



Figure 4.8. Lateral view (profile) of the 20 day embryo of C. destructor.

$a^1$, first antenna; $a^2$, second antenna; ab, abdomen; bs, branchiostegite; h, heart; lab, labrum; $p^1$ - $p^5$; pereiopods; r, rostrum.

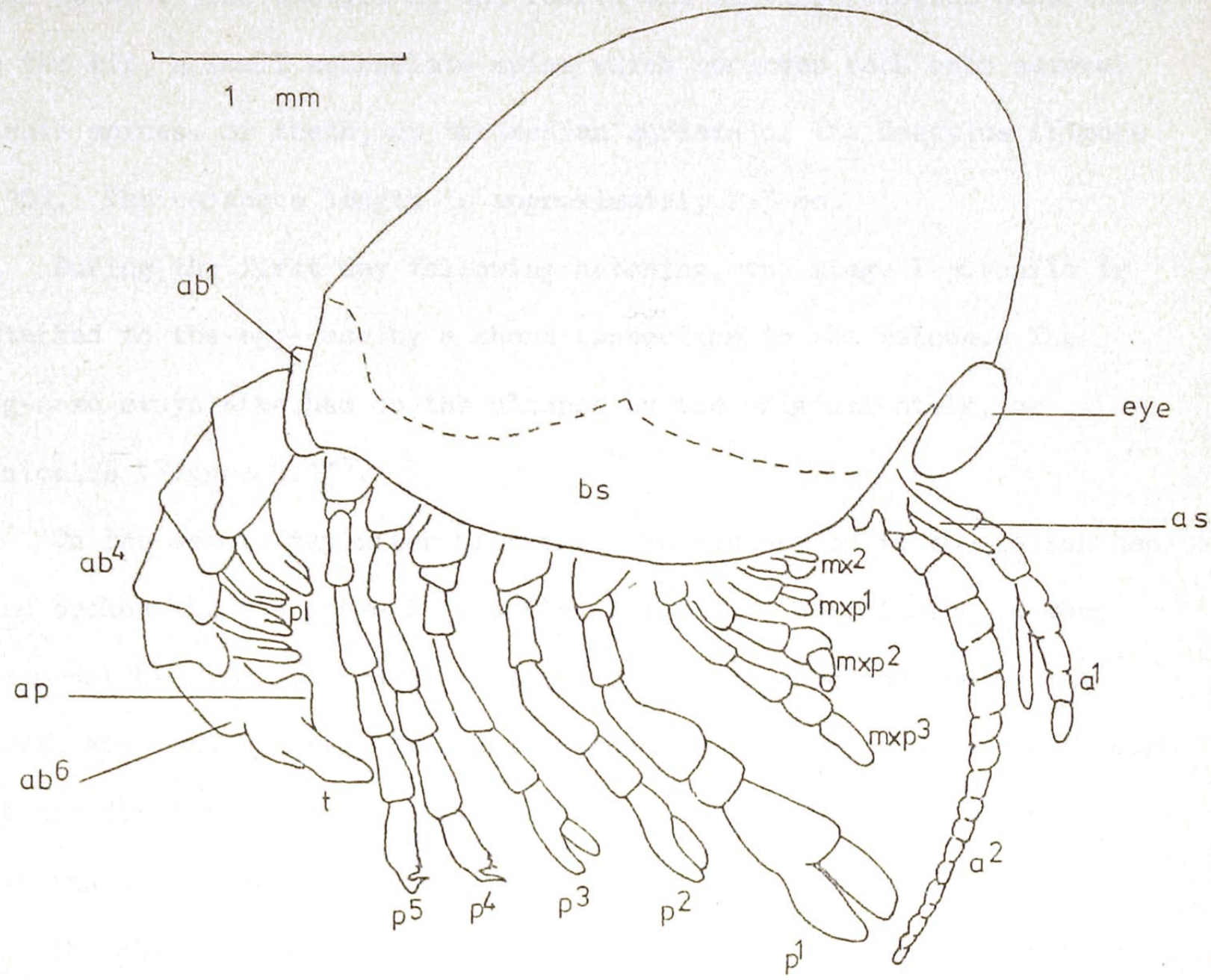



Figure 4.9. Stage I juvenile of C. destructor, one day after hatching.

$a^1$, first antenna; $a^2$, second antenna; $ab^1$ - $ab^6$, abdominal segments; a.p, anal papilla; a.s, antennal scale; bs, branchiostegite; $mx^2$, second maxilla; $mxp^1$ - $mxp^3$, maxillipeds; $p^1$ - $p^5$, pereiopods; pl, pleopods; t, telson.

the propodi and dactyli of the chelate pereiopods are rounded rather than spined. The dactyli of the fourth and fifth pereiopods each bear, on the tip, a small subchelate spine which recurves back onto several minute spines, or teeth, on the median surface of the dactylus (Figure 4.10). The carapace length is approximately 2.3 mm.

During the first day following hatching, the stage I juvenile is attached to the egg-case by a short connection to the telson. The egg-case stays attached to the pleopod by the original stalk, or funiculus (Figure 4.11).

On the second day after hatching, the connection to the telson has been broken and the stage I juvenile is found to be clinging to the pleopodal hairs of the adult by means of the recurved spines on the fourth and fifth pereiopods. This method of holding on persists throughout the first and second juvenile stages, during which time there is essentially no juvenile activity.

The stage I juvenile persists for three days and, by the third day, the first signs of bright red pigmentation of the cuticle appear on the margins of the orbit and on the posterior margin of the carapace. The lateral crescent of visual pigment has thickened and appears to occupy about 20 percent of the orbit when viewed from the front. Discarded egg-cases are no longer to be found, presumably having been removed by the mother.

Stage II juvenile.

The first juvenile moult now takes place with the emergence, on the fourth day, of a second juvenile stage (Figure 4.12). This stage is morphologically more like the adult and has the following characteristics. The carapace is elongated and shows the beginnings of a carapace groove. The rostrum is elevated and the eyes are stalked. The abdomen is still relatively undeveloped, although the pleopods are

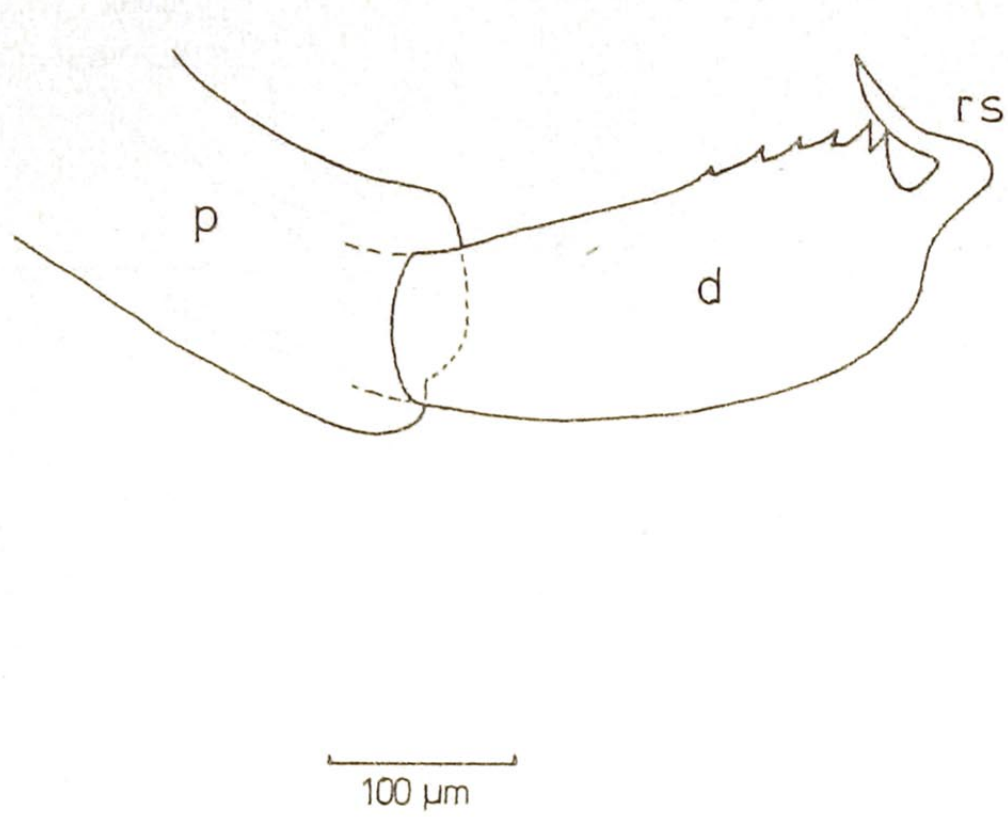



Figure 4.10. Dactyl of the fifth pereiopod of a Stage I juvenile of C. destructor, showing the terminal recurved spine.

d, dactylus; p, propodus; r.s, recurved spine.

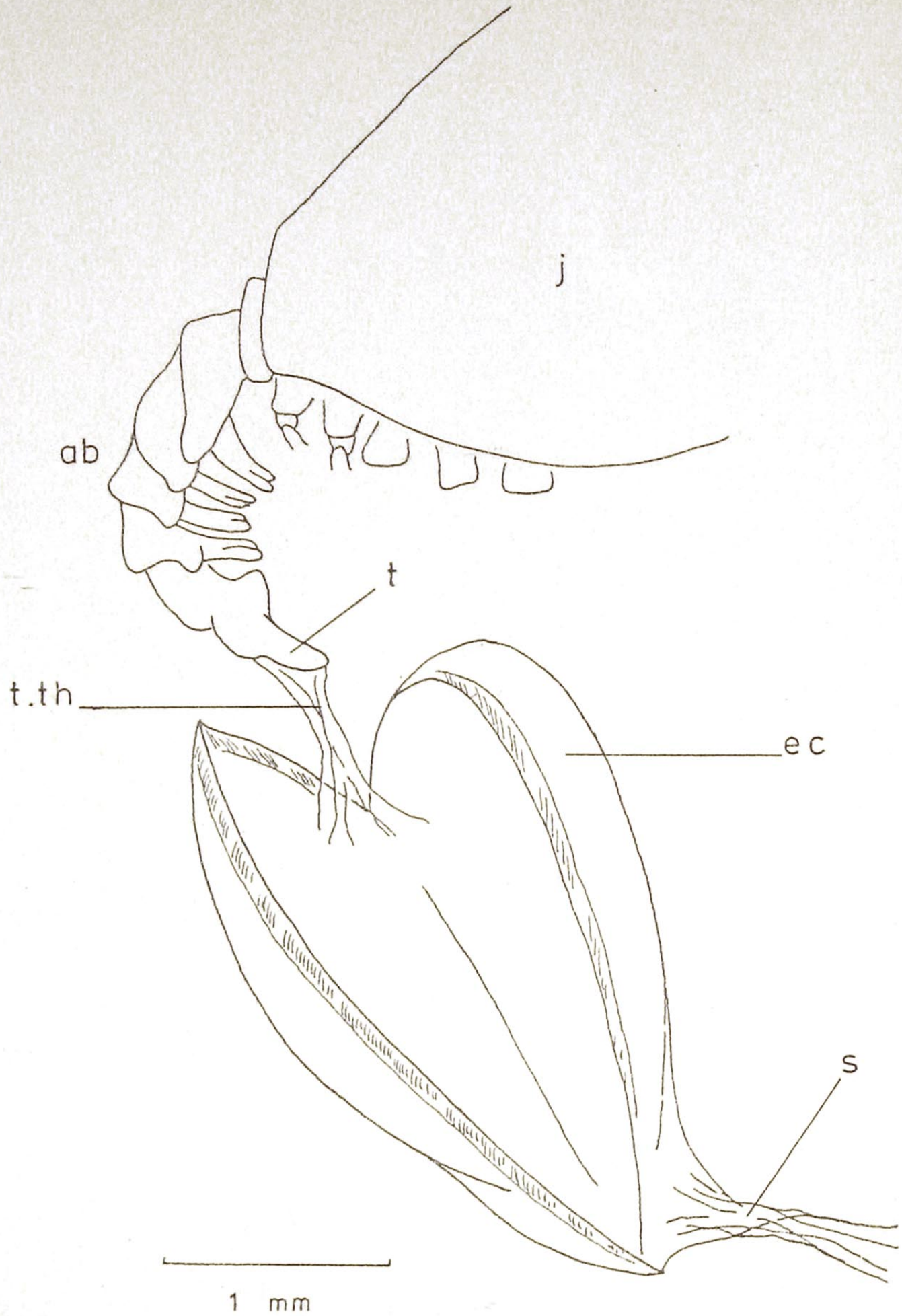
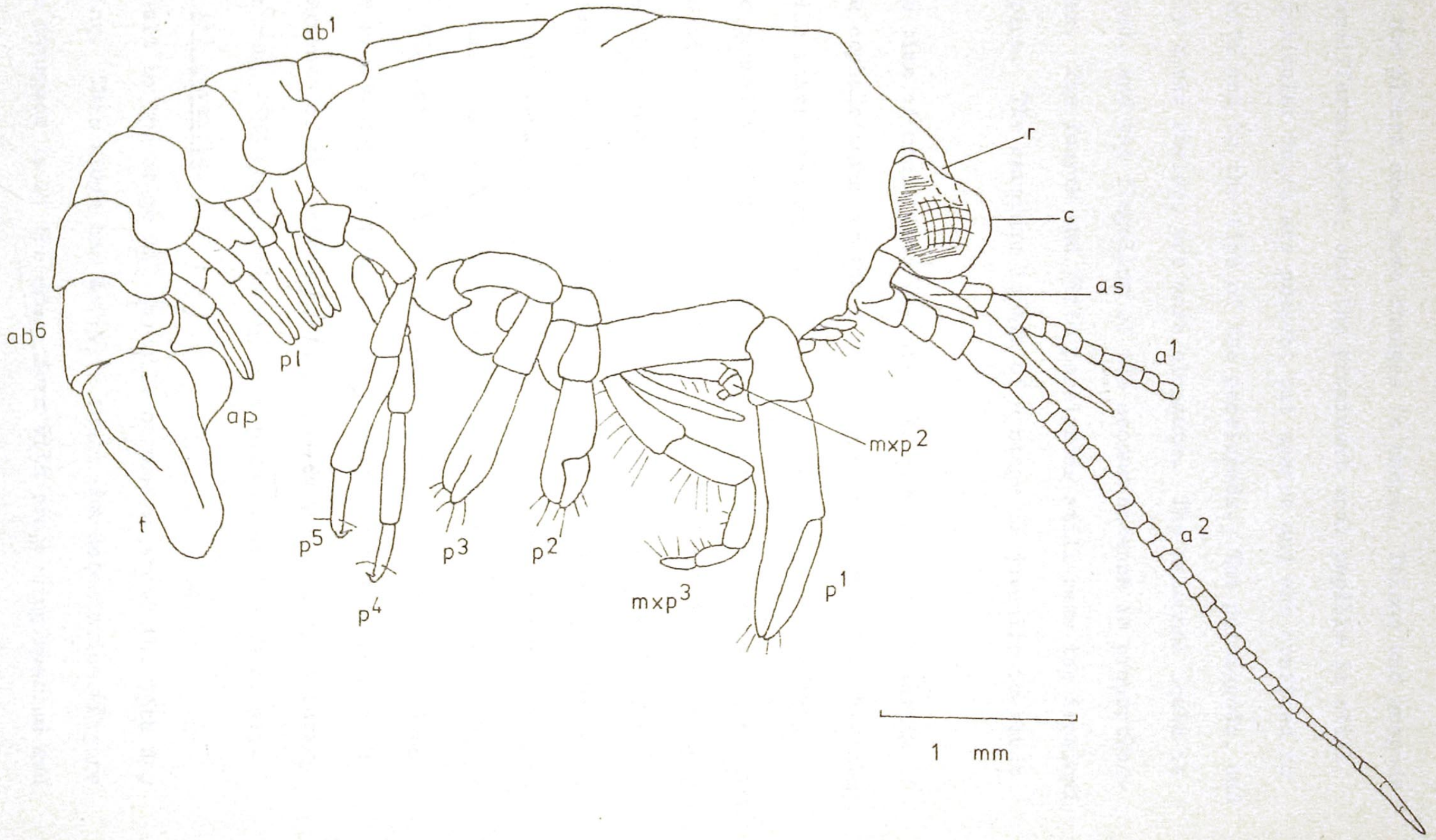



