REPRODUCTION IN *GAMMARUS* (CRUSTACEA, AMPHIPODA): BASIC PROCESSES

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Introduction

Amphipod crustaceans of the genus *Gammarus* (Fig. 1) are common throughout much of the world and are almost universally characteristic of the surface waters of northern Eurasia and America. In fresh waters, particularly running waters, they have a central position in food webs, being responsible (along with some cased-caddisfly larvae) for shredding and comminuting all kinds of decomposing leaf litter; hence their classification as detritivores in the trophic economy of lowland rivers.

Because *Gammarus* is so widespread, frequently abundant, and an important and obvious member of most freshwater and saltwater communities, it has been extensively studied from a variety of viewpoints. Even so, only a few general aspects of its ecology have been occasionally reviewed in depth (e.g. Marchant 1981) and, unlike some other common and ubiquitous animals, it has not been the subject of a handy textbook. Some years ago I began such a task and, although the work is only half completed, I have summarised research on the reproduction of *Gammarus.* As *Freshwater Forum* is seeking review-type articles to help fill its pages, I thought it would be useful to publish most of my review of the literature, in three parts. The second and third parts deal with reproductive strategies in males and females of *Gammarus.* The latter, in particular, draws attention to the approaches used by some marine biologists in examining aspects of fecundity that have been largely ignored by freshwater biologists. If these were more widely known and adopted they would undoubtedly illuminate some central problems in the ecology of closely-related freshwater species. First, however, it seems useful and appropriate to summarise what is known about the reproductive processes that are the basis for adaptive changes in the strategies of males and females, especially in their reproductive responses to various natural temperature-regimes. I attempt to do this here for the commoner species of *Gammarus,* including those from marine and brackish habitats; to artificially treat these separately from freshwater species is both unscientific and unnecessarily restrictive.

One technical point must be made. Most if not all of the species mentioned in this article have been or are placed in the genus

Gammarus. At one time this was split into *Rivulogammarus* and *Marinogammarus,* although the division was not popularly embraced. More recently the genus has been extensively revised by taxonomists, so that some species are now (or have been or may be) allocated to genera such as *Chaetogammarus, Eulimnogammarus, Lagunogammarus* and *Pectenogammarus,* to name only a few. Having noted that these changes exist and are scientifically necessary, for the sake of simplicity I shall ignore the generic names and refer only to the specific names, which do not alter.

The female and male reproductive systems

The gonads are paired, tubular organs sited in a dorsolateral position immediately above the midgut and below the heart, in pereon (thoracic) segments 2 to 7 (Cussans 1904; LeRoux 1933; Clemens 1950; Schmitz 1967). The gonads, and eggs, are often brightly coloured by carotenoid pigments.

Ovaries

The ovaries vary in length, depending on the state of maturation. The narrow oviducts arise in pereon segment 5. They coil round the midgut and ventral caeca before opening ventrally at the bases of the fifth pair of pereopods (i.e. the third pair of large limbs behind the two pairs of gnathopods).

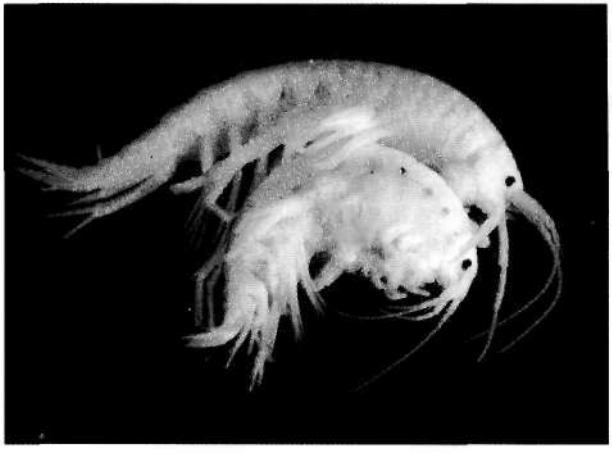

FIG. 1. A male freshwater *pulex* carrying a female in precopulatory amplexus. Photographed from preserved specimens.

Inside each ovary a single strand of large oocytes develops from a layer of oblong cells, the oogonia. The diameter of the oocytes is several times greater than that of the oviducts. The latter stretch to allow the passage of eggs as they are ejected into the ventral marsupium or broodpouch; stretching can only occur when the oviduct wall is flexible, immediately after the moult. This anatomical fact determines, very precisely, the moment when ovulation and subsequent external fertilisation can occur.

In amphipods, a "permanent ovarian hormone" is probably secreted by the primary follicular cells of the ovary (Fingerman 1987). The hormone controls development of the oostegites which form the broodpouch; they are a permanent secondary sexual characteristic of females. A "temporary ovarian hormone", which is probably secreted by the secondary follicular cells, controls the development of temporary sexual features such as the long ovigerous setae on the oostegites. These long setae appear after a moult when secondary vitellogenesis is occurring and the female is ready to mate.

Testes

In pereon segment 6 of males, each testis expands to form a seminal vesicle which in turn expands into a small canal, the vas deferens, lined with endothelial cells. These secrete a viscous substance (LeRoux 1933) that is ejected with the spermatozoa during copulation, via two genital papillae which lie in the ventral midline of pereon segment 7.

The development of eggs (oocytes) in the ovaries

Groups of oocytes develop sequentially from oogonia in the ovaries. A detailed account of the process is given by Wourms (1987). At the distal, blind end of the ovary, primordial germ cells or gonocytes differentiate by mitosis and proliferate into oogonia. These cells undergo meiotic division, unique to eggs and sperms, and become oocytes. The oocytes develop slowly during a previtellogenic stage, in which meiosis passes through prophase but stops short at diplotene; the chromosomes remain dispersed throughout the nucleoplasm. The nucleus then enlarges (the germinal vesicle stage) and the oocyte synthesises plasmic organelles, e.g. mitochondria, during a period of intense RNA synthesis. This is followed by a vitellogenic stage during which yolk is formed and the oocyte rapidly grows to its full size. The primary oocyte undergoes the first meiotic division of metaphase, at the time of ovulation (Table 1), forming a secondary oocyte in which the second meiotic division of metaphase produces the haploid condition of the unfertilised oocyte, ovum or egg.

During the second telophase of meiosis, some 2-3 hours after ovulation into the broodpouch, sperms can penetrate and fertilise the egg. Metaphase of the first subsequent mitosis (i.e. cleavage by ordinary cell division) in the diploid egg occurs about 5-7 hours after ovulation and shortly after fertilisation (Table 1).

Table 1. Time-scale (minutes) of cytological events in the oocytes of brackishwater *duebeni* at 16°C (LeRoux 1933) and freshwater *pulex* at 15°C (Orian & Callan 1957).

Stage and major events	*Time from ovulation (min)* duebeni	pulex	*Time from pre-oviposition moult of female* pulex
Diplotene: start of meiosis	–	–	15
Diakinesis	–	–	30
Prometaphase I	–	–	45-60
Metaphase I: ovulation	0	0	75
Anaphase I	0	15	90
Telophase I	15	25	100
Prometaphase II	45	35	110
Metaphase II	60	40-120	115-195
Anaphase II	75	135	210
Telophase II: sperm penetrates the egg	90-135	150	225
Approximation of pronuclei	240	150-250	225-325
Metaphase of 1st cleavage (mitosis)	300	350-450	425-525

In *duebeni* the time taken for oocytes in the ovaries to mature into eggs ready for laying is equivalent to two moult cycles of the adult female (Fig. 2). Groups of oocytes develop in sequence within each ovary; when one set has matured another is partly developed and a third set is newly-forming from oogonia in the same ovary. The largest ovaries, producing the biggest number of oocytes, occur in large mature females that have ovulated more than once (Sheader & Chia 1970).

The mature oocyte of *duebeni* is about 400 m in diameter, similar in size to the oocytes *of* some other species. The biggest (about 650 um diameter) occur in *wilkitzkii,* a large, circumpolar arctic marine species (Steele & Steele 1975a). The sizes of oocytes in *pulex* and other freshwater species appear to be unknown.

Chromosome numbers

Most species of *Gammarus* have 26 haploid and 52 diploid chromosomes. These are the usual numbers in *pulex, lacustris* and *wautieri,* which also exhibit chromosomal polymorphism, i.e. they sometimes have

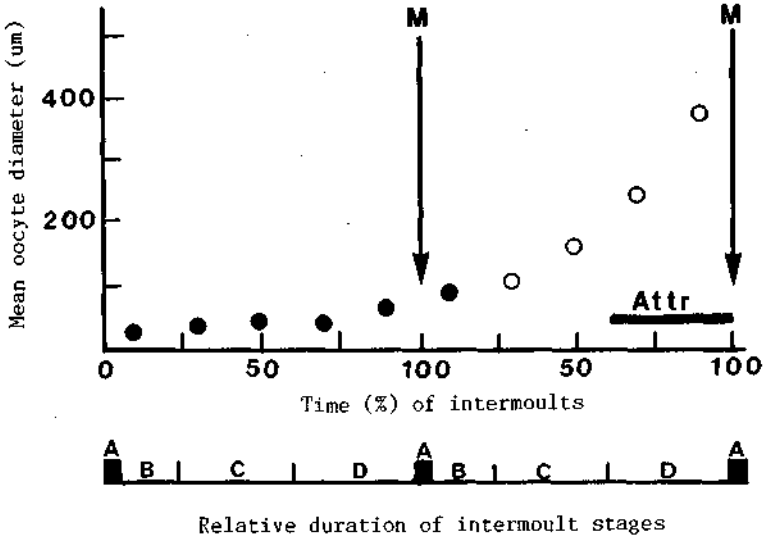

FIG. 2. Increase in mean diameter (μm) of unyolked (●) and yolked (O) oocytes through two intermoults of brackishwater *duebeni*. (After Hartnoll & Smith 1978). Subdivisions of the moult cycle (A-D) are based on values given by Martin (1965). The period when the female is sexually attractive to males is also shown (Attr); M indicates the moults.

27 haploid chromosomes. Both 26 and 27 haploid numbers can occur in oocytes from the same female, producing 52, 53 or 54 diploid chromosomes in fertilised eggs. A similar polymorphism exists in *marinus* and *pirloti,* the former having slightly low diploid numbers of 50, 51 or 52, the latter having larger than usual diploid chromosome numbers, from 59 up to 63. The variable numbers of chromosomes may be due to the presence of extra "supernumeraries", rather than originating from "fragmentation-fusion" or recombination of broken pieces of chromosomes (LeCalvez & Certain 1951; Orian & Callan 1957; Roux 1971a,b).

In a group of seven species that are restricted to Lake Ohrid in Yugoslavia, *salemaai* has only 12 haploid chromosomes, *macedonicus* has 21, *ochridensis, parechiniformis, stankokaramani* and *solidus* all have 25, and *lychnidensis* has 34 haploid chromosomes (Karaman & Pinkster 1987).

Determination of sex (gender)

Experimental evidence indicates that an individual's gender is not simply determined by "X" and "Y" chromosomes, even if these actually exist in

Gammarus — which is rather uncertain (Legrand et al. 1987). In *duebeni, zaddachi* and *pulex,* maleness and femaleness are determined by a balanced polyfactorial system of allelic sex genes held on several pairs of chromosomes (Bulnheim 1972, 1978a). The progeny of some pairs are either all males or all females. Temperature and photoperiod during development can modify the gender of some individuals. Juveniles of *duebeni,* and to a lesser extent *zaddachi,* are sensitive to diel fluctuations in daylength; more males develop after exposure to long daylight hours whereas more females develop at short daylight hours. The critical period in development is about 3 weeks after the young leave the broodpouch, at about the third moult. At 15°C, a daylength of 13-14 hours represents the photoperiod at which males and females develop in equal numbers, in European populations of *duebeni* (Bulnheim 1978b; Naylor et al. 1988). But photoperiod had little if any effect on *duebeni* from Newfoundland, Canada (Bulnheim 1978a).