Figure 4.11. The connection (telson thread) between telson and ruptured egg-case, in the newly-hatched C. destructor juvenile. ab, abdomen; e.c, egg-case; j, juvenile; s, stalk; t, telson; t.th, telson thread.

Figure 4.12. Stage II juvenile of C. destructor.

$a^1$, first antenna; $a^2$, second antenna; $ab^1$ - $ab^6$, abdominal segments; a.p, anal papilla; a.s, antennal scale; c, cornea; $mxp^2$, second maxilliped; $mxp^3$, third maxilliped; $p^1$ - $p^5$, pereiopods; pl, pleopods; r, rostrum; t, telson.


ab1
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1 mm

longer and the pleura show some changes in shape. Telson and uropods are still undifferentiated, and the prominent anal papilla is still present. Cuticular hairs are present but sparse and are confined to the mouthparts and to the tips of the pereiopods. The pereiopods, and particularly their chelae, are more elongate. The antennal scale is now flattened, and the flagellum of the second antenna is proportionately longer. The fourth and fifth pereiopods still bear the terminal recurved spine. The carapace length of a stage II juvenile is about 2.7 mm.

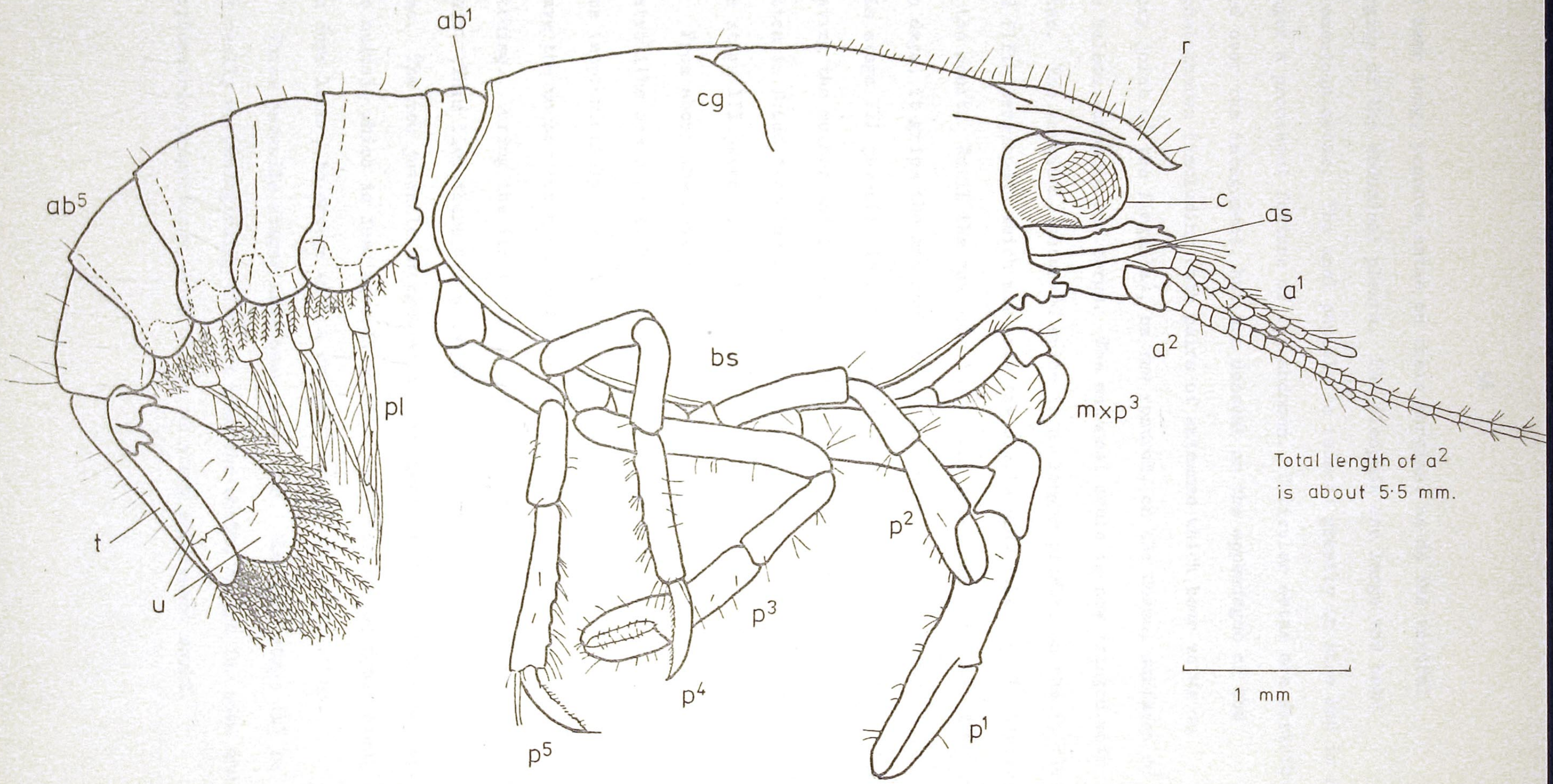
At the time of moult into the stage II juvenile, the quantity of yolk in the cephalothorax is about two thirds that present at hatching. This juvenile stage persists for about nine days and, during this period, the quantity of yolk gradually diminishes, disappearing entirely by the time of the moult into the stage III juvenile. For about four days before this moult, gastroliths can be seen developing in the cephalothorax. They are clearly visible, and unobscured by the small quantity of yolk remaining. Visual pigment has been gradually increasing during this stage and, after this second moult, the eye is fully pigmented. Pigmentation of the integument has also been proceeding and, by the second day of the stage II juvenile, has occurred in the proximal segments of the antennae and in the large chelae, colouring them bright red. The pereiopods begin to pigment on the third day.

Stage III juvenile.

The moult to the stage III juvenile occurs on about the 13th day from hatching. This stage has definite adult characteristics (Figure 4.13). The carapace is of the adult form with projecting rostrum and pronounced grooves, although the deep cervical groove of the adult has not yet formed. The most obvious developmental changes are those which have taken place in the abdomen. The telson and uropods have differentiated

Figure 4.13. Stage III juvenile of C. destructor.

$a^1$, first antenna; $a^2$, second antenna; $ab^1$ - $ab^6$, abdominal segments; a.s, antennal scale; bs, branchiostegite; c, cornea; c.g, carapace grooves; $mxp^3$, third maxilliped; $p^1$ - $p^5$, pereiopods; pl, pleopods; r, rostrum; t, telson; u, uropods.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mxp3
p2
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p4
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p1
Total length of a2
is about 5·5 mm.
1 mm

and bear long, pinnate setae on their margins, as do the ventral margins of the abdominal pleura. The pleopods are large and bear filamentous setae. The anal papilla is reduced greatly in size and is no longer a prominent feature of the abdomen. Cuticular setae are distributed over the integument, but are densest on the appendages of the cephalothorax (including both pairs of antennae which bear setae on every joint of the flagella), on the rostrum, on the dorsal surfaces of the telson, uropods and terga. The antennal scale is now fringed with setae. The terminal, recurved spine is no longer present on the fourth and fifth pereiopods which now have the simple claw-like dactyli typical of the adult. Until the juvenile leaves the parent in a further five to ten days, it grips the maternal, pleopodal setae with the large chelae. This stage III juvenile is active and is exploratory from the first day, leaving the mother and feeding for short periods. Bright red pigmentation is found over the whole dorsal surface. The carapace length of the stage III juvenile is about 3.0 mm.

From about the seventh day of the stage III juvenile's existence, gastroliths are seen to be developing in the cephalothorax. At this time (approximately the 19th day from hatching) the juveniles begin to leave the mother for good, and have all left by the 23rd day from hatching. During the first few days of independence, the juvenile moults again. This time there are no major morphological changes except in size. The new juvenile emerges with a carapace length of about 3.3 mm. The animal, which is feeding well by this time, moults yet again about ten days later, to emerge with a carapace length of about 4.0 mm.

Three juvenile stages have been described. However, stage III is not really morphologically distinct from the adult, except for size and morphometric proportions which change at every subsequent moult.

## 4.4. DISCUSSION

### 4.4.1. Spermatophore transfer

The Parastacidae, like the Astacidae, do not have the annulus ventralis of the Cambaridae. Parastacid and astacid spermatophores are deposited on the female's sternal plates. Males of Orconectes nais (Cambaridae), depositing sperm in a small receptacle, spend longer in copulation (up to four hours) than do Pacifastacus trowbridgii (Astacidae) whose copulation lasts 8 to 20 minutes (Pippitt 1977). Although mating was not observed in C. destructor, it is likely to be similar to that of the Astacidae, rather than the Cambaridae, except that the pleopods of the C. destructor male are not used in sperm transmission.

In Cherax tenuimanus, the gelatinous spermatophoric mass is deposited on the sternal keel close to the oviducts (Shipway 1951), as is the case in C. destructor.

The female of C. destructor does not moult before mating, as is common in many decapods (Green 1961). External deposition of the spermatophoric mass is consistent with the lack of a spawning moult.

### 4.4.2. Spawning, fertilization and egg attachment

The process of spawning and egg attachment, as observed in a single C. destructor female, is similar to that in other macrurans, especially Astacus fluviatilis, described by Huxley (1880), and Pacifastacus trowbridgii, described by Mason (1970).

The period between spermatophore deposition and the start of spawning is short (no more than a few hours) in comparison with other macrurans (e.g. Astacus fluviatilis, 10-45 days, Huxley 1880; Panulirus argus, 4-6 hours, Crawford and De Smidt 1921-22; P. homarus, about a week, Berry 1970; Jasus lalandii, 15-32 days, Silberbauer 1971b). Spermatophore deposition, and therefore mating, does not usually occur in C. destructor without subsequent spawning; thus the short period between

mating and spawning indicates that mating is initiated, or at least accepted, by the female as a behavioural response to the final maturation of oocytes in the ovaries, provided that other conditions required for mating are fulfilled.

The position taken up by the spawning C. destructor female (lying on the side) differs slightly from that described for A. fluviatilis (Huxley 1880) and Pacifastacus trowbridgii (Mason 1970) which lie on the back to spawn. Panulirus argus (Crawford and De Smidt 1921-22) and Jasus lalandii (von Bonde 1936, Silberbauer 1971a) spawn in an upright position, but Panulirus homarus spawns in a vertical position (clinging to a support), or on her back (Berry 1970). All these macrurans, however, form the cupped abdomen to receive the eggs in the same fashion as described for C. destructor.

Fertilization in C. destructor is presumed to occur while the eggs are being extruded from the oviducts and passed over the spermatophoric mass on their way to the pleopods. Internal fertilization is unlikely in view of the position of the spermatophoric mass on the female, and the short time between deposition and spawning. Nevertheless, internal fertilization was thought to occur in Jasus lalandii which has external spermatophore deposition (Silberbauer 1971b), although Paterson (1969) believed fertilization to be external in this species. Fertilization in Astacus fluviatilis is external, according to Huxley (1880) and later investigators (Bieber 1940).