Cytoplasmic inheritance

In some populations of *duebeni* a proportion of the females produce offspring that are nearly all females, and these also produce daughters. This particular form of inheritance for femaleness is cytoplasmic, due to the transmission of microsporidia (parasitic protozoans) via the egg cytoplasm. The microsporidia apparently prevent, or subdue (producing intersexes), the development of genetically-determined males. Moreover, the expression of this cytoplasmic thelygeny is affected by low water temperatures and high salinities, which inhibit the parasite's feminising influence during development (Bulnheim 1975, 1978a, b; Legrand et al. 1987). This cytoplasmic determination of sex appears to be peculiar to *duebeni;* it is not found in *pulex, salinus* or *locusta* (Bulnheim 1975)

Courtship and precopulatory amplexus: the amorous Gammarus

When a male makes bodily contact with a sexually attractive female he chases her, touches her with his divergent antennae, catches her by an appendage, "beats" against her with his abdomen and finally places her lengthwise beneath him in the position of precopulatory amplexus (Fig. 1). The male uses his first pair of gnathopods to grasp the female, by inserting the dactylus (finger) of one gnathopod between the head and first free thoracic segment; the other dactylus is inserted between the fifth and sixth free thoracic segments. The male can swim whilst carrying the female; she may remain curled up but sometimes assists by straightening her body and beating her pleopods. (Heinze 1932; Hartnoll & Smith 1978; Birkhead & Pringle 1986; Borowsky 1984; Ward 1985).

In some instances, especially when a female is larger than the male attempting to hold her, she straightens and flexes her abdomen very vigorously, breaks free from the male's grasp and swims away. Apparently the female is exercising a choice over her potential mate.

Pheromone attraction by females

In eight British species *(pulex, duebeni, locusta, salinus, zaddachi, marinus, obtusatus, pirloti)* the sexual attractiveness of females becomes prominent at oostegite stage 2.5, early in premoult (Hartnoll & Smith 1978). In *pulex* and *duebeni* the urine of females contains a chemical substance (pheromone) that makes them attractive to males. It may be the moulting hormone, ecdysone (Hammoud et al. 1975; Ducruet 1982), or another substance produced at the same time. When the pheromone is released onto the female's external body cuticle, it elicits in males the courtship behaviour described above. The pheromone is detected by chemical receptors on the male's second pair of antennae. Courtship by males of *duebeni* does not occur when a small amount of an organic surfactant, TWEEN 80, is placed in the water (Lyes 1979).

Although nearly all accounts agree that females of *Gammarus* liberate a body-contact pheromone, there is disagreement on whether or not it can also operate at a distance, borne in water-currents to nearby males (Dahl et al. 1970; Harnoll & Smith 1980; Borowsky & Borowsky 1987). However, *Gammarus* can detect other water-borne chemicals produced by a variety of invertebrates and fish (Williams & Moore 1985).

Stimulation of the male

The sexual stimulation of males by ecdysone (crustecdysone) has probably developed because the hormone controls the impending moult in the female, and this is the only time when eggs can pass down the oviduct for external fertilisation. It is therefore vital for the male to be ready to release his sperms as soon as the eggs enter the broodpouch. The detection of ecdysone indicates to the male that a female's moult is imminent. Nevertheless, perhaps an additional substance is also produced by the female, characteristic for each species and enabling males to recognise conspecific females. This could be important in habitats where several species coexist. A pheromone of this kind might be released during maturation of the oocytes.

Cross-pairing between species

Species recognition is not always perfect. Pair formation between males and females of different species occurs frequently in the laboratory when animals are confined together in small vessels, although interspecific

pairing is thought to be relatively uncommon in natural populations. The "pair-bond" between male and female in an interspecific precopula is generally weaker than it is in normal conspecific pairs.

In experiments, males and females of *pulex* and *fossarum* form mixed couples and females of both species produce eggs during interspecific matings. But the eggs are infertile, failing to develop after early gastrulation (Meijering 1972; Kolding 1986). Similar results have been obtained in crosses between the freshwater species *pulex, duebeni, fossarum, wautieri* and *gauthieri* (Dennert 1974; Goedmakers & Roux 1975), and between the saltwater species *locusta, salinus* and *zaddachi* (Kolding 1986). Unfertilised eggs are shed from the broodpouch.

The effect of temperature on precopulatory mate-guarding

In laboratory experiments, precopula time (P, days) is dependent on water temperature (7, °C), lasting for a proportionally longer time at low temperatures (Fig. 3). For *pulex* at 4.5 to 18.5°C the relationship is approximately but conveniently described by a power equation:

$$P = 168.3\ T^{-1.284}$$

For *duebeni* at 10 to 18°C, $P = 213.8T^{-1.302}$ (Steele & Steele 1969); at 6 to 17°C (Kinne 1959), $P = 97.5\ \ T^{-0.45}$.

From these equations the calculated mean time for precopula at 10°C is 8.8 days for *pulex* and 8.8-10.7 days for *duebeni.* At 18°C the respective mean times are 4.1 and 4.8-5.0 days. Quantitative information of this kind is lacking for most species.

Ward (1985, 1986) estimated precopula time in natural populations of *pulex* and *duebeni.* It varied seasonally, ranging from about 1 week in summer to about 1 month in autumn and winter, largely due to the seasonal variation in temperature.

The effect of behaviour on precopulatory mate-guarding

In experiments with *pulex* (Ward 1984), larger males guarded females for longer periods and larger females were guarded for longer periods, although there are some exceptions. Inexplicably, guarding time increased as the ratio of male to female body size (length) increased. In these experiments, at 10°C, the guarding time of 11-12 days for large males (14-15mm body length) was approximately double that of small males (10-11 mm body length).

Females in precopula sometimes "kick" by vigorously straightening the body. When this occurs repeatedly the male has difficulty in retaining his grasp, and the female may escape. She then may be taken over by another male (Ward 1984).

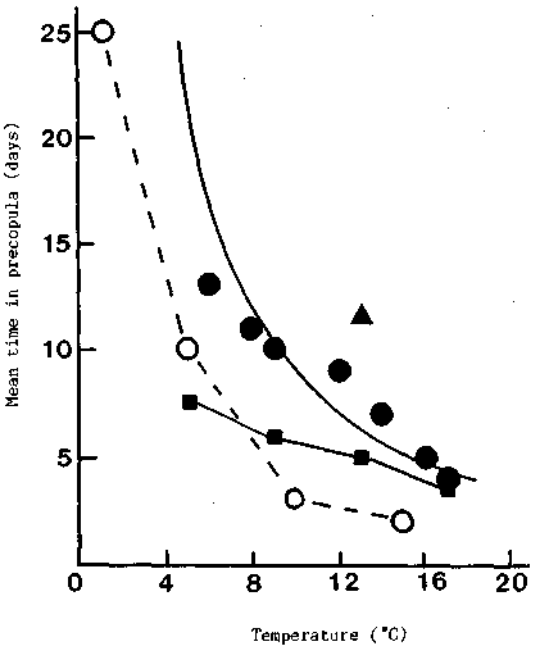

FIG. 3. Mean duration of precopula (P, days) plotted against water temperature (T, °C) in the laboratory. The solid curve is drawn from $P = 168\ T^{-1.284}$, calculated from results on 35 specimens of *pulex*; ■, means for *roeseli* (Koch-Kallnback & Meijering 1977); O, means for *pulex* (Nilsson 1977); ●, means for *duebeni* (Kinne 1959); ▲, mean for *duebeni* (Hartnoll & Smith 1978).

The effect of parasites on precopulatory mate-guarding

The normal behaviour of *Gammarus* is altered by the presence of larvae and cystacanths of acanthocephalan parasites. Infected males are less likely to guard females, infected females often do not breed (Ward 1986) and males are castrated (LeRoux 1933; Bulnheim 1978a,b).

Mating and the production of sperms

Precopula ends when the female moults and sheds the exuviae. The guarding male then turns the female ventral side up to face him, and repeatedly places sperms onto her ventral surface whilst holding her in a cross-wise position. Fertilisation is external, in the female's broodpouch (Embody 1911; Heinze 1932; LeRoux 1933; Clemens 1950; Hynes 1955; Kinne 1959; Birkhead & Clarkson 1980). Spermatozoa are ejected from a

pair of copulatory papillae, in irregular masses, mixed with a viscous substance. The male's pleopods thrust the bundles of sperms close to the openings of the female's oviducts. This is done with rapid bursts of pumping actions that usually last for less than a minute. There may be several consecutive bouts of copulatory activity. Heinze (1932) observed seven matings between one pair of *pulex,* at intervals of about 30 min; similar observations have been recorded for *duebeni* (Kinne 1959) and *fasciatus* (Clemens 1950).

Multiple matings

After mating the guarding male abandons the female. She remains sexually attractive for a short time during which she may mate with other males. However, in experiments with *pulex* the first mate-guarding male fertilised about 90% of the eggs that subsequently produced offspring (Birkhead & Pringle 1986). A high success rate for the first male may depend on his ability to place bundles of sperms adjacent to the female's oviducts, next to the gelatinous egg masses. Sperms will then not have to travel very far to fertilise the eggs. Success will also be maximised by repeated bursts of copulatory activity over a period of 2-3 hours. Penetration of the eggs by spermatozoa is possible only for a short period some 2-3 hours after ovulation (Table 1), after the mucilaginous coating disintegrates but before the eggs are enveloped in the egg-sac. Thus if the first guarding male remains with his mate until this process is more or less completed, the sperms from a second male will not be able to penetrate the eggs.

Egg laying

Eggs pass singly down each oviduct and are pushed forwards as they emerge into the broodpouch. Mucous, secreted from glands in the ovaries, immediately surrounds the eggs; the batch from each oviduct is contained in a separate gelatinous mass. Several hours later (ca. 2-3h at 15-16°C; see Table 1) the two gelatinous masses dissolve and the eggs are fertilised. Each batch of eggs is then enclosed by a sac within which the eggs move freely and expand, becoming oval in shape. Some hours later again the sacs dissolve and the eggs become distributed along the broodpouch. By this time the new cuticle of the freshly-moulted female is hardening and the long setae fringing the oostegites have extended and interlaced to prevent the eggs from falling out of the broodpouch. (Embody 1911; Sexton 1928, 1935; Heinze 1932; LeRoux 1933; Clemens 1950; Weygoldt 1958; Kinne 1959; Sheader & Chia 1970).

When females are kept without males in the laboratory, some species lay eggs but they fail to develop beyond the early stages of cleavage, like eggs that have been fertilised by males of another species. Two European

saltwater species, *plumicornis* and *aequicauda,* are interfertile in laboratory experiments and they produce live young, but interspecific pairs in precopula rarely occur in nature (Stock 1969).

Embryonic development and hatching

Detailed accounts of egg maturation and development, with numerous drawings, are given by LeRoux (1933), Weygoldt (1958), Sheader & Chia (1970) and Scholtz (1990). Amphipods differ from many other crustaceans, including isopods (e.g. *Asellus),* by having holoblastic cleavage during early development of the embryo. After fertilisation in the female's broodpouch the germinating egg takes up water and swells, changing from rounded to ovoid in shape. The egg changes colour as it increases in size and passes through a series of embryonic developmental stages (Table 2) before the fully-grown embryo hatches, at a length of about 1.5-2.0 mm in *pulex* and *duebeni.* When hatching the animal breaks through the external chorion of the egg, using spines on the posterior urosome to rupture the membranes (Weygoldt 1958; Sheader & Chia 1970).

Table 2. Stages of egg development in freshwater *pulex* at 11°C. (After McCahon & Pascoe 1988). Similar developmental characteristics have been used to describe slightly different "stages" of embryonic development of freshwater *fossarum* and *roeseli,* and saltwater *aequicauda, duebeni, insensibilis, marinus, obtusatus, oceanicus* and *stoerensis.*

Stage	Mean Age (days)	Mean width (mm)	length (mm)	Major characteristics
1	1–3	0.47	0.62	Newly fertilised, oval, black, undifferentiated egg with two membranes – the outer chorion and an inner embryonic layer enclosing the yolk.
2	2–7	0.50	0.63	Cleavage cells divide to form large non-nucleated pigment yolk cells; blastoderm forms.
3	6–13	0.52	0.66	Thorax and abdomen separated by a groove; dorsal organ present.
4	9–15	0.56	0.68	Comma-like shape, orange–red thorax; midgut and appendages developing.
5	16–19	0.55	0.70	Dorsal organ has regressed, eyespot pigmented; fully developed appendages but immobile.
6	17–22	0.62	0.75	Fully formed animal with compound eye; movements of limbs, heart and midgut (peristalsis).
7 (Hatching)	20–23	–	–	Embryo hatches, emerging in ca. 1 min, telson first, by vigorous body movements; length 1.6–1.8 mm, with 5 antennal segments.