The arrangement of pleopodal setae in the breeding C. destructor female does not differ from that in the non-breeding female. This, also, is consistent with the lack of a spawning moult. Descriptions of pleopodal setae of other parastacids differ markedly from that of C. destructor, and close attention to the arrangement of pleopodal setae, in parastacids generally, is indicated.

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According to Suter (1977), the eggs of the Tasmanian crayfish, Engaeus cisternarius, are bound to plumose (= pinnate, Roberts 1968) setae. Gurney (1942) stated, however, that the ovigerous setae of decapods are usually quite smooth. In Paranephrops planifrons (Hopkins 1967) and Cherax tenuimanus (Morrissy 1975), the pleopodal setae of the breeding females are non-pinnate, whereas the setae of the non-breeding females are pinnate. Woodland (1967) has said that the female Cherax davisi has filamentous setae on the endopodites, and pinnate setae on the exopodites of the pleopods, while all setae on the male pleopods are pinnate (it is the filamentous setae to which the eggs are attached).

In view of the close relationship between C. davisi and C. destructor (Riek (1969) suspected that davisi may be a sub-species of destructor), it is possible that Woodland missed the pinnate setae on the endopodite of the female, and the filamentous setae in the male.

The clear, viscous liquid accompanying the newly-spawned eggs of C. destructor is the "glair" described in the spawning of Astacus by Andrews (1906, cited by Cheung 1966). Glair is found in other freshwater crayfishes (Huxley 1880, Suko 1962, LaCaze 1970, Mason 1970) but has apparently not been described previously for parastacids. The palinurid lobster, Jasus lalandii does not have glair (Silberbauer 1971a). According to Suko (1962), glair, which is secreted from the glandular cells of the oviduct (Terao 1929 and Zehnder 1934, cited by Suko 1962) forms the chorion, the middle egg-membrane; whereas the outer egg-membrane, that which is continuous with the stalk, is secreted by the cement gland (Zehnder 1934, cited by Suko 1962, Stephens 1952). Origin and structure of the egg-membranes in C. destructor were not investigated in this study.

The thin outer membrane enclosing the egg of C. destructor and forming a stalk, or funiculus, attached to the pleopod, is similar to

that in Astacus (Huxley 1880, Bieber 1940) and in the parastacids, Euastacus kershawi (Clark 1937) and Paranephrops planifrons (Hopkins 1967).

The timing of some events in the spawning process of C. destructor is similar to that of the astacid crayfish, Pacifastacus trowbridgii, as described by Mason (1970). Spawning took about 45 minutes in C. destructor and about 20 minutes in P. trowbridgii. The completion of egg-attachment took a further 4½ hours approximately in C. destructor, and 4 hours in P. trowbridgii.

The frequent changing of position, observed in the C. destructor female during spawning, is identical with the phenomenon of "turning", originally described by Andrews (1904, cited by Mason 1970) for the crayfish, Orconectes limosus, and also by Mason (1970) for P. trowbridgii. However, "turning" in C. destructor occurred during spawning, whereas Andrews and Mason described "turning" as post-spawning behaviour. Mason suggested that "turning" results in placement of the eggs on the pleopods; this is thought to be the case in C. destructor also.

Eggs are attached in bunches in animals with large numbers of eggs, as in E. kershawi with 1,200 eggs (Clark 1937) and Panulirus argus with 500,000 eggs (Crawford and De Smidt 1921-22), whereas in C. destructor with 350 eggs, they are usually attached singly. The attachment of the egg-stalk to several pleopodal setae in C. destructor also occurs in Astacus (Cheung 1966).

The eggs are attached mostly to the endopodite in C. destructor as is usual in egg-bearing decapods (J. lalandii, von Bonde 1936; E. kershawi, Clark 1937; P. planifrons, Hopkins 1967; Pacifastacus trowbridgii, Mason 1970).

The berried C. destructor female aerates the eggs by moving the pleopods continuously as is also the case in E. kershawi (Clark 1937) and A. fluviatilis (Huxley 1880). The aggressive defence by the berried

C. destructor female was said by Clark (1936a) to be characteristic of parastacids in general; but Woodland (1967) found berried females of C. davisi to be docile. Woodland (1967) also noticed a reluctance in the berried female, as was found in C. destructor, to use the abdominal escape reflex.

That mating is not a necessary spawning stimulus is indicated by the spawning of several C. destructor females, apparently without mating. This phenomenon has also been described for Panulirus homarus by Berry (1970) and for P. argus by Sutcliffe (1953). In all cases, the eggs were removed from the pleopods, probably by the adult, within several days.

4.4.3. Egg and embryonic development

The egg of C. destructor is typical of freshwater decapods, being large and elongate (up to 2.5 x 2.0 mm), and with a great amount of yolk.

The eggs of other Cherax species are similar to the C. destructor egg. The egg of C. tenuimanus varies in size from 2.4 x 1.6 mm to 2.8 x 2.1 mm (Shipway 1951). The egg of C. davisi has an "average diameter" of about 2 mm and, as in C. destructor, is green in colour (Woodland 1967). The egg of Engaeus is orange when spawned (Suter 1977). Astacus has a 2.8 x 2.4 mm egg (Zehnder 1934, cited by Anderson 1973).

The superficial anatomical description of the embryonic development of C. destructor is identical to that of Astacus fluviatilis (Reichenbach 1886, described in Macbride 1914). Woodland (1967) did not describe the development of C. davisi but simply stated that it was the same as that of A. fluviatilis. As in all freshwater astacurans (Anderson 1973, Barnes 1974), development in C. destructor is direct, larval stages are fully embryonized, and hatching occurs as a juvenile.

The embryonized larval stages of C. destructor, typically found in

astacuran development (Anderson 1973) are the embryonized (i) nauplius, which occurs on about the fifth day after spawning, (ii) protozoea, occurring just before the eighth day, and (iii) mysis, shortly after the fourteenth day. The juvenile hatches on about the twenty-first day.

In the closely related C. davisi, which developed over 31 days, Woodland (1967) observed a "late nauplius" stage at 16-17 days; i.e. halfway through the development time. The embryonized nauplius in C. destructor was seen much earlier, after only one quarter of the development time had elapsed.

4.4.4. Hatching, and juvenile stages

The mechanism of hatching was not determined for C. destructor. In Procambarus clarkii, the egg is burst by extension of the abdomen of the embryo (Suko 1962). Hatching in Homarus americanus results when the egg-case splits due to intake of water and swelling of the entire embryo (Davis 1966).

The juvenile stages of C. destructor are morphologically typical of the Parastacidae (Huxley 1880, Stebbing 1893, Hale 1925, 1927, Clark 1937, Shipway 1951, Hopkins 1967, Woodland 1967, Lake and Newcombe 1975, Suter 1977).

The outstanding difference between parastacid juveniles and those of the northern hemisphere crayfishes is the presence of a terminal, recurved spine on the dactyli of the last two pairs of pereiopods in parastacids. These spines, absent in the Astacidae and Cambaridae, are used by the juvenile to cling to the pleopodal setae of the mother. The spines were first described by Wood-Mason (1876, cited by Huxley 1880, and Stebbing 1893) for the New Zealand crayfish Paranephrops, and are typical of parastacid juveniles. This method of juvenile attachment is not restricted to the Parastacidae; in the carid prawn, Sclerocrangon ferox, the fourth and fifth pereiopods bear claws for

clinging to the parent (Wolleback 1906, cited by Gurney 1942).

Three juvenile stages, similar to those found in C. destructor, have also been found in Paranephrops planifrons (Hopkins 1967), C. davisi (Woodland 1967) and Engaeus cisternarius and E. fossor (Suter 1977). Hale (1927) briefly described the second and third stages of C. destructor but appears to have been unaware of the first stage, as was pointed out by Woodland (1967). Clark (1937) described two juvenile stages for Euastacus kershawi, equivalent to stages I and II of C. destructor, but did not mention a third stage. As the stage III juvenile is basically of the adult form, Clark may have reasonably considered there to be only two juvenile stages.

The occurence, in C. destructor, of three juvenile stages, separated by two moults, and of the undifferentiated telson and uropods in the first two stages, is typical of decapods with direct development (Gurney 1942).

Stage I juvenile.

The newly hatched juvenile of C. destructor is attached to the egg-case by a short connection from the undifferentiated caudal segment; this connection lasts 24 hours or less.

The connection is identical with the "telson thread" secreted by the juvenile and formed a short time before hatching in Astacus (Zehnder 1934, cited by Suko 1962) and Procambarus clarkii (Suko 1962). The telson thread has been observed in the parastacids Cherax preissii (Gurney 1942), C. davisi (Woodland 1967) and Engaeus (Suter 1977); however, the description by Clark (1937) of the hatching process in Euastacus kershawi implies that a telson thread does not exist in this species. As in C. destructor, the breaking of the telson thread occurs in the first few days after hatching in Procambarus (Suko 1962) and in Engaeus (Suter 1977).

The stage I juvenile of C. destructor is similar to those found in other parastacids, C. tenuimanus (Shipway 1951), Paranephrops planifrons (Hopkins 1967), C. davisi (Woodland 1967) and Engaeus cisternarius and E. fossor (Suter 1977). A major difference, however, is seen in the stage I juvenile of Euastacus kershawi in which, according to Clark (1937), the tips of the chelae of the first three pairs of pereiopods are sharply incurved to form hooks, and the terminal spine of the antennal scale is bent to form a large hook. These hooks are less evident at each subsequent moult and are not present in the adult. Clark (1937) could not explain their function but stated that they were not used for clinging to the mother. The juvenile of the South American parastacid Parastacus pilimanus bears similar incurved spines on the great chelae which it uses for clinging, in addition to the fourth and fifth pereiopods (Gurney 1942).

In C. destructor the first juvenile stage lasts 3 days compared with "a few days" in Euastacus kershawi (Clark 1937), 20-30 days in Paranephrops planifrons (Hopkins 1967), 3 days in C. davisi (Woodland 1967) and 12-24 days in Engaeus (Suter 1977). By comparison, the first postembryonic moult occurs after 10-17 days in Astacus (Chantran 1870, cited by Huxley 1880; Fioroni 1969). The stage I juveniles of the parastacids are similar in size, the carapace length of C. destructor being 2.3 mm, C. davisi, 2.5 mm (Woodland 1967), and Engaeus, 1.5-2 mm (Suter 1977).

Stage II juvenile.

The stage II juvenile of C. destructor is similar to those described for other parastacids (Hopkins 1967, Woodland 1967, Suter 1977). The stage II juvenile of Euastacus kershawi still differs from those of the other species, but the hooks on the antennal scale and first three pairs of pereiopods are much less curved than in the stage I

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juvenile (Clark 1937).

This stage lasts 9 days in C. destructor, 20 days in P. planifrons (Hopkins 1967), 11-16 days in C. davisi (Woodland 1967) and 40-50 days in Engaeus cisternarius and E. fossor (Suter 1977). Carapace lengths are 2.7 mm in C. destructor, 3.1 mm in C. davisi (Woodland 1967) and 2.5-3.5 mm in Engaeus (Suter 1977).

The final disappearance, during this stage, of the yolk in the cephalothorax is also seen in most other parastacids (Hale 1927, Clark 1937, Hopkins 1967, Suter 1977), but Woodland (1967) described the persistence of yolk for two or three days into the third juvenile stage of C. davisi. In Astacus, however, all the yolk has disappeared by the time of the first embryonic moult (Fioroni 1969).

Stage III juvenile.

The stage III juveniles of P. planifrons (Hopkins 1967), C. davisi (Woodland 1967) and Engaeus cisternarius and E. fossor (Suter 1977) closely resemble that of C. destructor. All have the basic adult characteristics and have lost the distinctive sub-chelate spines on the fourth and fifth pereiopods. In C. destructor, P. planifrons (Hopkins 1967), Engaeus cisternarius and E. fossor (Suter 1977) the juvenile is now observed to cling to the mother with the great chelae; but Woodland (1967) described the stage III C. davisi juvenile as clinging with the second and third pereiopods.

As in all freshwater crayfish, the uropods and telson of C. destructor remain undeveloped until the third juvenile stage. The appearance of the uropods after the second moult is found in all normal larval series (Gurney 1942).

The stage III juvenile of C. destructor is active and exploratory and starts feeding immediately. In C. davisi the juvenile does not begin feeding until two or three days after the moult (at which time the

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yolk has finally disappeared) (Woodland 1967). Feeding in Engaeus, however, does not begin until the first week of independence of the juvenile, i.e. over three weeks from the time of the moult into stage III (Suter 1977); this appears to be inconsistent with the disappearance of the yolk during the second juvenile stage. Juveniles of Astacus do not begin feeding until the yolk is absorbed (Huxley 1880).

The stage III juvenile remains on the mother 6-10 days in C. destructor, 10-16 days in P. planifrons (Hopkins 1967), 5-8 days in C. davisi (Woodland 1967) and 21 days in Engaeus (Suter 1977). The stage III juvenile of C. destructor, with a carapace length of 3.0 mm, is smaller than those of P. planifrons, 3.4-3.8 mm (Hopkins 1967), C. davisi 3.8 mm (Woodland 1967), Parastacoides tasmanicus 3.5-3.8 mm (Lake and Newcombe 1975) and Engaeus 4.0-6.0 mm (Suter 1977).

Development of the embryos and juveniles of C. destructor is synchronous throughout the brood, all the juveniles taking up an independent existence within a few days of each other (after a total period of about 42 days from spawning). The eggs of the lobster Panulirus longipes cygnus all hatch within a few hours of each other, after 25 to 58 days incubation (Chittleborough 1974). Hopkins (1967) stated that the development of Paranephrops planifrons juveniles is not uniform, the most precocious young leaving the parent many days before the last. This, however, may be simply the normal variability in development times to be expected over the comparatively long, total development period of eight months.

Development times, both of the embryo and the juvenile, are specific for each species. However, in addition to genetic control, the environment, especially temperature, plays an important part in regulating speed of development; and this must be taken into consideration when comparing development times.

From the distribution of parastacids (Riek 1969), it can be seen that those species in which development times are short, such as C. destructor, are usually found in areas where the water temperature is relatively high (15-25°C) during most of the spring and summer. Conversely, species with long development times tend to occur in areas where the water temperature is low all year.

# 5. SEASONAL REPRODUCTIVE ACTIVITY

## 5.1. INTRODUCTION

Apart from some superficial observations by Hale (1925), the breeding cycle of Cherax destructor has not been previously examined; and, amongst parastacids, only in C. davisi (Woodland 1967) and C. tenuimanus (Morrissy 1975), has seasonal ovarian maturation been examined.

This section of the study examines various aspects of breeding activity in C. destructor, including the breeding cycle of mature animals, environmental regulation of the breeding cycle, the incubation period, repetitive breeding, the attainment of sexual maturity, and fecundity.

The only comparable study of breeding activity in parastacids has been carried out by Woodland (1967) on C. davisi.

## 5.2. MATERIALS AND METHODS

Specimens of C. destructor which were collected routinely from the study dam, as described in Section 2.4.4., were also used in the study of seasonal reproductive activity. Morphometric measurements were carried out as described in Section 2.4.2. Dissection of the animals and examination of the gonads was carried out as described in Section 3.2.2. Information for some parts of this study was provided by the observation of captive animals.

5.2.1. Breeding cycle of mature animals

The adult animals, about four of each sex, collected during routine sampling of the study dam (Section 2.4.4.), were dissected. The reproductive state of the gonads was assessed by visual inspection, by measurement of gonosomatic index, and by histological examination.

Males were examined only for the presence or absence of spermatophoric masses in the vasa deferentia. The criteria used to assess ovarian maturation stages in the females are described in Section 4.3.3. Adult females have a carapace length of 36.5 mm or more (this criterion is established in Section 5.3.5). The incidence of berried females was recorded as described in Section 2.4.3.

5.2.2. Environmental regulation of the breeding cycle

Water temperature, daylength, food availability and water level were recorded throughout the study period.

5.2.2.1. Temperature. As yabbies are benthic animals, only bottom water temperatures were considered of significance to the study and were therefore the only temperatures recorded. Temperatures were recorded at a depth as close as possible to the average depth of the dam.

From September 1974 to August 1975, water temperatures were measured by a maximum-minimum thermometer which was read and reset at each visit. The indicated maximum and minimum temperatures were averaged and this value was accepted as the average bottom water temperature for the period. This average temperature is shown, in the results, at the mid-point of the related period. Temperature records for the period 9th October to 18th December 1974 are missing because the thermometers were stolen. The probable continuity was estimated from temperature records of nearby ponds with similar water temperature regimes.

In August 1975, a recording chart thermometer was installed at the site and continuous temperature records were kept for the remainder of the study. The averages of daily mean bottom water temperatures are calculated for weekly periods.

Temperatures were measured in degrees Celsius, to the nearest tenth of a degree. Thermometers were calibrated periodically against a standard thermometer.

5.2.2.2. Daylength. Daylength was taken to be the time, in hours, between sunrise and sunset. A simple sine curve represents change in daylength with time.

5.2.2.3. Food availability. The specimens taken routinely for dissection were also examined for the presence of food in the stomach and hindgut. Since crayfish are opportunistic scavengers, the presence or absence of food was able to be used as an index of food availability.

5.2.2.4. Water height. The height of water in the study dam was measured on a stake driven into the bottom of the dam. The height, measured in metres, to the nearest centimetre, was recorded at each visit throughout the study period. Water height is approximately equal to the average depth of water in the dam.

5.2.2.5. Aquarium observations. During the winter and spring of 1975, several captive animals were maintained (equal numbers of both sexes together) in aquaria at ambient temperatures. In 1976, in addition to control animals kept in aquaria at ambient water temperatures, several breeding pairs were kept in aquaria at a constant water temperature of 20-21°C.

5.2.3. Period of incubation

As described in Section 4.2.3., 22 berried females were held in the laboratory during the 1975/76 breeding season to observe development of the embryo and the juvenile. The spawned eggs were examined daily

and colour and external appearance recorded. Water temperatures experienced by the incubating eggs were ambient, and ranged from 20°C to 25°C.

5.2.4. The pattern of ovarian regeneration and repetitive breeding

Twenty five berried females, collected during the 1975/76 breeding season, as described in Section 2.2.2, were dissected and the reproductive state of the ovaries assessed by visual inspection, by measurement of gonosomatic index, and by histological examination. The time elapsed since spawning was estimated from the colour of the eggs (see Section 5.3.3 for age/colour key). The values of gonosomatic index were regressed on time elapsed since spawning and a regression line was fitted to the data by the method of least squares.

Captive animals were examined regularly during the 1974/75 and 1975/76 breeding seasons to observe the occurrence of repetitive breeding. Marked animals in the study pond were checked at each sampling date for signs of repetitive breeding. Animals were marked using a leather punch to produce a combination of holes in the uropods. The marks are readily identifiable and appear to have little effect on the survival of the individual.

5.2.5. Attainment of sexual maturity

5.2.5.1. Size of animal at spawning. The incidence of berried females in each size class (Section 2.4.2.1), in samples collected during the breeding season, was noted. Carapace length was measured as described for routine samples (Section 2.4.2.1).

5.2.5.2. Size of animal at entry into the adult gonadal cycle. Specimens taken during routine sampling of the study dam, for dissection (Section 2.4.4), were used in investigating the attainment of sexual maturity. Males were examined for the presence of spermatophoric masses in the vasa deferentia. The ovaries of females were examined to

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determine whether the adult ovarian cycle (stages IV - VII, as described in Section 3.3.3) had begun.

5.2.6. Fecundity

Twenty berried females were collected during the breeding season, as described in Section 2.2.2. The carapace length of each individual was measured (Section 2.4.2.1) and the total number of eggs attached to the pleopods was removed and counted. Numbers of eggs were regressed on carapace lengths, for 20 individuals, and a regression line was fitted to the data by the method of least squares.

## 5.3. RESULTS

### 5.3.1. Breeding cycle of mature animals

Examination of the gonads of C. destructor at intervals throughout the study period revealed a well-defined annual reproductive cycle. During late autumn and early winter (April, May and June) most males were devoid of semen. At all other times, the vasa deferentia of most adult males contained semen.

Seasonal changes in the ovaries of non-spawning adult females are shown in Table 5.1. Only ovarian maturation stages IV - VI are found in non-spawning adult females. Spawning periods are indicated by the incidence of berried females, as shown in Figure 5.1.

Table 5.1. Seasonal changes in ovaries of non-spawning adult females of C. destructor. Numbers of ovaries in each stage are shown for each sample during the study period.

| Date of sample | Maturation stage IV | V | VI |
|---|---|---|---|
| 9 Aug 1974 | | | |
| 17 Sep | | | |
| 26 Sep | | 2 | |
| 9 Oct | 1 | | 1 |
| 25 Nov | | 4 | |
| 18 Dec | 1 | 1 | |
| 7 Jan 1975 | 2 | | 1 |
| 29 Jan | 2 | 3 | |
| 18 Feb | 4 | 1 | |
| 11 Mar | 4 | | |
| 1 Apr | 2 | 4 | |
| 29 Apr | 3 | 1 | |
| 5 Jun | 1 | 2 | |
| 23 Jul | 1 | 2 | |
| 1 Sep | 1 | 1 | |

| Date of sample | Maturation stage IV | V | VI |
|---|---|---|---|
| 30 Sep 1975 | 1 | 2 | |
| 22 Oct | | 3 | 1 |
| 4 Nov | 1 | 4 | 1 |
| 19 Nov | | 3 | 6 |
| 2 Dec | | 1 | 1 |
| 17 Dec | 1 | 4 | |
| 31 Dec | 1 | 2 | 2 |
| 14 Jan 1976 | | 4 | 1 |
| 28 Jan | | 2 | 2 |
| 11 Feb | | 2 | |
| 25 Feb | 2 | 1 | |
| 11 Mar | 2 | 1 | |
| 18 Mar | | | |
| 24 Mar | 3 | | |
| 14 Apr | 1 | 5 | |
| 3 Sep | | 2 | 1 |

The study pond was visited for the first time on 9th August 1974 and a few crayfish were examined. One female was seen to be carrying a spermatophore which implied that the breeding season was either beginning or about to begin. This spermatophore deposition may, however, have been premature (see Section 5.3.2).

In the first ovarian sample taken in late September 1974, both females were found to have ovaries in the second maturing stage (V). Mature ovaries (stage VI) were found in one individual of the October sample. Spawning had begun at this time, and berried females were found up to the end of January 1975. Approximately 25

percent of the adult female population was found to be carrying eggs at any one time during this four-month period. The incidence of berried females amongst adult females, in each sample taken throughout the study period, is shown graphically in Figure 5.1 (see also Appendix 4).

For most (October to January) of the 1974/75 breeding season, the ovaries of non-berried females were found in all three stages (IV - VI) of the ovarian cycle, with a slight majority in stage V. In late January, mature ovaries were no longer seen, and all were in the first and second maturing stages (IV and V). From February to April most individuals were in stage IV with some in stage V. From May to September 1975 the majority of ovaries were in the second maturing stage (V). In late October/early November some individuals had mature ovaries (stage VI) and by mid-November the majority were mature, and spawning had begun. In late September a few animals were found to have spawned, but this is thought to have been premature, as all the eggs died (Section 5.3.2). Spawning began somewhat later in 1975 than in 1974; however, as berried females were found up to mid-March 1976, the breeding season still occupied four months. Again, about 25 percent of adult females were berried at any one time during this period (except for the very beginning and end of the season). The peak occurred in early February 1976, when 54 percent of adult females sampled were carrying eggs (Figure 5.1).

All three stages (IV - VI) of ovarian maturation were found amongst non-berried adult females throughout most of the breeding season, as was seen the previous year. Towards the end of the season (February 1976), mature ovaries were no longer observed. From late February and throughout March, most ovaries were in stage IV with some in stage V (as was observed the previous year). During April, the majority of individuals had developed to stage V. Mature ovaries (stage VI) were

found in September 1976.

These observations, together with those on repetitive breeding, are summarised in Figure 5.3.

5.3.2. Environmental regulation of the breeding cycle

Four environmental factors were considered as possible regulators of the breeding cycle of C. destructor. These factors were food availability, water level, daylength and water temperature.

Food was always available as indicated by a continual excess of food in the dam and the year-round presence of food in yabby stomachs. Food availability therefore played no part in regulating the breeding cycle of the crayfish in the study dam.

The relationships between the breeding cycle (indicated by the incidence of berried females) and water level, daylength and bottom water temperature are illustrated in Figure 5.1. Coefficients of correlation, r, are respectively 0.62, 0.62 and 0.49; all correlations are significant at the 95 percent level. Values for water level, daylength and bottom water temperature throughout the study period are tabulated in Appendices 1, 2 and 3 respectively.

That water level was unimportant in regulating the breeding cycle was indicated from observations of captive animals. Spawning, amongst crayfish held in aquaria, was seen to occur on numerous occasions, without change in water level, or addition or change of water.

Both daylength and temperature, however, appear to be involved in regulation of the breeding cycle of C. destructor.

Figure 5.1 shows that, in both 1974 and 1975, the onset of spawning coincided approximately with a rise in water temperature to around 15°C. A similar sequence of events was observed amongst laboratory animals held in aquaria at ambient water temperatures during the winter and spring of 1975.