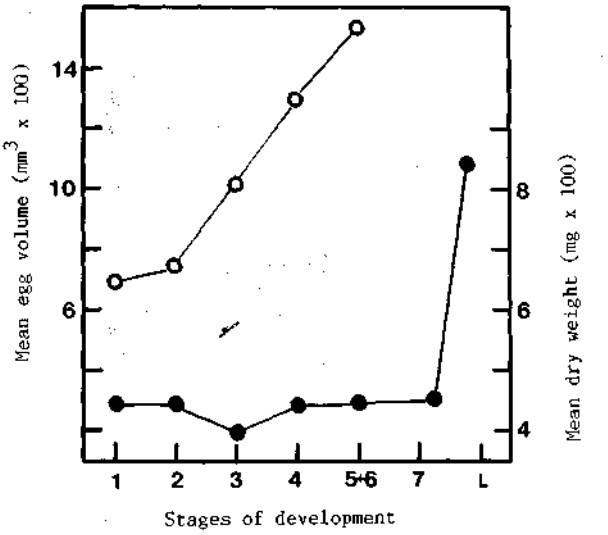

FIG. 4. Egg volume (O) of brackishwater *duebeni* increases greatly after stage 2 of embryonic development (Table 2) as the egg takes up water and swells. Dry weight (●) remains approximately constant until after the egg has hatched at stage 7. The dry weight of hatched juveniles increases markedly before they leave (L) the broodpouch, indicating that the young had fed whilst still in the broodpouch. (After Sheader 1983).

Large increases in volume due to water uptake (Fig. 4) occur after the egg has passed through stage 2 of development (Table 2). Before this occurs the ovoid eggs are relatively uniform in size (Sheader & Chia 1970); therefore eggs in stage 2 of development are normally used for measuring the sizes of eggs for comparative purposes. The volumes of eggs have been calculated in several ways by different authors, but the best method is to measure the longest axis or diameter *(L)* and the shortest *(S)* and then apply the formula for a prolate sphere:

egg volume = n x (4/3) x $(L/2)^2$ x (S/2).

Lipids and fatty acids in eggs and embryos

Reproducing females accumulate and store lipids as the ovaries mature. In *oceanicus* and *marinus,* the rates of accumulation and the amounts stored by the female are greater in spring than in winter. Lipids accumulate in the second batch of oocytes after the first batch has matured and passed into the broodpouch. Winter eggs of *oceanicus* are larger and contain more lipid and fatty acids than eggs laid in spring; they also have a slightly higher energy content (Clarke et al. 1985).

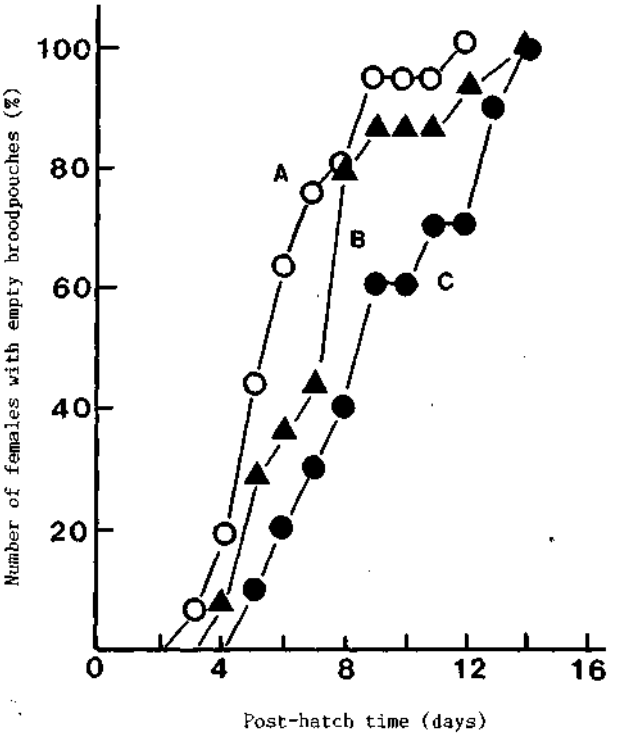

FIG. 5. Posthatch time is related to the body size of female saltwater *obtusatus* kept at 8–11°C in the laboratory. The number of females that have released all of their young from the broodpouch is expressed as a cumulative frequency (%) for (A), 16 females of ca. 9.2–10.4 mm body length; (B), 14 females of ca. 10.5–11.6mm length; (C), 10 females of ca. 12–14mm length. (After Sheader & Chia 1970).

Length of time required for embryonic development

Development time in the broodpouch consists of a period of embryonic development (egg incubation), from fertilisation of the egg to emergence of the hatchling (Table 2), and a posthatch period before the juveniles leave the broodpouch. The latter event is easier to observe and is usually used to experimentally determine the end of the period of embryonic development. The durations of both incubation and posthatch periods are chiefly dependent on water temperature, although other environmental factors such as salinity and oxygen content may also be important, as is the size of the eggs. Posthatch periods are relatively short, representing