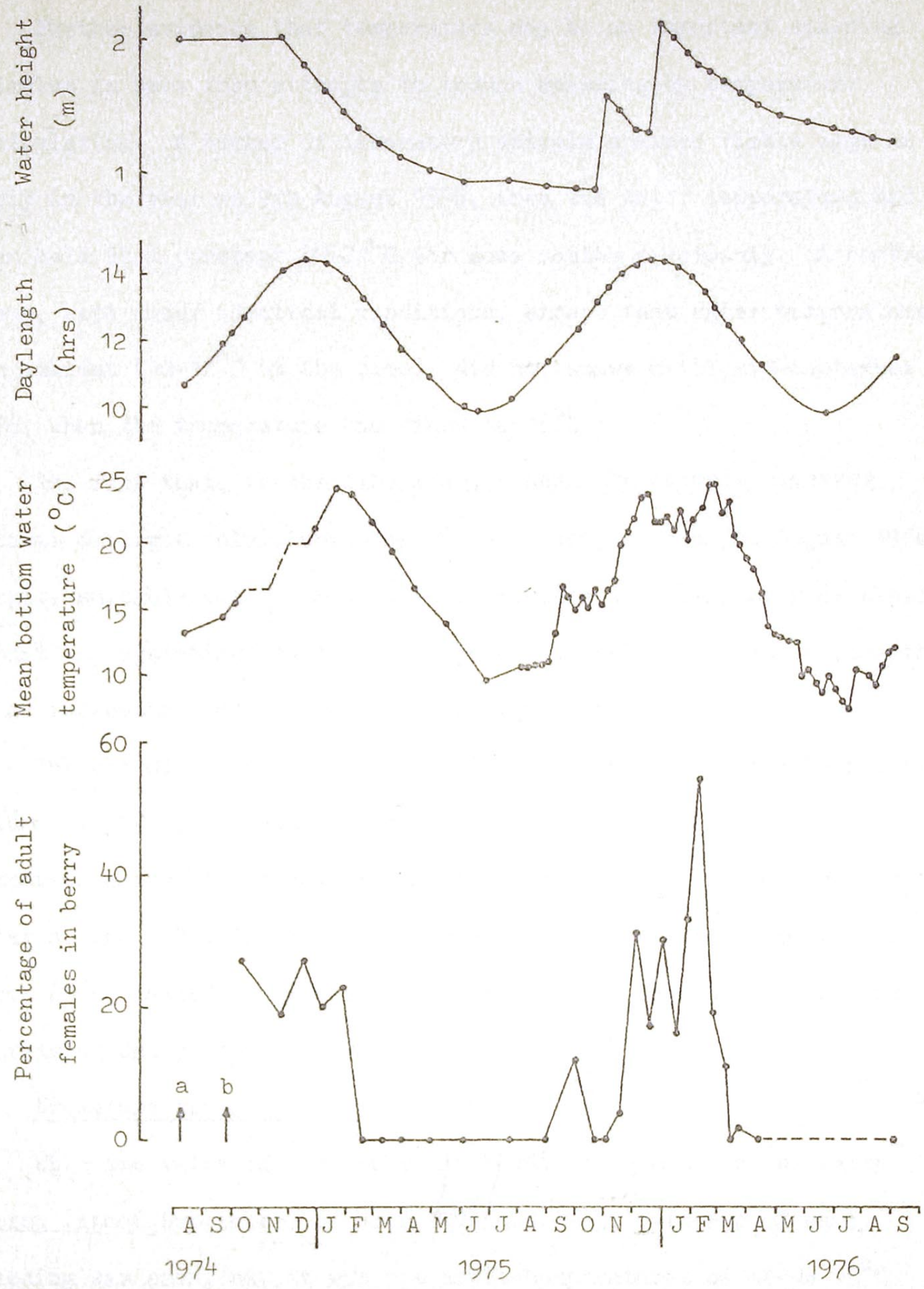


Figure 5.1. The breeding cycle of C. destructor (indicated by the incidence of berried females) compared with water height, daylength, and mean bottom water temperature throughout the study period (see Appendices 1,2,3,4).

a. Premature spawning (see text).

b. Spawning had definitely begun by this time (see text).

Further evidence that temperature may be an important spawning stimulus is seen from attempts to induce spawning by temperature manipulation. A number of laboratory animals spawned viable eggs as early in the year as 9th August 1976, when the water temperature had been held at a constant 20-21°C for some months previously. A control group, kept under identical conditions, except that water temperature was ambient (10-12°C at the time), did not spawn until mid-September 1976, when the temperature had risen to 16°C.

The fact that, in the laboratory (where the animals received natural daylight only), spawning did not occur before 9th August 1976, despite suitable water temperatures, suggests that time of year plays a part in regulating the breeding cycle. A certain minimum daylength or an increasing daylength may be necessary for spawning to occur.

The end of the breeding season in the late summer of 1975 and early autumn of 1976 coincided, in both years, with a decrease in water temperature from the summer peak. Attempts to prolong breeding into later autumn 1976, in laboratory animals maintained at temperatures above 20°C, failed, suggesting the influence of daylength which was decreasing daily at this time.

Premature spawning.

When the water of the study dam began to warm up in the early spring (about September) of both 1974 and 1975, evidence of early breeding was seen, but it was not until temperatures of above 15°C were maintained that spawning became established.

Observed episodes of early breeding appear likely to have been premature. The sample of 30th September 1975 contained two berried females; the eggs of one were dead (yellow-brown discolouration) and the newly-spawned eggs (spermatophore still attached) of the other were found to be dead three weeks later. (The death of the latter

batch may have been due to mechanical damage in the net when first caught. Amongst animals held in aquaria it was frequently seen that the soft, newly-spawned eggs would die if the crayfish was handled roughly. The eggs are much harder after two or three days). Nevertheless, it is thought that this particular spawning may have been premature, and was probably stimulated by the sudden rise in temperature during September. This phenomenon of spawning of non-viable eggs, which are probably unfertilised, was observed amongst laboratory animals at this same time, when the aquarium water temperature rose to about 16.5°C for two days, and then fell (see also Section 4.3.2).

The 9th August 1974 sample contained one female bearing a spermatophore but no spawn. It is likely that this spermatophore deposition was premature.

5.3.3. Period of incubation

Observations of 13 berried females showed that the time from spawning to hatching is 20-22 days, when kept at temperatures varying between 20°C and 25°C. Colour changes in the incubating eggs were observed and are recorded in Table 5.2, against the mean number of days elapsed since spawning.

Stages of the embryo, described in Section 4.3.3, are also shown in the table. The embryo is visible to the naked eye, for the first time, around the 13th day, when it appears as a whitish crescent occupying a small percentage (5-10%) of the egg in profile. Just before hatching, the visible parts of the embryo, which are white in appearance, occupy about 50 percent of the egg, in profile.

After hatching, maternal care continues for a further 21 days approximately, when the juveniles take up an independent existence.

Table 5.2. Changes in the colour of C. destructor eggs against time elapsed since spawning. Temperature of incubation varied between 20°C and 25°C.

| Time since spawning (days) | Colour of egg | Embryo stage |
|---|---|---|
| 0 | Light olive-green. Spermatophore present. | Fertilisation. |
| 1 | " " " " absent. | |
| 2 | Olive-green. | |
| 3 | " " | |
| 4 | Olive to dark olive-green. | |
| 5 | Dark olive-green. | Early naupliar. |
| 6 | " | |
| 7 | " | |
| 8 | " | Post naupliar (late embryonized protozoea). |
| 9 | " Some turning black. | |
| 10 | Most eggs black. | |
| 11 | All eggs black. | 1 mm (long) embryo. |
| 12 | Black, dark brown or dark red. | |
| 13 | " , 5 - 10% white. * | |
| 14 | " , 10- 15% " | 2 mm embryo (embryonized mysis). |
| 15 | " , 20% " | |
| 16 | " , 30% " | |
| 17 | " ,30 - 35% " | |
| 18 | " , 40% " | |
| 19 | " ,40 - 45% " | |
| 20 | " , 50% " | 5 mm embryo. |
| 21 | Hatching takes place. | Stage I juvenile. |

5.3.4. The pattern of ovarian regeneration and repetitive breeding

At the beginning of the breeding season, the majority of adult females have mature ovaries and are ready for breeding (Section 5.3.1).

Examination of 25 berried females indicated that, upon spawning, the ovary immediately begins regenerating in preparation for a further spawning. The observations show that the degree of regeneration, as

(* From now on the embryo appears as a whitish crescent occupying an increasingly greater percentage of the egg in profile.)

indicated by both maturation stage and gonosomatic index, is proportional to the time elapsed since spawning (Figure 5.2 and Appendix 5). The correlation between gonosomatic index and time elapsed since spawning was found to be significant ($r = 0.86$, $P < 0.05$). The slope of the line was shown to be significantly different from zero,

$(t = 8.16) > (t_{(0.05,\ 23^{o}F)} = 2.07)$.

Immediately following spawning, ovaries are in the spent/regenerating stage (VII), but after approximately two days they have entered stage IV of the cycle. By the time spawned eggs have hatched (approximately 21 days from spawning), the ovary is in stage V. Mature ovaries (stage VI) were never seen in a berried female; it is not until the juveniles have all left the mother (at about six weeks from spawning) that the ovary reaches maturity and the female is ready to breed again.

An individual may breed two or three times in a season. This was observed in laboratory animals as well as in tagged animals in the study pond.

In individuals which spawn towards the end of the breeding season, the regeneration sequence described above is seen to occur. In this case, however, it occurs during colder months and proceeds at a much slower rate.

Figure 5.3 summarises the pattern of maturation found in C. destructor, based on these observations and those recorded in Section 5.3.1. Breeding activity takes place over a discrete period of time, which coincides with the annual period of high water temperature, and which is generally from October to March. During this period, the regenerative process of ovarian maturation is accelerated and may pass through two or three cycles, spawning occurring each time.

There is no evidence for synchronous spawning throughout

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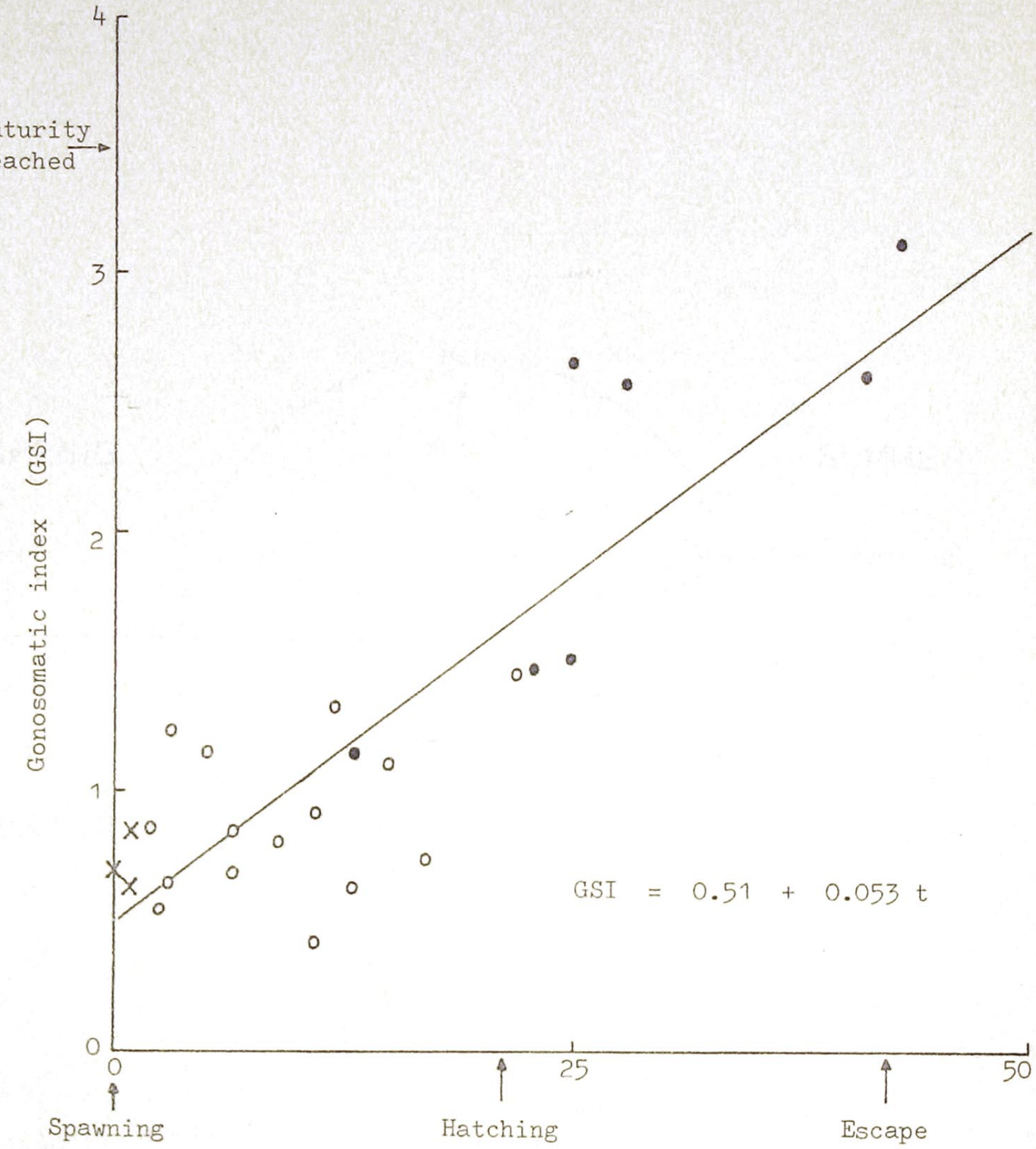


Figure 5.2. Regeneration of ovaries of C. destructor during the breeding season. Major events in development are shown. (Data as in Appendix 5).

× Maturation stage VII. Spent/regenerating.
o " " IV. First maturing stage.
● " " V. Second maturing stage.

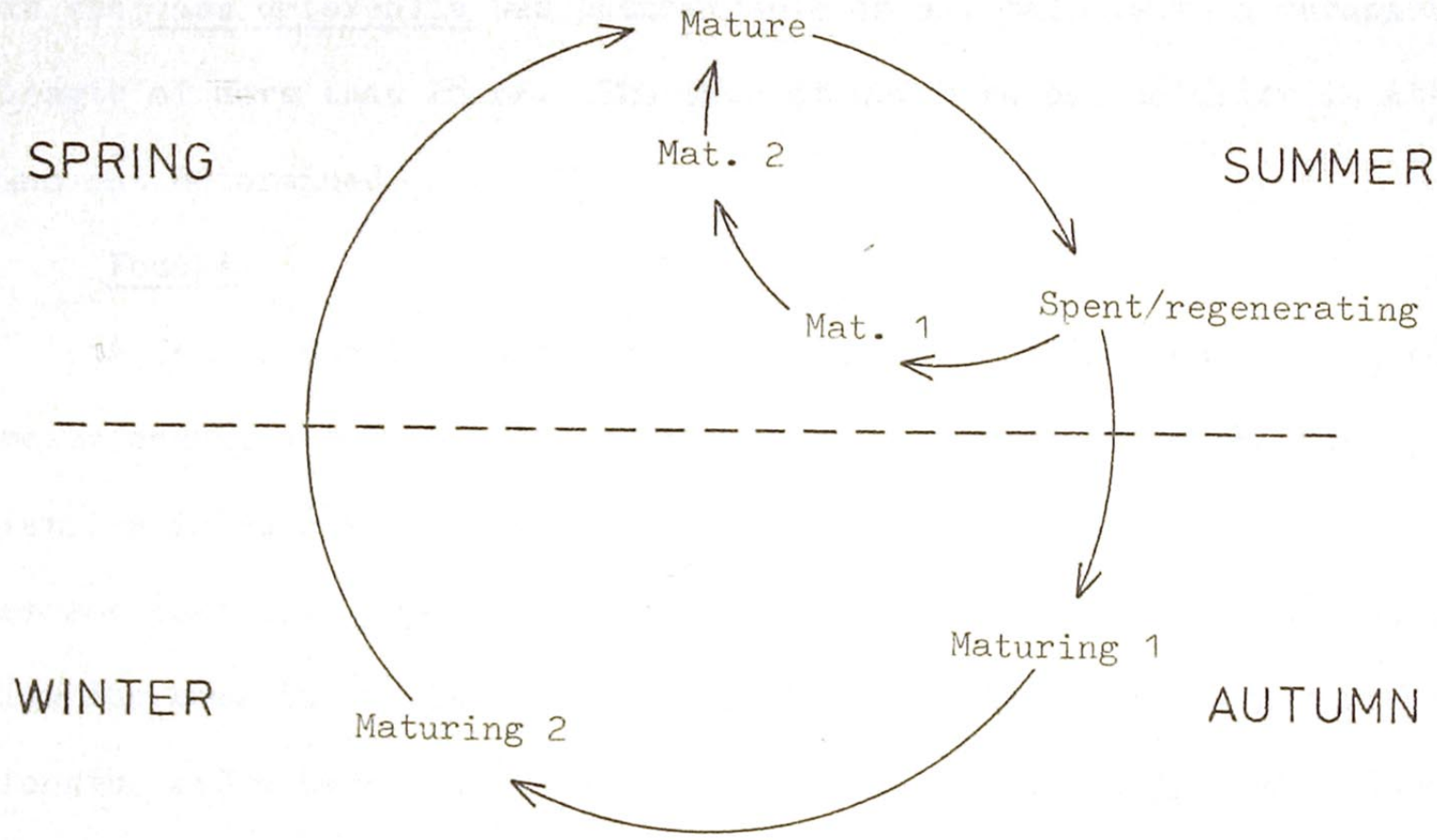


Figure 5.3. Reproductive phases of the ovary of C. destructor.

the population although, at certain times, a large percentage may be taking part in spawning activity.

5.3.5. Attainment of sexual maturity

Male.

Most males of all sizes had empty vasa deferentia during the three-month period (April - June) immediately following the breeding season. Throughout the rest of the year, the presence of spermatophoric masses in the vasa deferentia was demonstrable in all males with a carapace length of more than 28 mm. The size at which sexual maturity is attained was not determined.

Female.

A female bearing fertilised eggs must, by definition, be regarded as being sexually mature. Figure 5.4 shows the incidence of berried females in each size class in samples collected during the breeding season (see also Appendix 6). It can be seen that there is a progressive increase in the incidence of egg-bearing with increasing carapace length, and a marked increase at a carapace length of 36.5 mm. These results indicate that while some females are capable of breeding at a size of about 33.5 mm carapace length, the majority do not reach sexual maturity until a carapace length of about 36.5 mm is reached.

Ovarian state can also be used as a criterion of the attainment of sexual maturity, since immature females have ovaries in stages I - III only, and these stages are not found in mature animals. Table 5.3 shows, for each size-class, the incidence of females with ovaries in the adult cycle (stages IV to VII).

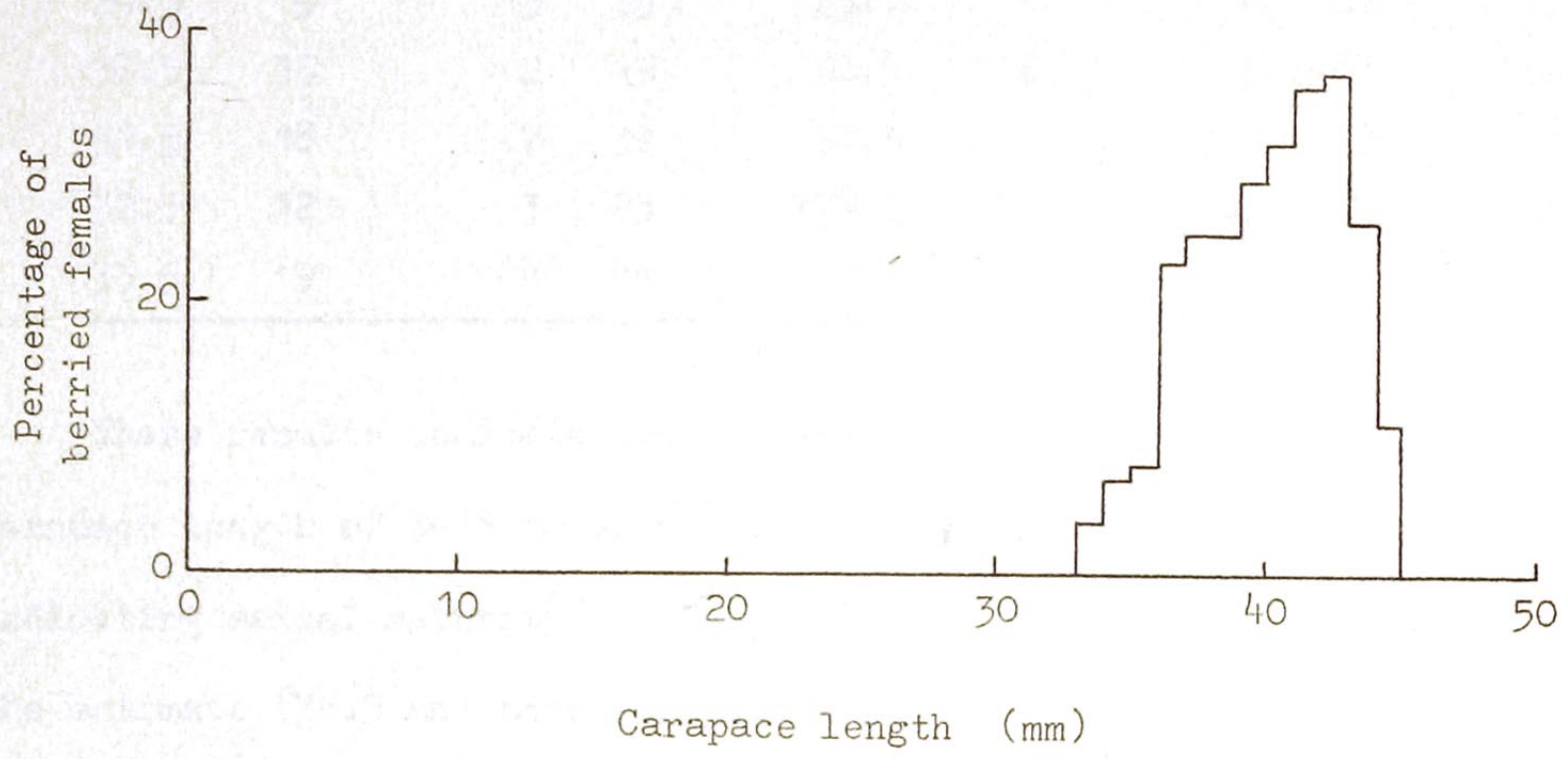


Figure 5.4. Percentage of berried females in each size-class, in samples from the study dam, collected during the breeding season. Range of carapace length: 0 - 45 mm. (Data from Appendix 6).

Table 5.3. Incidence of C. destructor females, for each size class, with ovaries in the adult cycle (stages IV-VII). Samples were collected throughout the year.

| CL (mm) | No. of females | Females in stages IV-VII | | CL (mm) | No. of females | Females in stages IV-VII | |
|---|---|---|---|---|---|---|---|
| | | No. | % | | | No. | % |
| ≤28.5 | 41 | 0 | 0 | 34.5 | 7 | 6 | 86 |
| 29.5 | 9 | 3 | 33 | 35.5 | 9 | 8 | 89 |
| 30.5 | 15 | 2 | 13 | 36.5 | 6 | 5 | 83 |
| 31.5 | 18 | 7 | 39 | 37.5 | 9 | 8 | 89 |
| 32.5 | 12 | 3 | 25 | ≥38.5 | 83 | 83 | 100 |
| 33.5 | 9 | 4 | 44 | | | | |

These results indicate that the majority of females, with a carapace length of 34.5 mm or more, have ovaries in stages IV-VII, indicating sexual maturity or its approach. This size is smaller than the estimate (36.5 mm) based on the incidence of berried females, but the difference can be shown to be due to the time needed for development of the ovary from the first maturing stage (IV) to maturity (VI).

Table 5.4 shows the predominant ovarian stage, and the percentage of females with ovaries in that stage, for size classes 33.5 to 36.5 mm carapace length. Progressive development of the ovary to maturity can be seen to occur over this range. This supports the evidence, obtained from analysis of the incidence of berried females in each size-class, that the majority of females are capable of spawning at a carapace length of about 36.5 mm.

Table 5.4. Maturation of the ovary over the size range 33.5 to 36.5 mm carapace length (CL) as indicated by the percentage of females in each ovarian stage for each size class. Based on same data as used for Table 5.3.

| CL (mm) | Percentage of females in each stage | | | | |
|---|---|---|---|---|---|
| | I-III | IV | V | VI | VII |
| 33.5 | 56 | 22 | 11 | | 11 |
| 34.5 | 14 | 58 | 14 | 14 | |
| 35.5 | 11 | 11 | 78 | | |
| 36.5 | Mixture of stages, including many spawning animals. Maturity reached. | | | | |

5.3.6. Fecundity

Egg counts, varying between 124 and 498, were made on twenty berried females with carapace lengths in the range 31-58 mm (see Appendix 7). The data were subjected to statistical analysis, the fecundity (number of eggs per brood), F, being regressed on carapace length, CL.

Estimates of fecundity need only be approximate, and therefore, because of its simplicity, and because of the small range of measurements involved, a linear relationship was chosen to represent the data. The relationship is:

$$F = 13.0\,CL - 181$$

where coefficient of correlation, r = 0.65.

The relationship, which is significant at the 95 percent confidence level, is presented graphically in Figure 5.5. The slope of the line was shown to be significantly different from zero,

$(t = 3.6) > (t_{(0.05,\ 18^{o}F)} = 2.1)$.

Fecundity, therefore, appears to be directly proportional to the size of the female.

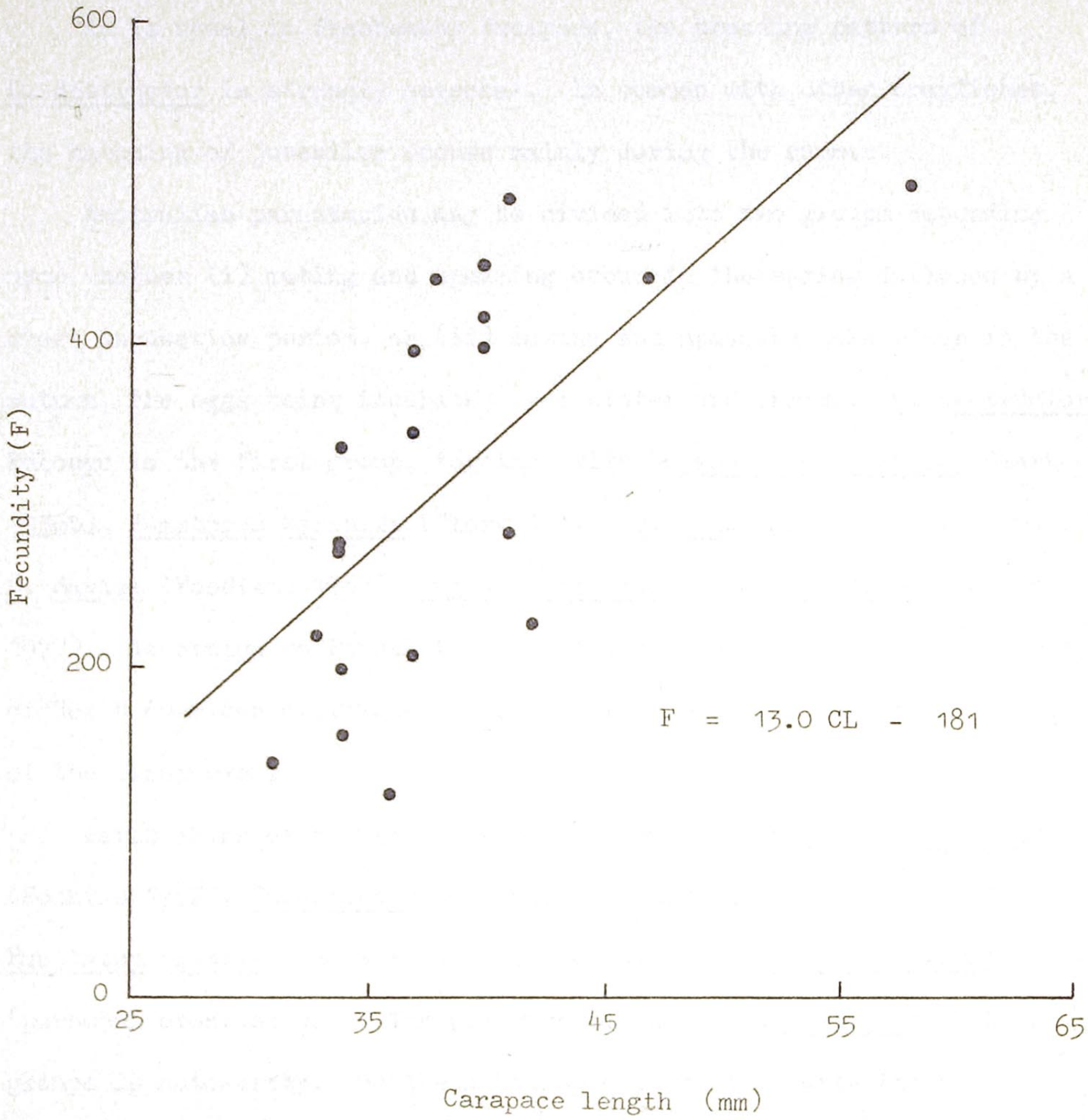


Figure 5.5. Fecundity versus size of female in C. destructor from farm dams near Narrandera. (Data from Appendix 7).