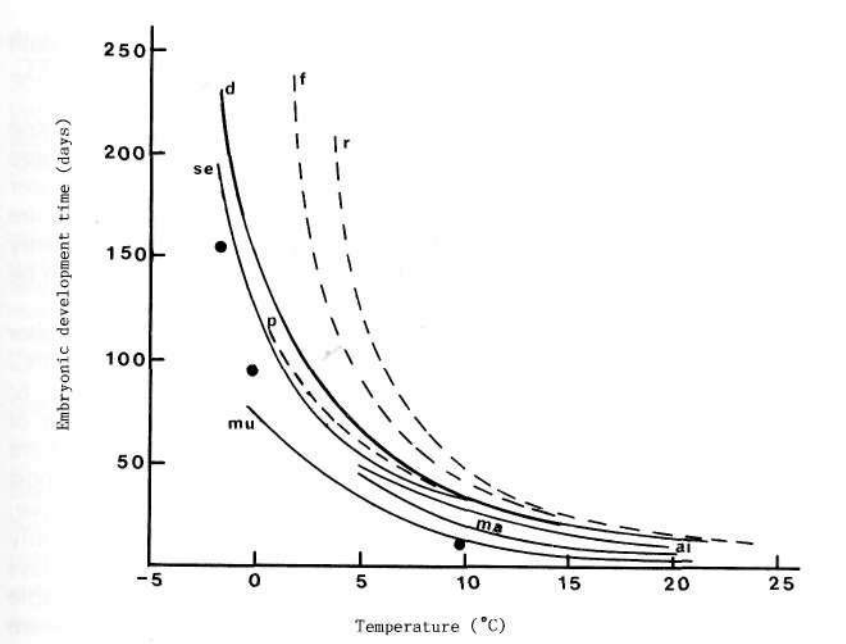

FIG. 6. Embryonic development times of some saltwater species (solid curves) and freshwater species (broken curves) related to temperature. ●, data points for *oceanicus*; ai, *aequicauda* and *insensibilis*; d, *duebeni;* f, *fossarum*; ma, *marinus*; mu, *mucronatus*; p, *pulex*; r, *roeseli*; se, *setosus*. Drawn from numerous sources.

about 10% of incubation times in *pulex* and lasting for 0-2 days at 15-25°C but two or more weeks at 1-5°C. In *obtusatus* parental body size affects the posthatch time. At 8-11°C, the median post-hatch time, when 50% of females have released all of their young, is about 6 days for small females and 9 days for large females (Fig. 5).

Effect of constant temperatures on development time of embryos

The number of days required for incubation and emergence from the broodpouch is proportionally greater at low temperatures (Fig. 6), and this is characteristic for all species of *Gammarus* that have been studied. For quantitative and statistical comparisons of development times, both within and between species, the curvilinear relationships shown in Fig. 6 have been manipulated by fitting a variety of mathematical equations to the original data (Welton & Clarke 1980; Pockl & Timischl 1990). Of these equations the simplest, though not necessarily the best fit, is a log-log regression, used to calculate the mean development times shown in Table

3. However, second-order equations should be used when dealing with results that encompass ranges of temperature greater than about 5 to 20°C, particularly at temperatures near zero Celcius.

Development times of saltwater species are generally faster than those of freshwater species, especially at temperatures near 0°C where development of the embryos may virtually cease in some freshwater species. Reasons for the faster development of saltwater species require thorough investigation on a wide range of species, but may be partly related to smaller sizes of eggs and broods; saltwater species appear to be better adapted for growth and development in cold water.

Embryonic development times are sometimes expressed as the number of degree-days above a threshold temperature, usually assumed to be 0°C. In fact the development of some saltwater species occurs at -1.5°C (Fig. 6; Steele & Steele 1973). For other (technical) reasons the calculation of degree-days for comparative purposes is best restricted to development times determined over the temperature range 5 to 20°C or, for the most accurate comparisons, at 10 to 20°C (Table 4). As indicated above, saltwater species have much faster development times, requiring only 117-270 degree-days at 10 to 20°C, compared with 313-363 degree-days in the four freshwater species for which detailed information is available (Table 3). Thus in low-salinity habitats where summer temperatures are between 10 and 20°C, broods of *tigrinus* and *zaddachi* can develop in approximately 10 to 20 days, whereas European freshwater species require some 17 to 35 days to develop from fertilisation of the egg to emergence from the broodpouch.

Effect of fluctuating temperatures on development time of embryos

The results in Fig. 6 and Tables 3 and 4 are based on experiments done at constant temperatures. However the duration of intermoults in breeding females, and hence the brood development time, is slightly reduced when specimens are kept at temperatures which fluctuate in a more natural manner. The fecundity, growth and production of *lacustris* are all greater in habitats with relatively large, natural diel fluctuations compared with relatively constant temperature regimes (Sarviro 1983/4).

Effect of egg size on development time of embryos

Smaller eggs develop faster than large eggs at a given temperature, ranging from about 17 days to 35 days at 10°C for eggs of 0.41 to 0.69mm diameter, respectively (Fig. 7). Thus species with small eggs can produce more broods per year than species with large eggs. The effects of this on breeding strategies and life cycles will be discussed later when reviewing female reproductive strategies.

Natural mortality and survival of developing embryos

Some researchers have suggested that when large numbers of eggs are laid, they could overcrowd the broodpouch and fall out of it as the eggs swell with water and greatly increase their total volume. Skadsheim (1982) and Sheader (1983) think that accidental loss of eggs and developing embryos

Table 3. Mean embryonic development times (days) of some saltwater and freshwater species, calculated from log–log regressions of mean development time versus mean temperature over the range 4 to 24°C. The table shows 95% confidence limits of calculated mean values at each of four temperatures; *n* is the number of mean values obtained from various sources. Further details are given by Welton & Clarke (1980) for *pulex*, and Pöckl & Humpesch (1990) for *fossarum* and *roeseli*.

		Embryonic development time (days)			
Species	*n*	*5°C*	*10°C*	*15°C*	*20°C*
aequicauda and *insensibilis*	8	47-68	25-30	17-19	11-15
duebeni	11	59-70	28-30	17-19	12-13
pseudolimnaeus	5	65-83	31-35	18-23	13-17
pulex	12	54-80	32-38	22-26	16-20
fossarum	9	56-95	30-41	19-27	13-21
roeseli	6	116-176	40-51	20-26	12-17

Table 4. Calculated mean numbers of degree-days required for embryonic development of some saltwater and freshwater species at 5–20°C and 10–20°C, based on mean times given in Table 3 or calculated from the literature.