## 5.4. DISCUSSION

### 5.4.1. Breeding cycle of mature animals

As is usual in freshwater decapods, the breeding pattern of C. destructor is strongly seasonal. In common with other crayfishes, the hatching of juveniles occurs mainly during the summer.

Australian parastacids may be divided into two groups depending upon whether (i) mating and spawning occur in the spring followed by a short incubation period, or (ii) mating and spawning take place in the autumn, the eggs being incubated over winter and spring. C. destructor belongs to the first group, together with Engaeus victoriensis (Clark 1936b), Euastacus kershawi (Clark 1937), C. tenuimanus (Shipway 1951), C. davisi (Woodland 1967), Engaeus cisternarius and E. fossor (Suter 1977). According to Pennak (1953, cited by Hopkins 1967), the majority of North American crayfishes have a similar breeding pattern to those of the first group.

Parastacids of the second group include Paranephrops planifrons (Hopkins 1967), Parastacoides tasmanicus (Lake and Newcombe 1975), Euastacus spinifer (Turvey, unpublished data) and Euastacus armatus (personal observation). The presence of the genus Euastacus in both groups is noteworthy. Northern hemisphere crayfish with the characteristics of the second group include Astacus (Huxley 1880) and Pacifastacus (Riegel 1959, Mason 1970, cited by Lake and Newcombe 1975). Orconectes rusticus is unusual as, according to Langlois (1936), it usually spawns in the spring, but sometimes a second spawning will occur in the autumn. This phenomenon has not been recorded for any parastacids.

Woodland (1967) and Morrissy (1975) have given details of seasonal ovarian maturation in C. davisi and C. tenuimanus, respectively. C. davisi appears to be similar to C. destructor in that maturing

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oocytes are usual during the autumn (following the breeding season) and, similarly, oocytes continue to develop slowly throughout the winter, and reach maturity in the spring. In C. tenuimanus, a similar process occurs a few months earlier, with ovarian development beginning in late summer, and mature oocytes being apparent by July. Nevertheless, breeding begins in C. tenuimanus at the same time as in C. destructor and C. davisi, i.e. around October. A similar pattern of oocyte maturation is seen in the crayfish Cambarus virilis (Stephens 1952), and also in the marine spiny lobster, Panulirus homarus (Berry 1971).

The presence, during the breeding season, of maturing, mature and spent/regenerating ovaries in different individual females, indicates that breeding is not synchronous (see also Section 5.3.4).

Throughout the breeding season, approximately 25 percent of adult C. destructor females were berried at any one time. Comparable figures for other parastacids are 75 percent (of females greater than 42 mm carapace length) for C. davisi (Woodland 1967), 40 percent for Parastacoides tasmanicus (Lake and Newcombe 1975) and 75 percent (of females over two years old) for C. tenuimanus (Morrissy 1976b).

5.4.2. Environmental regulation of the breeding cycle

The strong seasonality found in the breeding cycle of C. destructor is usual in freshwater crayfishes (Section 5.4.1). The timing of spawning and hatching of the young is related to external events, so that the young are produced at a time most favourable for their survival. Factors likely to be important in regulating the breeding cycle of a crayfish include food availability, rainfall, daylength and temperature. These seasonal variables, however, are confounded and, without a set of controlled experiments, it is impossible to separate out the importance of specific components.

A sufficiency of the appropriate food is necessary to bring many

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animals into breeding condition (Andrewartha 1970). Although this is probably true for C. destructor also, food availability was not a factor limiting breeding in the study dam population, as was shown by the year-round excess of food in the dam, and its continual presence in crayfish stomachs. In general, breeding in natural populations is probably not restricted by food shortages. This is indicated by the feeding habits of C. destructor (it is an opportunistic scavenger) and by the occurrence of breeding in every population of C. destructor examined during the breeding season.

Springtime flooding is a necessary stimulus to spawning in several species of Australian freshwater fishes (Lake 1967, Llewellyn 1973), all of which are associates of C. destructor. The breeding cycle of C. destructor in the study dam was found to be significantly correlated with changes in water level. Nevertheless, since spawning occurred amongst aquarium animals at the same time, flooding was not a necessary stimulus to spawning.

The influence of daylength in regulating breeding cycles is well-documented for many animals; and the effect of temperature, especially as a spawning stimulus in ectotherms, is also well-documented. Physiological rhythms in decapod crustaceans are greatly dependent upon light and temperature (Brown 1961). The importance of light in influencing events in the reproduction of decapod crustaceans is described by Adiyodi and Adiyodi (1970). Cummings (1961) showed that correlation exists between spawning events in the shrimp, Penaeus duorarum, and bottom temperatures; Rao (1968) reported correlations between water temperature and spawning activity for a number of penaeid prawns. Similar correlations have been found for freshwater crayfishes (Cambarus rusticus, Langlois 1935; Orconectes virilis, Stephens 1952, Aiken 1969; Cambarellus shufeldti, Lowe 1961;

Cherax davisi, Woodland 1967; Astacus astacus, Abrahamsson 1972; Orconectes propinquus, Capelli and Magnuson 1975).

The events of the breeding cycle in C. destructor show significant correlation with both daylength and water temperature. The pattern of ovarian maturation throughout the year was similar for both years of the study, whereas the timing of the breeding season differed. These observations suggest that the stages in the cycle of ovarian maturation are related to the time of year, and thus to the daylength; whereas the timing of the breeding season is more likely influenced by more changeable environmental factors, especially water temperature. Aiken (1969) found that daylength and temperature exert control over ovarian maturation in Orconectes virilis, and that increased water temperature induces spawning in the spring.

Spawning in C. destructor began in the spring when the bottom water temperature reached a threshold of about 15°C, as shown by both aquarium and field observations. Aiken (1969) found a temperature threshold (of 10-11°C) for spawning in Orconectes virilis, and Capelli and Magnuson (1975) found that 7°C was sufficient to cause spawning in O. propinquus. Both C. destructor and O. virilis spawn in the spring, (being examples of the first group described in Section 5.4.1), and therefore respond to rising temperatures, whereas those crayfishes that spawn in autumn (second group) respond to falling temperatures.

However, although temperature appears to be an important spawning stimulus in C. destructor, the results indicate that the time of year, and therefore daylength, critically influence the events of the breeding cycle. This was seen from the unsuccessful attempts to (i) induce early spawning before late winter and (ii) maintain spawning past mid-autumn with the water temperature held above threshold. The implication is that a certain minimum daylength, or an increasing

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daylength, may be necessary for spawning to occur, or that, as Aiken (1969) found in Orconectes virilis, a certain minimum time (4-5 months in O. virilis) of low temperature and short daylength is necessary for complete ovarian maturation to occur. Young (1974) suggested that a certain time, a refractory period, must elapse, following the activity of summer, before spermatogenesis in the crab, Uca pugnax, could begin again.

The occurrence of early spawning (or sometimes just spermatophore deposition), which appeared to be premature, and the consequent death of spawned eggs, was observed both in the field and in aquarium animals.

One such occurrence of premature spawning was seen in the study dam in the early spring of 1975 when the temperature first rose to about 15°C. The spawned eggs of one of two berried females were already dead, and those of the other died, despite the temperature in the study pond remaining at 15-16°C for about eight weeks after the initial rise. It may be that temperatures of around 15-16°C, although high enough to stimulate spawning, are not high enough to sustain egg development. It may also be that spawning occurred without mating, as appears to have been the case in aquarium animals.

5.4.3. Period of incubation

The period of incubation of an egg is dependent upon the speed of development of the embryo which, in turn, depends, among other things, upon the temperature of incubation. The C. destructor eggs described were incubated at ambient, mid-summer water temperatures of 20-25°C which are roughly equivalent to the temperatures occurring in the study dam at that time. As water temperatures higher than 25°C are not commonly found in water bodies containing resident C. destructor populations, the time of approximately 21 days from spawning to hatching is likely to be the shortest incubation period found under natural

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conditions.

Incubation times, comparable to that of C. destructor, have been reported for other freshwater crayfishes (Procambarus clarki, 14-21 days, and P. blandingi acutus, 17-29 days, LaCaze 1970; C. davisi, 31 days, Woodland 1967). Eggs of the spiny lobster, Panulirus longipes cygnus, incubate for 20-30 days at 25°C (Chittleborough 1974).

However, the period of incubation of C. destructor eggs spawned in early spring (the beginning of the breeding season) was much longer than that of eggs spawned in mid-summer, as would be expected in view of the lower water temperatures occurring in the spring. The first spawning in the study dam in 1974 occurred on 9th October, but it was not until 7th January that hatched juveniles were seen. Eggs of C. destructor spawned in early spring may therefore require an incubation period of up to three months, much longer than the 21 days necessary for eggs spawned in mid-summer.

Other crayfish species, in which the eggs spawned in spring have an incubation period of approximately three months, have already been mentioned (Section 5.4.1).

A much longer incubation period (six to eight months) is seen in those crayfish species which incubate their eggs over winter (see Section 5.4.1).

5.4.4. The pattern of ovarian regeneration and repetitive breeding

C. destructor, at least in the study area, has a discrete breeding season, lasting four or five months, and occurring between spring and autumn. During this time, an individual adult female may spawn a number of times. Regeneration of the ovary begins within a few days of spawning, the speed of oocyte maturation matching that of development in the spawned eggs so that, a few days after the juveniles have reached independence, the mother is ready to breed again.

The phenomenon of repetitive breeding is not unusual in decapods (Adiyodi and Adiyodi 1970). According to Ortmann (1906, cited by Woodland 1967), repetitive breeding occurs in the crayfish Cambarus obscurus. It is found in the lobster Panulirus homarus (Berry 1971) but not in Jasus lalandii although ovarian regeneration begins within a day or two of spawning (Heydorn 1969). Woodland (1967) considered that repetitive breeding in C. davisi was possible but unlikely.

The process of regeneration of the ovary in C. destructor during the winter appears to be similar to that occurring between sequential spawnings in the breeding season, except for the length of time taken. However, in many animals it is commonly found that, when fertilization of the ova does not occur, the unfertilized ova are broken down and resorbed. Oocyte breakdown occurs in the crayfish Orconectes virilis (Stephens 1952) and in the lobster Jasus lalandii (Heydorn 1969). Morrissy (1975) found unusual ova, which he considered to be breaking down, in ovaries of C. tenuimanus, in the months following the breeding season. Therefore it may be that resorption of the ovary, involving oocyte breakdown, occurs in some individuals of C. destructor during the weeks following the end of the breeding season, but this could not be properly demonstrated.

### 5.4.5. Attainment of sexual maturity

The size at which C. destructor males reach maturity was not determined; however, it appears that males are mature at a smaller size than females. This was also found for C. davisi by Woodland (1967) who suggested that males mature earlier because of the lower energy requirement to produce sperm, compared with that needed to produce oocytes.

C. destructor females in the study dam population were found to reach sexual maturity at a carapace length of about 36.5 mm. Growth

rate studies by the author (unpublished data) indicate that a female C. destructor of this size is about two years old. Suter (1977) suggested that Engaeus females reach maturity in their second year.

The criteria used to establish sexual maturity were (i) spawning, and (ii) the ovary being in some stage of the adult maturation cycle.

The smallest berried C. destructor female from the study dam had a carapace length of 33.5 mm, but several smaller berried females (one with a carapace length of 22 mm) were collected from other farm dams in the area. This intraspecific difference in size at maturity, even though the dissimilar specimens were taken from different populations of C. destructor, is likely to be phenotypic rather than genotypic. Differences in habitat and environment can affect physiological processes, especially growth. Chittleborough (1974) showed that the spiny lobster Panulirus longipes cygnus matures sexually at an age of about five years, relatively independent of size. He suggested that relatively small mature (Panulirus) females found on certain reefs are more likely to be stunted in growth rather than to have matured at an early age. This is possibly true for C. destructor also.

In the closely related C. davisi, Woodland (1967) found the smallest berried female to have a carapace length of 39.6 mm, which is somewhat larger than the 33.5 mm of C. destructor. This difference may be genotypic. However, in view of the close relationship between the species (Riek (1969) suspected davisi of being a sub-species of destructor), and the similar biology and morphometry (Woodland 1967), it is possible that this difference is predominantly phenotypic. Another possible explanation is that small berried females of C. davisi did not come to Woodland's notice because his samples may have been too small (although his reported sample sizes are comparable to those of this study).

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5.4.6. Fecundity

Freshwater crayfishes produce relatively small numbers of large yolky eggs which hatch at an advanced stage.

As in other crayfishes (Abrahamsson 1966, Hopkins 1967, Lake and Newcombe 1975), the number of eggs laid in C. destructor was found to increase with the size of the female. Egg counts ranged from approximately 120-500, with an average of about 320.

The numbers of eggs carried by other species of freshwater crayfishes are similar to those of C. destructor (Astacus fluviatilis, about 120, Dröscher 1906, cited by Bieber 1940; Cambarus rusticus, 80-570, Langlois 1935; Euastacus kershawi, 1000-1200, Clark 1937; Cherax tenuimanus, 200-360 (up to 670 in large animals), Shipway 1951; Paranephrops planifrons, about 100, Hopkins 1967; Cherax davisi, 200-440, average 320, Woodland 1967; Procambarus clarki, about 400, LaCaze 1970; Parastacoides tasmanicus, 38-80, Lake and Newcombe 1975; Engaeus cisternarius, 45-75, Engaeus fossor, 30-100, Suter 1977; Euastacus spinifer, 800-1300, Turvey, unpublished data; Euastacus armatus, about 800, personal observation).