	Embryonic development time (degree-days)	
Species	*5–20°C*	*10–20°C*
mucronatus	134	117
marinus	190	181
tigrinus	—	203
zaddachi	215	212
aequicauda and *insensibilis*	273	268
duebeni	283	270
pseudolimnaeus	327	313
pulex	351	357
fossarum	355	347
roeseli	455	363

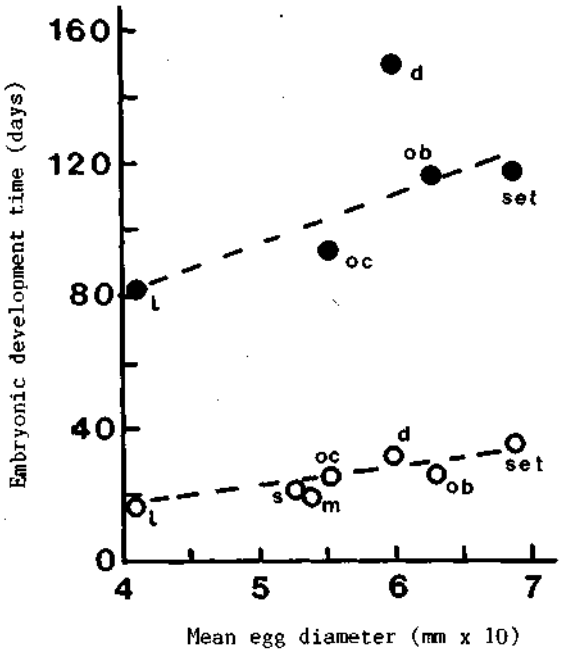

FIG. 7. Development time increases linearly with increasing egg size of saltwater species at 0°C (O) and 10°C (●). d, *duebeni;* l, *lawrencianus;* m, *marinus;* ob, *obtusatus;* oc, *oceanicus;* s, *salinus;* set, *setosus.* (Data from Steele & Steele 1975).

by this means is unlikely, at least in *duebeni, marinus* and *stoerensis,* where the presence of dead, undeveloped eggs in the broodpouch indicates that it is not easy for them to fall out; the long setae on the oostegites form an efficient barrier. Hynes (1955) found no evidence for accidental loss of embryos from *duebeni* and *pulex.* Goedmakers (1981) also found no evidence for loss of embryos during development in *pulex* and *fossarum,* except perhaps in the last stage prior to hatching, but only one-third of *berilloni* embryos hatched into live young.

Sheader & Chia (1970) record a mortality of about 30% during embryonic development of *obtusatus,* which has a relatively small number of large eggs. In *marinus,* mortality ranged from 16-38% at 15-20°C increasing to 63-84% at 9-12°C (Vlasblom 1969). High mortalities also occurred in *aequicauda* and *insensibilis* (Janssen et al. 1979). Skadsheim (1982) reported brood mortalities of 31% in *marinus* and 16-20% in *stoerensis.* The causes of death are not known for these saltwater species, but in *duebeni* high fatalities can occur at high temper-

atures and low salinity (Kinne 1959), and at very low temperatures and very high salinities (Sheader 1983; Naylor et al. 1988). Hungry females of *duebeni* apparently eat their developing broods (Sheader 1983).

Very high or low temperatures are generally a major cause of death for developing embryos. Those of marine species are particularly prone to suffer high mortalities when air temperatures are around 0°C. The effect of water temperature on two freshwater species is examined in more detail below.

Effect of temperature on mortality and survival of embryos in two freshwater species

The most detailed study of egg/embryo mortality is that of Pockl & Humpesh (1990) on *fossarum* and *roeseli* from streams in Austria. Batches of eggs were carefully removed from broodpouches of pregnant females, and eggs at stage 2 of development were then cultured in petri-dishes containing streamwater held at a series of constant temperatures, ranging from 2 to 26°C. (This *in vitro* technique has also been used by others studying the development of eggs and embryos). The number of embryos that hatched into live young was greatest at 8 to 12°C, for *fossarum,* where some 70-80% of the embryos survived, and at 10 to 16°C for *roeseli,* where some 40-50% survived (Fig. 8). At higher and lower temperatures, mortality of the embryos increased, being 100% at 26°C in both species.

Very similar results were obtained in experiments where females carried the developing embryos in their broodpouches, in the normal manner. The number of *in vivo* broods producing live young was expressed as a percentage of the total number of brood-bearing females kept at each experimental temperature (Fig. 8). The optimum mean temperature for this "reproductive success" was 12°C for *fossarum,* where *77%* of the females hatched live young; 50% or more hatched at 4.5 to 19.1°C. The optimum mean temperature was 14°C for *roeseli,* where 76% hatched live young; 50% or more hatched at 7.0 to 21.0°C (Fig. 8).

Ovarian diapause and the non-breeding resting period

At some period of the year, usually between mid summer and early winter, mature ovigerous females fail to pair with males in readiness for mating at the next moult. Instead, when the newly-hatched young are released from the broodpouch and the female moults, her oostegites are slightly reduced in size and the long fringing setae are absent. These behavioural and morphological changes are preceded by changes in activity of the ovaries: Oogonia, which normally enlarge in the ovary whilst the female is brooding the previous batch of eggs, do not develop; regression of the oostegites (which form the broodpouch) then follows at the next moult and breeding stops.

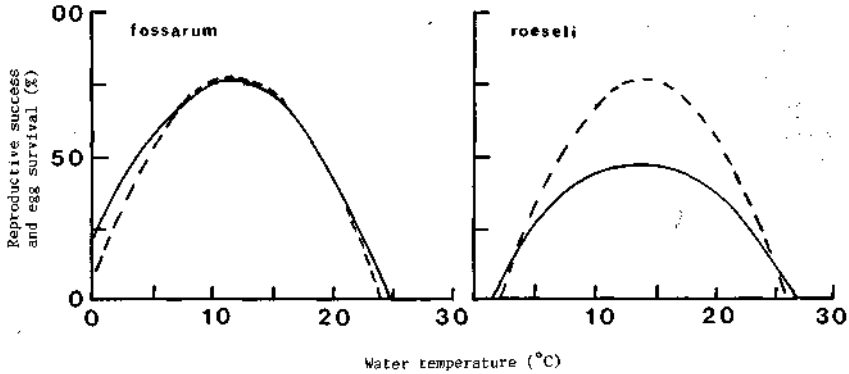

FIG. 8. The survival of developing embryos from stage 2 to hatching is similar when experimentally kept in petri-dishes (solid curves) and in the mother's broodpouch (broken curves). In *fossarum* (left) survival was maximal at about 12°C and in *roeseli* (right) at about 14°C. (After Pöckl & Humpesch 1990).

Diapause

The cessation of ovarian activity may be a true diapause in which normal development is suspended and cannot be resumed — even when conditions appear to be favourable — until the diapause is "broken" or terminated by a specific stimulus.

At the appropriate time of year for the resting period, females that otherwise would become sexually mature at the next moult do not do so; instead they also enter the ovarian diapause and fail to develop full-sized oostegites with fringing setae. Males also become sexually inactive and may lose some of the long setae that are secondary sexual characteristics (Hynes 1955), suggesting that there is a testicular diapause, at least in *pulex* and in *tigrinus* (studied by Hynes under the name *fasciatus).* Interestingly, in these two species Hynes found that males begin and end the resting period earlier than the females, ensuring that the latter have mates to guard them in precopula when the development of oogonia recommences. The incidence of non-breeding is 100% in some populations (or localities) but a few breeding females may occur in other populations of the same species. Nevertheless the majority of females have a resting period and a diapause that lasts for some 2-4 months or longer in large Arctic marine species.

The role of ovarian hormones in the resting period

The loss of setae and partial regression of the oostegites is directly linked to ovarian activity. When the development of oocytes and vitellogenesis

are stopped by sterilisation with radium or by parasitic castration, females of *duebeni* exhibit the same morphological changes that occur naturally during the resting period (LeRoux 1933). When the ovaries become active again and oocytes start to enlarge, the female regains fully-developed oostegites with fringing setae at her next moult. These changes are regulated by "primary" and "secondary" ovarian hormones produced by the ripening ovaries (see the section on *Ovaries,* p. 104).

Induction of ovarian diapause and resting periods

For several freshwater and saltwater (brackish) species of *Gammarus,* falling temperatures have been suggested as environmental stimuli responsible for inducing the resting period (Kinne 1959; Hynes 1955). However, experimental exposure to low temperatures did not induce a resting period in *tigrinus* from England (Hynes 1955) and experiments on *lacustris, duebeni, lawrencianus, setosus, oceanicus* and *obtusatus* (see below) indicate that changes in photoperiods (i.e. changing daylengths) are important for the onset and termination of the resting period and the ovarian diapause that controls it. In crustaceans the endogenous control of gametogenesis is brought about by hormones, including ecdysone, which are activated via neurosecretory centres in the head; these are known to be sensitive to photoperiods.

Experimental manipulation of the diapause

Specimens of *lawrencianus* were collected in May from a population breeding in a Newfoundland estuary, and were induced to enter ovarian diapause and a resting period in the laboratory (Steele & Steele 1986). This was done by exposing cultures of the naturally reproducing *lawrencianus* to short-day photoperiods for various lengths of time. Diapause was not induced by short exposure times lasting for only a few days, but after 61 days at 12 hours of light and 12 hours of darkness (12L: 12D), breeding had stopped in 20% of the females. This increased to 95% after 92 days at 12L:12D and 100% after 123 days at 8L:16D, i.e. for the majority of females the stimulus to enter diapause required continuous exposure to short daylengths for more than 2 months at 10°C (Fig. 9). After 169 days at short daylengths, some females terminated the diapause and began breeding again. Females induced to diapause and then transferred to long daylengths (18L:6D) started breeding within 42 days. Breeding females kept in constant darkness continued to reproduce for 169 days before some of them entered diapause. In constant light and at 18L:6D, reproduction continued for more than 199 days without pause.

Thus reproduction of *lawrencianus* is continuous at long daylengths (in summer), but short days (in autumn) induce an ovarian diapause; this is

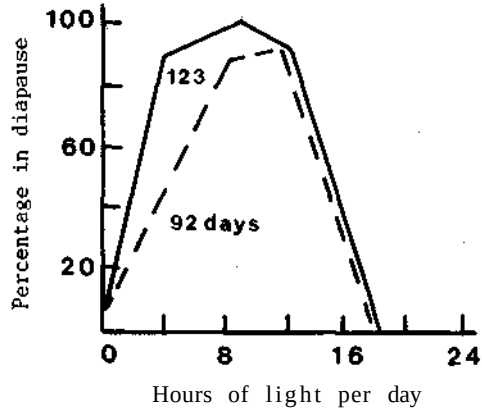

FIG. 9. Ovarian diapause in breeding females of saltwater *lawrencianus* is experimentally induced by exposure to short photoperiods at 10°C. The percentage that stopped breeding and entered diapause was maximal at 12L : 12D after 92 days and 8L: 16D after 1 23 days of exposure. Breeding continued for at least 199 days in females exposed to long photoperiods (>18L:6D). (After Steele & Steele 1986).

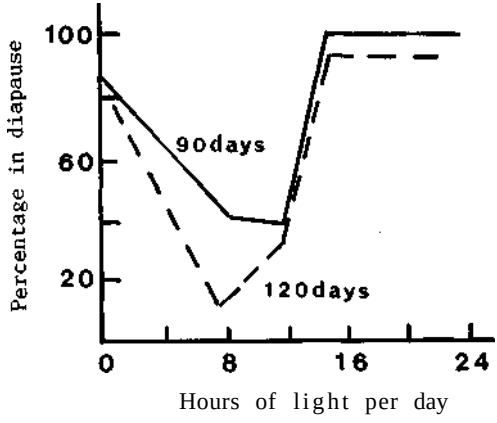

FIG. 10. Ovarian diapause in females of saltwater *setosus* is experimentally terminated by exposure to short photoperiods at 10°C, although not all of the females started to breed. The percentage remaining in diapause was minimal at 12L: 12D after 90 days and 8L: 16D after 120 days. Diapause continued in 90-100% of females kept for at least 160 days at long photoperiods (14L:10D and 20L:4D). (After Steele & Steele 1986).

terminated relatively quickly by subsequent exposure to long days in spring. In addition, diapause ends spontaneously after lengthy periods of

time (169 days), even at short daylengths. Therefore ovarian diapause appears to be basically under endogenous control, with photoperiod (changing daylength) acting as an environmental "primer" that sets the clock for the annual reproductive cycle.

The natural summer ovarian diapause of *setosus,* a northern Arctic intertidal species, is terminated by short daylengths (Fig. 10). This ensures that ovulation occurs in autumn and the embryos develop during the dark winter months to hatch in spring, when food is readily available. Although the breeding cycle is the opposite of that found in *lawrencianus,* the basic reproductive cycle of *setosus* is also driven endogenously (Steele & Steele 1986).

Mature specimens of the freshwater species *lacustris,* from Manitoba, Canada, also need exposure to short daylengths (<12 hours) or dim light before starting to reproduce in laboratory cultures (DeMarch 1982).

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