The close agreement between C. destructor egg counts and those made on C. davisi by Woodland (1967) serves further to illustrate the close relationship between these two species.

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## APPENDIX 1

### WATER HEIGHT IN STUDY DAM

(Height is in metres. See section 5.2.2.4)

| Date | Height | Date | Height |
|---|---|---|---|
| 9 Aug 74 | 2.00 | 2 Dec 75 | 1.30 |
| 25 Nov 74 | 2.00 | 17 Dec 75 | 1.28 |
| 18 Dec 74 | 1.80 | 31 Dec 75 | 2.10 |
| 7 Jan 75 | 1.62 | 14 Jan 76 | 1.99 |
| 29 Jan 75 | 1.45 | 28 Jan 76 | 1.88 |
| 18 Feb 75 | 1.33 | 11 Feb 76 | 1.80 |
| 11 Mar 75 | 1.21 | 25 Feb 76 | 1.75 |
| 1 Apr 75 | 1.11 | 11 Mar 76 | 1.68 |
| 29 Apr 75 | 1.00 | 24 Mar 76 | 1.58 |
| 5 Jun 75 | 0.91 | 14 Apr 76 | 1.49 |
| 23 Jul 75 | 0.93 | 6 May 76 | 1.41 |
| 1 Sep 75 | 0.89 | 2 Jun 76 | 1.36 |
| 30 Sep 75 | 0.87 | 30 Jun 76 | 1.32 |
| 22 Oct 75 | 0.86 | 22 Jul 76 | 1.29 |
| 4 Nov 75 | 1.55 | 14 Aug 76 | 1.24 |
| 19 Nov 75 | 1.46 | 3 Sep 76 | 1.21 |

## APPENDIX 2

DAYLENGTH (SUNRISE TO SUNSET) FOR EACH SAMPLING DATE DURING THE STUDY PERIOD, 9TH AUGUST 1974 TO 3RD SEPTEMBER 1976

| Date | Day length (hrs) | Date | Day length (hrs) |
|---|---|---|---|
| 9 Aug 74 | 10.66 | 22 Oct 75 | 13.12 |
| 17 Sep 74 | 11.91 | 4 Nov 75 | 13.57 |
| 26 Sep 74 | 12.23 | 19 Nov 75 | 13.98 |
| 9 Oct 74 | 12.68 | 2 Dec 75 | 14.25 |
| 25 Nov 74 | 14.10 | 17 Dec 75 | 14.42 |
| 18 Dec 74 | 14.42 | 31 Dec 75 | 14.38 |
| 7 Jan 75 | 14.32 | 14 Jan 76 | 14.20 |
| 29 Jan 75 | 13.83 | 28 Jan 76 | 13.88 |
| 18 Feb 75 | 13.22 | 11 Feb 76 | 13.45 |
| 11 Mar 75 | 12.47 | 25 Feb 76 | 13.00 |
| 1 Apr 75 | 11.75 | 11 Mar 76 | 12.47 |
| 29 Apr 75 | 10.82 | 18 Mar 76 | 12.23 |
| 5 Jun 75 | 10.00 | 24 Mar 76 | 12.02 |
| 23 Jul 75 | 10.25 | 14 Apr 76 | 11.28 |
| 1 Sep 75 | 11.38 | 3 Sep 76 | 11.45 |
| 30 Sep 75 | 12.37 | | |

## APPENDIX 3

MEAN BOTTOM WATER TEMPERATURES IN THE STUDY DAM

(Readings are in degrees Celsius. See Section 5.2.2.1)

1. Temperatures measured by maximum-minimum thermometer

(Periods are irregular in length)

| Period | Mid-point of period | °C |
|---|---|---|
| 9 Aug 74 | 9 Aug 74 | 13.1 |
| 9 Sep 74 - 26 Sep 74 | 21 Sep 74 | 14.4 |
| 26 Sep 74 - 9 Oct 74 | 2 Oct 74 | 15.5 |
| *( 9 Nov 74) | | (16.5) |
| *(25 Nov 74) | | (20) |
| 18 Dec 74 - 1 Jan 75 | 28 Dec 74 | 21.0 |
| 1 Jan 75 - 29 Jan 75 | 18 Jan 75 | 23.9 |
| 29 Jan 75 - 18 Feb 75 | 8 Feb 75 | 23.5 |
| 18 Feb 75 - 11 Mar 75 | 28 Feb 75 | 21.5 |
| 11 Mar 75 - 1 Apr 75 | 22 Mar 75 | 19.2 |
| 1 Apr 75 - 29 Apr 75 | 15 Apr 75 | 16.5 |
| 29 Apr 75 - 5 Jun 75 | 17 May 75 | 13.8 |
| 5 Jun 75 - 23 Jul 75 | 29 Jun 75 | 9.5 |

* Temperature records for the study pond are missing for the period 9th October to 18th December 1974 because the thermometers were stolen. The temperatures shown here are estimated from temperature records of nearby ponds with water temperature regimes similar to the study pond.

....cont.

2. Temperatures measured by recording chart thermometer

(Periods are of one week's duration)

| Mid-point of period | °C | Mid-point of period | °C |
|---|---|---|---|
| 6 Aug 75 | 10.5 | 3 Mar 76 | 24.4 |
| 13 Aug 75 | 10.6 | 10 Mar 76 | 22.2 |
| 20 Aug 75 | 10.8 | 17 Mar 76 | 23.1 |
| 27 Aug 75 | 10.7 | 24 Mar 76 | 20.4 |
| 3 Sep 75 | 11.0 | 31 Mar 76 | 19.8 |
| 10 Sep 75 | 13.1 | 7 Apr 76 | 18.7 |
| 17 Sep 75 | 16.5 | 14 Apr 76 | 17.9 |
| 24 Sep 75 | 15.8 | 21 Apr 76 | 16.3 |
| 1 Oct 75 | 14.9 | 28 Apr 76 | 13.8 |
| 8 Oct 75 | 15.8 | 5 May 76 | 13.1 |
| 15 Oct 75 | 15.0 | 12 May 76 | 12.9 |
| 22 Oct 75 | 16.4 | 19 May 76 | 12.6 |
| 29 Oct 75 | 15.2 | 26 May 76 | 12.6 |
| 5 Nov 75 | 16.4 | 2 Jun 76 | 9.9 |
| 12 Nov 75 | 17.0 | 9 Jun 76 | 10.3 |
| 19 Nov 75 | 19.7 | 16 Jun 76 | 9.3 |
| 26 Nov 75 | 20.6 | 23 Jun 76 | 8.6 |
| 3 Dec 75 | 21.7 | 30 Jun 76 | 9.9 |
| 10 Dec 75 | 23.3 | 7 Jul 76 | 8.8 |
| 17 Dec 75 | 23.5 | 14 Jul 76 | 8.0 |
| 24 Dec 75 | 21.5 | 21 Jul 76 | 7.4 |
| 31 Dec 75 | 21.5 | 28 Jul 76 | 10.3 |
| 7 Jan 76 | 21.9 | 4 Aug 76 | -- |
| 14 Jan 76 | 20.8 | 11 Aug 76 | 9.8 |
| 21 Jan 76 | 22.3 | 18 Aug 76 | 9.1 |
| 28 Jan 76 | 20.1 | 25 Aug 76 | 10.6 |
| 4 Feb 76 | 21.6 | 1 Sep 76 | 11.7 |
| 11 Feb 76 | 22.6 | 8 Sep 76 | 12.0 |
| 18 Feb 76 | 24.4 | | |
| 25 Feb 76 | 24.6 | | |

## APPENDIX 4

### INCIDENCE OF BERRIED FEMALES AMONGST ADULT FEMALES OF *C. DESTRUCTOR* IN EACH SAMPLE TAKEN DURING THE STUDY PERIOD

| Date of sample | No. of adult females | Berried | | Date of sample | No. of adult females | Berried | |
|---|---|---|---|---|---|---|---|
| | | No. | % | | | No. | % |
| 9 Aug 74 | a few | 1 | - | 22 Oct 75 | 24 | 0 | 0 |
| 17 Sep 74 | 0 | - | - | 4 Nov 75 | 15 | 0 | 0 |
| 26 Sep 74 | 2 | 1 | (50) | 19 Nov 75 | 26 | 1 | 4 |
| 9 Oct 74 | 26 | 7 | 27 | 2 Dec 75 | 13 | 4 | 31 |
| 25 Nov 74 | 77 | 15 | 19 | 17 Dec 75 | 23 | 4 | 17 |
| 18 Dec 74 | 26 | 7 | 27 | 31 Dec 75 | 10 | 3 | 30 |
| 7 Jan 75 | 10 | 2 | 20 | 14 Jan 76 | 31 | 5 | 16 |
| 29 Jan 75 | 13 | 3 | 23 | 28 Jan 76 | 33 | 11 | 33 |
| 18 Feb 75 | 12 | 0 | 0 | 11 Feb 76 | 35 | 19 | 54 |
| 11 Mar 75 | 15 | 0 | 0 | 25 Feb 76 | 37 | 7 | 19 |
| 1 Apr 75 | 22 | 0 | 0 | 11 Mar 76 | 19 | 2 | 11 |
| 29 Apr 75 | 62 | 0 | 0 | 18 Mar 76 | 66 | 0 | 0 |
| 5 Jun 75 | 24 | 0 | 0 | 24 Mar 76 | 59 | 1 | 2 |
| 23 Jul 75 | 29 | 0 | 0 | 14 Apr 76 | 56 | 0 | 0 |
| 1 Sep 75 | 2 | 0 | 0 | 3 Sep 76 | 4 | 0 | 0 |
| 30 Sep 75 | 16 | 2 | 12 | | | | |

## APPENDIX 5

RELATIONSHIP BETWEEN REGENERATION OF THE OVARY IN C. DESTRUCTOR, AS INDICATED BY MATURATION STAGE AND GONOSOMATIC INDEX, AND THE TIME ELAPSED SINCE SPAWNING, AT INCUBATION TEMPERATURES RANGING BETWEEN 20°C AND 25°C

| Specimen No. | Time elapsed since spawning (days) | Maturation stage VII | IV | V | GSI |
|---|---|---|---|---|---|
| 1 | 0 | + | | | 0.70 |
| 2 | 1 | + | | | 0.63 |
| 3 | 1 | + | | | 0.85 |
| 4 | 2 | | + | | 0.86 |
| 5 | 2½ | | + | | 0.55 |
| 6 | 3 | | + | | 0.65 |
| 7 | 3 | | + | | 1.24 |
| 8 | 5 | | + | | 1.16 |
| 9 | 6½ | | + | | 0.69 |
| 10 | 6½ | | + | | 0.85 |
| 11 | 9 | | + | | 0.81 |
| 12 | 11 | | + | | 0.42 |
| 13 | 11 | | + | | 0.92 |
| 14 | 12 | | + | | 1.33 |
| 15 | 13 | | + | | 0.63 |
| 16 | 13 | | | + | 1.15 |
| 17 | 15 | | + | | 1.11 |
| 18 | 17 | | + | | 0.74 |
| 19 | 22 | | + | | 1.46 |
| 20 | 23 | | | + | 1.48 |
| 21 | 25 | | | + | 1.52 |
| 22 | 25 | | | + | 2.66 |
| 23 | 28 | | | + | 2.58 |
| 24 | 41 | | | + | 2.61 |
| 25 | 43 | | | + | 3.11 |

## APPENDIX 6

PERCENTAGE OF BERRIED FEMALES OF C. DESTRUCTOR IN EACH SIZE CLASS, IN SAMPLES FROM THE STUDY DAM DURING THE BREEDING SEASON

| CL (mm) | No. of females | Berried females | |
|---|---|---|---|
| | | No. | % |
| ≤32.5 | >200 | 0 | 0 |
| 33.5 | 48 | 2 | 4 |
| 34.5 | 43 | 3 | 7 |
| 35.5 | 38 | 3 | 8 |
| 36.5 | 39 | 9 | 23 |
| 37.5 | 32 | 8 | 25 |
| 38.5 | 36 | 9 | 25 |
| 39.5 | 45 | 13 | 29 |
| 40.5 | 53 | 17 | 32 |
| 41.5 | 25 | 9 | 36 |
| 42.5 | 30 | 11 | 37 |
| 43.5 | 19 | 5 | 26 |
| 44.5 | 19 | 2 | 11 |
| 45.5 | 10 | 0 | 0 |
| 46.5 | 7 | 3 | 43 |
| 47.5 | 2 | 1 | 50 |
| 48.5 | 4 | 2 | 50 |
| 49.5 | -- | -- | -- |
| 50.5 | 2 | 1 | 50 |
| 51.5 | 1 | 0 | 0 |
| 52.5 | -- | -- | -- |
| 53.5 | 1 | 0 | 0 |

## APPENDIX 7

NUMBER OF SPAWNED EGGS COUNTED ON BERRIED FEMALES OF *C. DESTRUCTOR* COLLECTED FROM FARM DAMS NEAR NARRANDERA, N.S.W.

| CL (mm) | Number of eggs |
|---|---|
| 31 | 143 |
| 33 | 221 |
| 34 | 160, 200, 274, 275, 337 |
| 36 | 124 |
| 37 | 209, 345, 395 |
| 38 | 440 |
| 40 | 398, 416, 450 |
| 41 | 285, 488 |
| 42 | 228 |
| 47 | 441 |
| 58 | 498 |